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1. Charakterystyka produktu leczniczego Rydapt 02/2021.
2. Zgodnie z aktualnym Obwieszczeniem Ministra Zdrowia w sprawie wykazu refundowanych leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych. Program lekowy: • Załącznik B.114. Leczenie chorych na ostrą białaczkę szpikową (ICD-10 C92.0) • Załącznik B.115. Leczenie agresywnej mastocytozy układowej, mastocytozy układowej z współistniejącym nowotworem układu krwiotwórczego oraz białaczki mastocytarnej (ICD-10: C96.2, C94.3, D47.9).

NOVARTIS

NOWA terapia dla pacjentów z przewlekłą anemią w przebiegu MDS

PRZYWRACA PRAWIDŁOWE DOJRZEWANIE KOMÓREK LINII ERYTROIDALNEJ

Wskazanie do stosowania u dorosłych pacjentów z:
(konieczne spełnienie wszystkich warunków)[1]

- anemią zależną od transfuzji
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- z obecnością pierścieniowatych syderoblastów
- u których wystąpiła niedostateczna odpowiedź na leczenie erytropoetyną lub niekwalifikujących się do takiego leczenia

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Nazwa produktu leczniczego: Reblozyl (luspatercept) 25 mg, 75 mg, proszek do sporządzania roztworu do wstrzykiwań. **Skład:** Każda fiolka zawiera odpowiednio 25 mg lub 75 mg luspaterceptu. Po rekonstytucji każdy ml roztworu zawiera 50 mg luspaterceptu. Luspatercept jest wytwarzany w komórkach jajnika chomika chińskiego (ang. *Chinese Hamster Ovary*, CHO) za pomocą technologii rekombinacji DNA. **Postać farmaceutyczna:** Proszek do sporządzania roztworu do wstrzykiwań (proszek do sporządzania płynu do wstrzykiwań). Biały lub białawy liofilizowany proszek. **Wskazania do stosowania**: Produkt leczniczy Reblozyl jest wskazany do stosowania w leczeniu dorosłych pacjentów, z niedokrwistością zależną od transfuzji z powodu zespołów mielodysplastycznych (ang. *myelodysplastic syndromes*, MDS) o ryzyku bardzo niskim, niskim i średnim z obecnością pierścieniowatych syderoblastów, u których wystąpiła niedostateczna odpowiedź na leczenie erytropoetyną, lub którzy nie kwalifikują się do takiego leczenia. **Dawkowanie i sposób podawania:** Leczenie produktem leczniczym Reblozyl powinien rozpoczynać lekarz doświadczony w leczeniu chorób hematologicznych. Dawkowanie: Przed każdorazowym podaniem produktu leczniczego Reblozyl należy ocenić poziom hemoglobiny (Hb) pacjentów. Jeśli przed podaniem dawki zostanie przeprowadzona transfuzja czerwonych krwinek, na potrzeby dawkowania należy wziąć pod uwagę poziom Hb sprzed transfuzji. *Zespoły mielodysplastyczne:* Zalecana dawka początkowa produktu leczniczego Reblozyl to 1,0 mg/kg raz na 3 tygodnie. W przypadku pacjentów, którzy nie są niezależni od transfuzji czerwonych krwinek po podaniu co najmniej 2 kolejnych dawek w dawce początkowej wynoszącej 1,0 mg/kg, dawka powinna zostać zwiększona do 1,33 mg/kg. Jeśli pacjenci nie są niezależni od transfuzji czerwonych krwinek po podaniu co najmniej 2 kolejnych dawek wynoszących 1,33 mg/kg, dawka powinna zostać zwiększona do 1,75 mg/kg. Zwiększanie dawki nie powinno następować częściej niż co 6 tygodni (2 podania) i nie powinno przekraczać maksymalnej dawki wynoszącej 1,75 mg/kg co 3 tygodnie. Nie należy zwiększać dawki bezpośrednio po opóźnieniu przyjęcia dawki. W przypadku pacjentów, u których stężenie Hb przed podaniem dawki będzie wynosić >9 g/dl i którzy jeszcze nie osiągnęli niezależności od transfuzji, może być wymagane zwiększenie dawki wedle uznania lekarza. Nie można wykluczyć ryzyka, że w przypadku jednoczesnego stosowania transfuzji, stężenie Hb wzrośnie do poziomu wyższego niż stężenie docelowe. Jeśli u pacjenta nastąpi utrata odpowiedzi (tj. niezależności od transfuzji), dawka powinna zostać zwiększona o jeden poziom dawki. *MDS Zmniejszenie dawki i opóźnione podanie dawki:* W przypadku zwiększenia poziomu Hb o wartość > 2 g/dl w ciągu 3 tygodni leczenia luspatercept przy braku transfuzji, dawka produktu leczniczego Reblozyl powinna zostać zmniejszona o jeden poziom dawki. Jeśli poziom Hb wynosi ≥ 11,5 g/dl przy braku transfuzji przez co najmniej 3 tygodnie, podanie dawki powinno zostać opóźnione do momentu, aż poziom Hb osiągnie wartość ≤11,0 g/dl. W przypadku towarzyszącego szybkiego wzrostu poziomu Hb (> 2 g/dl w ciągu 3 tygodni przy braku transfuzji) należy rozważyć zmniejszenie dawki do jednego poziomu w dół (minimum 0,8 mg/kg) po podaniu dawki z opóźnieniem. Nie należy zmniejszać dawki poniżej poziomu 0,8 mg/kg. Poniżej podano dane dotyczące zmniejszania dawki podczas leczenia luspaterceptem.

Tabela1: Dane dotyczące zmniejszania dawki w przypadku MDS

Aktualna dawka	Zmniejszenie dawki
1,75 mg/kg	1,33 mg/kg
1,33 mg/kg	1mg/kg
1mg/kg	0,8 mg/kg

Jeżeli u pacjenta wystąpią utrzymujące się działania niepożądane stopnia 3 lub wyższego związane z leczeniem, leczenie powinno zostać opóźnione do momentu poprawy w zakresie toksyczności lub powrotu do stanu wyjściowego. Po opóźnieniu podania dawki pacjenci powinni ponownie rozpocząć leczenie od poprzedniej dawki lub zmniejszoną dawką zgodnie z wytycznymi dotyczącym i zmniejszania dawki. *Pominięcie dawek:* W przypadku pominięcia lub opóźnienia w stosunku do zaplanowanego podania leku pacjentowi należy jak najszybciej podać produkt leczniczy Reblozyl, a dawkowanie powinno być kontynuowane zgodnie z zaleceniami, z odstępem co najmniej 3 tygodni pomiędzy dawkami. *Pacjenci, u których nastąpiła utrata odpowiedzi:* Jeżeli u pacjentów nastąpi utrata odpowiedzi na leczenie z zastosowaniem produktu leczniczego Reblozyl, należy ocenić czynniki przyczynowe (np. epizod krwawienia). Jeżeli typowe przyczyny utraty odpowiedzi hematologicznej zostaną wykluczone, należy rozważyć zwiększenie dawki w sposób opisany powyżej dla konkretnego wskazania leczniczego. *Przerwanie stosowania produktu leczniczego*: Stosowanie produktu leczniczego Reblozyl należy przerwać, jeżeli u pacjenta nie nastąpi zmniejszenie zależności od transfuzji po 9 tygodniach leczenia (3 dawkach) przy maksymalnym poziomie dawki, jeżeli nie stwierdzono innych przyczyn wyjaśniających niepowodzenie odpowiedzi (np. krwawienie, zabieg chirurgiczny, inne choroby współistniejące)lub w przypadku wystąpienia niedopuszczalnej toksyczności. Szczególne populacje: *Pacjenci w podeszłym wieku*: Dostosowanie dawki początkowej nie jest wymagane w przypadku produktu leczniczego Reblozyl. *Pacjenci z zaburzeniami czynności wątroby* Dostosowanie dawki początkowej nie jest wymagane w przypadku pacjentów, u których poziom bilirubiny całkowitej (BIL) wynosi > górnej granicy normy (GGN) i (lub) aminotransferazy alaninowej (ALT) lub aminotransferazy asparaginianowej (AST) wynosi < 3 x GGN. Nie można sformułować konkretnych zaleceń dotyczących dawkowania w przypadku pacjentów z ALT lub AST ≥ 3xGGN lub uszkodzeniem wątroby stopnia ≥3 wg CTCAE z powodu braku danych. *Pacjenci z zaburzeniami czynności nerek:* Dostosowanie dawki początkowej nie jest wymagane w przypadku pacjentów z zaburzeniami czynności nerek o nasileniu łagodnym do umiarkowanego [szacunkowy współczynnik przesączania kłębuszkowego (ang. *estimated glomerular filtration rate*, eGFR) od < 90 do ≥ 30 ml/min/1,73 m^2]. Nie można sformułować konkretnych zaleceń dotyczących dawkowania w przypadku pacjentów z ciężkimi zaburzeniami czynności nerek (eGFR <30 ml/min/1,73 m^2) z powodu braku danych klinicznych. Pacjenci z zaburzeniami czynności nerek w punkcie wyjściowym powinni być uważnie monitorowani pod kątem czynności nerek w ramach leczenia standardowego. *Dzieci i młodzież* Stosowanie produktu leczniczego Reblozyl u dzieci i młodzieży we wskazaniu zespoły mielodysplastyczne nie jest właściwe. Sposób podawania: Podanie podskórne. Po rekonstytucji roztwór produktu leczniczego Reblozyl należy wstrzyknąć podskórnie w górną część ramienia, udo lub brzuch. Należy obliczyć dokładną całkowitą objętość sporządzonego roztworu wymaganego dla pacjenta i pobrać ją powoli z jednodawkowej fiolki/fiolek do strzykawki. Zalecana maksymalna objętość produktu leczniczego w miejscu wstrzyknięcia wynosi 1,2 ml. Jeśli wymagane jest podanie więcej niż 1,2 ml, całkowita objętość powinna zostać podzielona na oddzielne wstrzyknięcia o podobnej objętości i podawana w różne miejsca. Jeśli wymagane są wielokrotne wstrzyknięcia, do każdego wstrzyknięcia podskórnego należy użyć nowej strzykawki i igły. Z fiolki należy podać nie więcej niż jedną dawkę. Jeśli roztwór produktu leczniczego Reblozyl został schłodzony po rekonstytucji, należy go wyjąć z lodówki 15–30 minut przed wstrzyknięciem, aby umożliwić osiągnięcie temperatury pokojowej. Zwiększy to komfort wstrzyknięcia. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą. Ciąża. **Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania** Identyfikowalność: W celu poprawienia identyfikowalności biologicznych produktów leczniczych należy czytelnie zapisać nazwę i numer serii podawanego produktu. Podwyższone ciśnienie krwi: W kontrolowanych badaniach klinicznych dotyczących MDS i β-talasemii u pacjentów leczonych luspatercept wykazano średni wzrost skurczowego i rozkurczowego ciśnienia krwi o 5 mm Hg w stosunku do wartości wyjściowej. Ciśnienie krwi należy monitorować przed każdym podaniem luspatercept. W przypadku utrzymującego się nadciśnienia lub zaostrzenia występującego wcześniej nadciśnienia pacjenci powinni otrzymywać leczenie nadciśnienia zgodnie z aktualnymi wytycznym i klinicznymi. Zawartość sodu Ten lek zawiera mniej niż 1 mmol (23 mg) sodu na dawkę, to znaczy lek uznaje się za „wolny od sodu". **Działania niepożądane:** Zarys profilu bezpieczeństwa: *Zespoły mielodysplastyczne:* Najczęściej zgłaszanymi działaniami niepożądanymi u pacjentów przyjmujących produkt leczniczy Reblozyl (u co najmniej 15% pacjentów) były zmęczenie, biegunka, osłabienie, nudności, zawroty głowy, ból pleców i ból głowy. Najczęściej zgłaszanymi działaniami niepożądanymi stopnia 3 lub wyższego (u co najmniej 2% pacjentów) były między innymi omdlenie/stan przedomdleniowy, zmęczenie, nadciśnienie tętnicze i osłabienie. Najczęściej zgłaszanymi ciężkimi działaniami niepożądanymi (u co najmniej 2% pacjentów) były zakażenie układu moczowego, ból pleców i omdlenie. Osłabienie, zmęczenie, zawroty głowy i ból głowy występowały częściej w trakcie pierwszych 3 miesięcy leczenia. Przerwanie leczenia z powodu działania niepożądanego nastąpiło u 2,0% pacjentów leczonych luspaterceptem. Działaniami niepożądanymi prowadzącymi do przerwania leczenia w grupie leczonej luspaterceptem były zmęczenie i ból głowy. Tabelaryczny wykaz działań niepożądanych Poniższa Tabela 2 zawiera dane dotyczące najwyższej częstotliwości występowania każdego działania niepożądanego, które zaobserwowano i zgłaszano w dwóch przed rejestracyjnych badaniach dotyczących MDS i β-talasemii. Działania niepożądane wymieniono poniżej według klasyfikacji układów i narządów i preferowanych terminów. Częstość występowania z definiowano w następujący sposób: bardzo często (≥ 1/10); często (≥ 1/100 do < 1/10); niezbyt często (≥ 1/1 000 do < 1/100); rzadko (≥ 1/10 000 do < 1/1 000) i bardzo rzadko(<1/10 000)

Tabela 2. Działania niepożądane u pacjentów leczonych produktem leczniczym Reblozylwprzypadku MDS

Klasyfikacja układów i narządów	Preferowany termin	Częstość (wszystkie stopnie)
Zakażenia i zarażenia pasożytnicze	zapalenie oskrzeli	Bardzo często
	zakażenie dróg moczowych	Bardzo często
	zakażenie górnych dróg oddechowych	Często
	grypa	Często
Zaburzenia układu immunologicznego	nadwrażliwość*	Często
Zaburzenia metabolizmu i odżywiania	hiperurykemia	Często
Zaburzenia układu nerwowego	zawroty głowy	Bardzo często
	ból głowy	Bardzo często
	omdlenie/stan przedomdleniowy	Często
Zaburzenia ucha i błędnika	zawroty głowy pochodzenia błędnikowego lub pozycyjne zawroty głowy pochodzenia błędnikowego	Często
Zaburzenia naczyniowe	nadciśnienie tętnicze~	Często
	zdarzenia zakrzepowo-zatorowe§	Często
Zaburzenia układu oddechowego, klatki piersiowej i śródpiersia	duszność	Bardzo często
Zaburzenia żołądka i jelit	biegunka	Bardzo często
	nudności	Bardzo często
Zaburzenia mięśniowo-szkieletowe i tkanki łącznej	ból pleców	Bardzo często
	bóle stawów	Często
	ból kości	Często
Zaburzenia ogólne i stany w miejscu podania	zmęczenie	Bardzo często
	osłabienie	Bardzo często
	reakcje w miejscu wstrzyknięcia#	Często

*Nadwrażliwość obejmuje obrzęk powiek, nadwrażliwość na lek, obrzęk twarzy, obrzęk około oczodołowy, obrzęk twarzy, obrzęk naczynioruchowy, obrzęk warg, wysypkę polekową. ~Reakcja nadciśnieniowa obejmuje samoistne nadciśnienie, nadciśnienie i przełom nadciśnieniowy. #Reakcje w miejscu wstrzyknięcia obejmują rumień w miejscu wstrzyknięcia, świąd w miejscu wstrzyknięcia, obrzęk w miejscu wstrzyknięcia i wysypkę w miejscu wstrzyknięcia. §Zdarzenia zakrzepowo-zatorowe obejmują zakrzepicę żył głębokich, zakrzepicę żył wrotnych, udar niedokrwienny i zatorowość płucną. Opis wybranych działań niepożądanych: *Ból kości*: Ból kości zgłoszono u 19,7% pacjentów z β-talasemią leczonych luspaterceptem (placebo 8,3%) oraz u 2,6% pacjentów z MDS leczonych luspaterceptem (placebo 3,9%). U pacjentów z β-talasemią leczonych luspaterceptem ból kości występował najczęściej w okresie pierwszych 3 miesięcy (16,6%) w porównaniu z miesiącami 4–6 (3,7%). Większość zdarzeń (41/44 zdarzeń) miała stopień nasilenia 1–2, przy czym 3 zdarzenia miały stopień nasilenia 3. Jedno z 44 zdarzeń było poważne, a 1 zdarzenie doprowadziło do przerwania leczenia. *Bóle stawów*: Bóle stawów zgłoszono u 19,3% pacjentów z β-talasemią leczonych luspaterceptem (placebo 11,9%) oraz u 5,2% pacjentów z MDS leczonych luspaterceptem (placebo 11,8%). W grupie pacjentów z β-talasemią leczonych luspaterceptem bóle stawów doprowadziły do przerwania leczenia u 2 pacjentów (0,9%). *Nadciśnienie tętnicze*: U pacjentów leczonych luspaterceptem wystąpił średni wzrost skurczowego i rozkurczowego ciśnienia krwi o 5 mm Hg w stosunku do punktu wyjściowego, którego nie zaobserwowano u pacjentów przyjmujących placebo. Nadciśnienie zgłoszono u 8,5% pacjentów z MDS leczonych luspaterceptem (placebo 9,2%) oraz u 8,1% pacjentów z β-talasemią leczonych luspaterceptem (placebo 2,8%). W grupie pacjentów z MDS zdarzenia o stopniu nasilenia 3 zgłoszono u 5 pacjentów (3,3%) leczonych luspaterceptem i u 3 pacjentów (3,9%) przyjmujących placebo. Nie przerwano leczenia w przypadku żadnego z pacjentów z powodu nadciśnienia. W grupie pacjentów z β-talasemią zdarzenia o stopniu nasilenia 3 zgłoszono u 4 pacjentów (1,8%) leczonych luspaterceptem (0,0% placebo).Nie przerwano leczenia w przypadku żadnego z pacjentów z powodu nadciśnienia. *Nadwrażliwość*: Reakcje typu nadwrażliwości (w tym obrzęk powiek, nadwrażliwość na lek, opuchlizna twarzy, obrzęk okołooczodołowy, obrzęk twarzy, obrzęk naczynioruchowy, obrzęk warg,wysypka polekowa) zgłoszono w przypadku 4,6% pacjentów z MDS (2,6% placebo) oraz 4,5% pacjentów z β-talasemią leczonych luspaterceptem (1,8% placebo). W badaniach klinicznych wszystkie zdarzenia miały stopień nasilenia 1/2. W grupie pacjentów z β-talasemią leczonych luspaterceptem nadwrażliwość doprowadziła do przerwania leczenia u 1 pacjenta (0,4%). *Reakcje w miejscu wstrzyknięcia*: Reakcje w miejscu wstrzyknięcia (w tym rumień w miejscu wstrzyknięcia, świąd w miejscu wstrzyknięcia, obrzęk w miejscu wstrzyknięcia i wysypka w miejscu wstrzyknięcia)zgłoszono w przypadku 3,9% pacjentów z MDS (placebo 0,0%) i u 2,2% pacjentów z β-talasemią otrzymujących luspatercept (placebo 1,8%). W badaniach klinicznych wszystkie zdarzenia miały stopień nasilenia 1, a żadne nie doprowadziło do przerwania leczenia. *Zdarzenia zakrzepowo-zatorowe*: Zdarzenia zakrzepowo-zatorowe (w tym zakrzepica żył głębokich, zakrzepica żył wrotnych, udar niedokrwienny i zatorowość płucna) wystąpiły u 3,6% pacjentów z β-talasemią otrzymujących luspatercept (placebo 0,9%). Wszystkie zdarzenia zgłoszono u pacjentów, którzy zostali poddani splenektomii, i u których występował co najmniej jeden inny czynnik ryzyka. Nie zaobserwowano różnic w zakresie zdarzeń zakrzepowo-zatorowych pomiędzy grupami otrzymującymi miluspatercept i placebo w przypadku pacjentów z MDS. *Immunogenność* W badaniach klinicznych dotyczących MDS analiza 260 pacjentów z MDS, którzy byli leczeni luspaterceptem i kwalifikowali się do oceny obecności przeciwciał przeciw luspaterceptowi, wykazała, że 23 (8,8%) pacjentów z MDS uzyskało dodatni wynik badania pod kątem obecności przeciwciał przeciw luspaterceptowi wytworzonych w toku leczenia, w tym u 9 (3,5%) pacjentów z MDS stwierdzono przeciwciała neutralizujące przeciw luspaterceptowi. W badaniach klinicznych dotyczących β-talasemi i analiza 284 pacjentów z β-talasemią, którzy byli leczeni luspaterceptem i kwalifikowali się do oceny obecności przeciwciał przeciw luspaterceptowi, wykazała, że 4 (1,4%) pacjentów z β-talasemią uzyskało dodatni wynik badania pod kątem obecności przeciwciał przeciw luspaterceptowi wytworzonych w toku leczenia, w tym u 2 (0,7%) pacjentów z β-talasemią stwierdzono przeciwciała neutralizujące przeciw luspaterceptowi. Stężenie luspaterceptu w surowicy wykazywało tendencję malejącą w obecności przeciwciał neutralizujących. Nie odnotowano ciężkich systemowych reakcji nadwrażliwości u pacjentów, u których występowały przeciwciała przeciw luspaterceptowi. Nie występował związek pomiędzy reakcjami typu nadwrażliwości lub reakcjami w miejscu wstrzyknięcia a obecnością przeciwciał przeciw luspaterceptowi.

▼Niniejszy produkt leczniczy będzie dodatkowo monitorowany. Umożliwi to szybkie zidentyfikowanie nowych informacji o bezpieczeństwie. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane.

Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nie przerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem: Departament Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, 02-222 Warszawa, Tel.: + 48 22 49 21 301, Faks: + 48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl

Podmiot Odpowiedzialny:
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Numery pozwoleń na dopuszczenie do obrotu wydanych przez Komisję Europejską:
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Kategoria dostępności:
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Przed zastosowaniem leku należy zapoznać się z Charakterystyką Produktu Leczniczego.
Przedstawiciel podmiotu odpowiedzialnego:
Celgene Sp. z o.o., Al. Armii Ludowej 26,
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▼Niniejszy produkt leczniczy będzie dodatkowo monitorowany. Umożliwi to szybkie zidentyfikowanie nowych informacji o bezpieczeństwie. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane. Aby dowiedzieć się, jak zgłaszać działania niepożądane – patrz punkt „Działania niepożądane".

Postać, skład: Kapsułka, miękka. Każda kapsułka miękka zawiera 25 mg midostauryny. Substancje pomocnicze o znanym działaniu: Każda kapsułka miękka zawiera około 83 mg bezwodnego etanolu i 415 mg hydroksystearynianu makrogloglicerolu. **Wskazania:** Lek Rydapt jest wskazany: • w skojarzeniu ze standardową chemioterapią indukcyjną daunarubicyną i cytarabiną oraz konsolidacyjną dużymi dawkami cytarabiny, oraz u pacjentów z całkowitą odpowiedzią, a następnie jako monoterapia podtrzymująca lekiem Rydapt u dorosłych pacjentów z noworozpoznaną ostrą białaczką szpikową (ang. *acute myeloid leukaemia* – AML) z mutacją genu FLT3 (patrz punkt 4.2 ChPL); • w monoterapii w leczeniu dorosłych pacjentów z agresywną mastocytozą układową (ASM), mastocytozą układową z nowotworem układu krwiotwórczego (SM-AHN) lub białaczką mastocytarną (MCL). **Dawkowanie:** Leczenie lekiem Rydapt powinno być rozpoczynane przez lekarza posiadającego doświadczenie w stosowaniu leków przeciwnowotworowych. Przed przyjęciem midostauryny, u pacjentów z AML należy potwierdzić występowanie mutacji FLT3 (wewnątrztandemowej duplikacji [ITD] lub mutacji w obrębie domeny kinazy tyrozynowej [TKD]) przy pomocy zwalidowanego testu. Dawkowanie: Lek Rydapt należy przyjmować doustnie dwa razy na dobę w odstępach około 12-godzinnych. Kapsułki należy przyjmować z pokarmem. Leki zapobiegające wymiotom należy podawać zgodnie z lokalnie obowiązującą praktyką medyczną, w zależności od tolerancji pacjenta. *AML:* Zalecana dawka leku Rydapt wynosi 50 mg doustnie dwa razy na dobę. Lek Rydapt jest podawany od 8 do 21 dnia cyklu chemioterapii indukcyjnej i konsolidacyjnej, a następnie u pacjentów z całkowitą odpowiedzią codziennie jako monoterapia podtrzymująca do nawrotu choroby przez maksymalnie 12 cykli po 28 dni każdy (patrz punkt 4.1). U pacjentów będących biorcami przeszczepu krwiotwórczych komórek macierzystych (ang. *stem cell transplant* – SCT) leczenie lekiem Rydapt należy przerwać 48 godzin przed kondycjonującym schematem leczenia poprzedzającym SCT. *Modyfikacje dawkowania w AML:* W Tabeli 1 przedstawiono zalecenia dotyczące modyfikacji dawkowania u pacjentów z AML.

Tabela 1. Zalecenia dotyczące przerwania, zmniejszenia dawki i zakończenia podawania leku Rydapt u pacjentów z AML

Faza	**Kryteria**	**Dawkowanie leku Rydapt**
Indukcji, konsolidacji i leczenia podtrzymującego	Nacieki w płucach stopnia 3/4	Przerwać podawanie leku Rydapt przez pozostałą część cyklu. Wznowić podawanie leku Rydapt w takiej samej dawce, gdy naciek zmniejszy się do stopnia ≤1.
	Inne niehematologiczne działania toksyczne stopnia 3/4	Przerwać podawanie leku Rydapt do czasu złagodzenia do stopnia ≤2 działań toksycznych uznawanych za posiadające przynajmniej możliwy związek z lekiem Rydapt, a następnie wznowić podawanie leku Rydapt.
	Odstęp QTc >470 ms i ≤500 ms	Zmniejszyć dawkę leku Rydapt do 50 mg raz na dobę przez pozostałą część cyklu. Wznowić podawanie leku Rydapt w dawce początkowej w następnym cyklu, jeśli odstęp QTc zmniejszy się do ≤470 ms na początku tego cyklu. W przeciwnym razie kontynuować podawanie leku Rydapt w dawce 50 mg raz na dobę.
	Odstęp QTc >500 ms	Wstrzymać lub przerwać podawanie leku Rydapt przez pozostałą część cyklu. Jeśli odstęp QTc zmniejszy się do ≤470 ms tuż przed rozpoczęciem kolejnego cyklu, wznowić podawanie leku Rydapt w dawce początkowej. Jeśli odstęp QTc nie zmniejszy się do czasu rozpoczęcia kolejnego cyklu, nie podawać leku Rydapt podczas tego cyklu. Podawanie leku Rydapt można wstrzymać na tyle cykli, ile to konieczne do czasu poprawy wartości QTc.
Tylko leczenie podtrzymujące	Neutropenia stopnia 4 (ANC <0,5 x 10^9/l)	Przerwać podawanie leku Rydapt do czasu, gdy ANC ≥1,0 x 10^9/l, następnie wznowić podawanie w dawce 50 mg dwa razy na dobę. Jeśli neutropenia (ANC <1,0 x 10^9/l) utrzymuje się przez >2 tygodnie i istnieją podejrzenia, że ma ona związek z lekiem Rydapt, podawanie leku Rydapt należy zakończyć.
	Utrzymujące się działania toksyczne stopnia 1/2	Utrzymujące się działania toksyczne o 1. lub 2. stopniu nasilenia, które pacjenci uznają za niemożliwe do zaakceptowania mogą spowodować przerwanie leczenia nawet na 28 dni.
ANC (ang. *Absolute Neutrophil Count*): bezwzględna liczba neutrofilów		

ASM, SM-AHN i MCL: Zalecana dawka początkowa leku Rydapt to 100 mg doustnie dwa razy na dobę. Leczenie należy kontynuować tak długo, jak długo obserwuje się korzyści kliniczne lub do czasu wystąpienia niemożliwych do zaakceptowania działań toksycznych. *Modyfikacje dawkowania w ASM, SM-AHN i MCL:* W Tabeli 2 przedstawiono modyfikacje dotyczące dawkowania leku Rydapt u pacjentów z ASM, SM-AHN i MCL.

Tabela 2. Zalecenia dotyczące przerwania, zmniejszenia dawki i zakończenia podawania leku Rydapt u pacjentów z ASM, SM-AHN lub MCL

Kryteria	**Dawkowanie leku Rydapt**
ANC <1,0 x 10^9/l przypisywane produktowi Rydapt u pacjentów bez MCL lub ANC poniżej 0,5 x 10^9/l przypisywane produktowi Rydapt u pacjentów z wyjściową wartością ANC wynoszącą 0,5-1,5 x 10^9/l	Przerwać podawanie leku Rydapt do czasu, gdy ANC wyniesie ≥1,0 x 10^9/l, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli mała liczba ANC utrzymuje się przez >21 dni i istnieją podejrzenia, że ma to związek z lekiem Rydapt.
Liczba płytek krwi mniejsza niż 50 x 10^9/l przypisywana produktowi Rydapt u pacjentów bez MCL lub liczba płytek krwi mniejsza niż 25 x 10^9/l przypisywana produktowi Rydapt u pacjentów z wyjściową liczbą płytek krwi wynoszącą 25-75 x 10^9/l	Przerwać podawanie leku Rydapt do czasu, gdy liczba płytek krwi wyniesie 50 x 10^9/l lub więcej, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli mała liczba płytek krwi utrzymuje się przez >21 dni i istnieją podejrzenia, że ma to związek z lekiem Rydapt.
Stężenie hemoglobiny poniżej 8 g/dl przypisywane produktowi Rydapt u pacjentów bez MCL lub zagrażająca życiu niedokrwistość przypisywana produktowi Rydapt u pacjentów z wyjściowym stężeniem hemoglobiny wynoszącym 8-10 g/dl	Przerwać podawanie leku Rydapt do czasu, gdy stężenie hemoglobiny wyniesie 8 g/dl lub więcej, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli małe stężenie hemoglobiny utrzymuje się przez >21 dni i istnieją podejrzenia, że ma to związek z lekiem Rydapt.
Nudności i (lub) wymioty stopnia 3/4 pomimo stosowania optymalnej terapii przeciwwymiotnej	Przerwać podawanie leku Rydapt na 3 dni (6 dawek), następnie wznowić podawanie w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, stopniowo zwiększać dawkę do 100 mg dwa razy na dobę.
Inne niehematologiczne działania toksyczne stopnia 3/4	Przerwać podawanie leku Rydapt do czasu, gdy nasilenie zdarzenia zmniejszy się do stopnia ≤2, następnie wznowić podawanie leku Rydapt w dawce 50 mg dwa razy na dobę i, jeśli leczenie będzie tolerowane, zwiększyć dawkę do 100 mg dwa razy na dobę. Podawanie leku Rydapt należy zakończyć, jeśli działanie toksyczne nie zmniejszy się do stopnia ≤2 w ciągu 21 dni lub jeśli dojdzie do nawrotu ciężkiego działania toksycznego przy stosowaniu zmniejszonej dawki leku Rydapt.
ANC: bezwzględna liczba neutrofilów. Stopień nasilenia wg CTCAE: stopień 1 = objawy łagodne; 2 = objawy umiarkowane; 3 = objawy ciężkie; 4 = objawy zagrażające życiu.	

Pominięcie dawki: W przypadku pominięcia dawki należy przyjąć kolejną dawkę o wyznaczonej porze. W przypadku wystąpienia wymiotów nie należy przyjmować dodatkowej dawki leku Rydapt, ale przyjąć kolejną dawkę o wyznaczonej porze. *Szczególne populacje pacjentów: Osoby w podeszłym wieku (≥65 lat):* Nie ma konieczności dostosowania schematu dawkowania u pacjentów w wieku powyżej 65 lat (patrz punkt 5.2 ChPL). Istnieje ograniczone doświadczenie ze stosowaniem midostauryny u pacjentów z AML w wieku 60-70 lat oraz brak jest doświadczenia ze stosowaniem u pacjentów z AML w wieku powyżej 70 lat. U pacjentów w wieku ≥60 lat, lek Rydapt powinien być stosowany wyłącznie u pacjentów kwalifikujących się do intensywnej chemioterapii indukcyjnej, o odpowiednim stanie sprawności i bez istotnych chorób współistniejących. *Zaburzenia czynności nerek:* Nie ma konieczności dostosowania dawki u pacjentów z łagodnymi lub umiarkowanymi zaburzeniami czynności nerek. Doświadczenie kliniczne u pacjentów z ciężkimi zaburzeniami czynności nerek jest ograniczone i brak jest dostępnych danych od pacjentów ze schyłkową niewydolnością nerek (patrz punkt 4.4 i 5.2 ChPL). *Zaburzenia czynności wątroby:* Nie ma konieczności dostosowania dawki u pacjentów z łagodnymi lub umiarkowanymi (stopnia A lub B w skali Child-Pugh) zaburzeniami czynności wątroby (patrz punkt 5.2 ChPL). Nie ukończono żadnego badania z udziałem pacjentów z ciężkimi (stopnia C wg Child-Pugh C) zaburzeniami czynności wątroby (patrz punkt 4.4 ChPL). *Ostra białaczka promielocytowa:* Lek Rydapt nie był badany u pacjentów z ostrą białaczką promielocytową i dlatego jego stosowanie nie jest zalecane w tej populacji pacjentów. *Dzieci i młodzież:* Produkt leczniczy Rydapt nie powinien być stosowany w skojarzeniu ze schematami intensywnej chemioterapii skojarzonej przeznaczonymi dla dzieci i młodzieży z AML, obejmującymi antracykliny, fludarabinę i cytarabinę z uwagi na ryzyko przedłużającej się normalizacji parametrów hematologicznych (na przykład przedłużającą się ciężką neutropenię i małopłytkowość) (patrz punkty 4.4 i 5.1). Sposób podawania: Lek Rydapt jest przeznaczony do podawania doustnego. Kapsułki należy połykać w całości, popijając szklanką wody. Nie należy ich otwierać, rozgryzać ani żuć, co zapewni dostarczenie odpowiedniej dawki leku i pozwoli uniknąć nieprzyjemnego smaku zawartości kapsułki. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą wymienioną w punkcie 6.1. Jednoczesne podawanie silnych induktorów CYP3A4, np. ryfampicyny, ziela dziurawca (*Hypericum perforatum*), karbamazepiny, enzalutamidu, fenytoiny (patrz punkt 4.5 ChPL). **Środki ostrożności/Ostrzeżenia:** Neutropenia i zakażenia: U pacjentów otrzymujących lek Rydapt w monoterapii i w skojarzeniu z chemioterapią występowała neutropenia (patrz punkt 4.8 ChPL). Ciężka neutropenia (ANC <0,5 x 10^9/l) była na ogół odwracalna po wstrzymaniu podawania leku Rydapt aż do powrotu liczby neutrofilów do wartości początkowych i odstawieniu leku w badaniach z ASM, SM-AHN i MCL. Należy regularnie kontrolować liczbę białych krwinek, zwłaszcza na początku leczenia. U pacjentów, u których wystąpi ciężka neutropenia o niewyjaśnionej etiologii, leczenie lekiem Rydapt należy przerwać do czasu, gdy ANC wyniesie ≥1,0 x 10^9/l, zgodnie z zaleceniami przedstawionymi w Tabelach 1 i 2. Lek Rydapt należy odstawić u pacjentów, u których wystąpi nawracająca lub przedłużająca się ciężka neutropenia, podejrzewana o związek z lekiem Rydapt (patrz punkt 4.2 ChPL). Przed rozpoczęciem podawania leku Rydapt w monoterapii należy opanować wszelkie czynne, ciężkie zakażenia. Należy monitorować pacjentów pod kątem przedmiotowych i podmiotowych objawów zakażenia, w tym wszelkich zakażeń związanych ze stosowaniem aparatury medycznej, a w przypadku rozpoznania zakażenia, należy szybko wdrożyć odpowiednie leczenie, w tym w razie konieczności, odstawić lek Rydapt. Zaburzenia czynności serca: Pacjenci z objawową zastoinową niewydolnością serca byli wykluczeni z badań klinicznych. W badaniach z ASM, SM-AHN i MCL występowały zaburzenia czynności serca, takie jak zastoinowa niewydolność serca (ang. *congestive heart failure* – CHF) (w tym przypadki śmiertelne) i przejściowe obniżenie frakcji wyrzutowej lewej komory (ang. *left ventricular ejection fraction* – LVEF). W randomizowanym badaniu z AML nie obserwowano różnic dotyczących CHF pomiędzy grupą otrzymującą Rydapt + chemioterapię a grupą otrzymującą placebo + chemioterapię. U pacjentów podlegających ryzyku Rydapt należy stosować z zachowaniem ostrożności, a pacjenci wymagają ścisłego monitorowania poprzez ocenę LVEF, jeśli wystąpią wskazania kliniczne (na początku leczenia i w czasie jego trwania). U pacjentów leczonych midostauryną odnotowano zwiększoną częstość wydłużenia odstępu QTc (patrz punkt 4.8 ChPL), jednak nie znaleziono mechanistycznego wyjaśnienia dla tej obserwacji. Należy zachować ostrożność u pacjentów z ryzykiem wydłużenia QTc (np. spowodowanym jednoczesnym stosowaniem produktów leczniczych i (lub) zaburzeniami równowagi elektrolitowej). Należy rozważyć ocenę odstępu QT w badaniu EKG, jeśli Rydapt jest przyjmowany jednocześnie z produktami leczniczymi, które mogą wydłużać odstęp QT.

Toksyczność płucna: U pacjentów leczonych lekiem Rydapt w monoterapii lub w skojarzeniu z chemioterapią występowała choroba śródmiąższowa płuc i zapalenie płuc, w niektórych przypadkach zakończone zgonem. Należy monitorować pacjentów pod kątem objawów płucnych wskazujących na chorobę śródmiąższową płuc lub zapalenie płuc oraz odstawić Rydapt u pacjentów z objawami płucnymi wskazującymi na chorobę śródmiąższową płuc lub zapalenie płuc, o nasileniu ≥stopnia 3 (wg NCI CTCAE). Toksyczne działanie na zarodek i płód oraz karmienie piersią: Należy poinformować kobiety w ciąży o potencjalnym ryzyku dla płodu; należy doradzić kobietom w wieku rozrodczym wykonanie testu ciążowego w okresie 7 dni przed rozpoczęciem leczenia produktem leczniczym Rydapt oraz stosowanie skutecznej antykoncepcji podczas leczenia produktem leczniczym Rydapt i przez co najmniej 4 miesiące po jego zakończeniu. Kobiety stosujące antykoncepcję hormonalną powinny dodatkowo stosować barierową metodę antykoncepcyjną. Z uwagi na możliwość wystąpienia ciężkich działań niepożądanych produktu leczniczego Rydapt u dzieci karmionych piersią, kobiety powinny zaprzestać karmienia piersią podczas leczenia produktem leczniczy Rydapt i przez co najmniej 4 miesiące po zakończeniu leczenia (patrz punkt 4.6 ChPL). Dzieci i młodzież: Produkt leczniczy Rydapt nie powinien być stosowany w skojarzeniu ze schematami intensywnej chemioterapii skojarzonej przeznaczonymi dla dzieci i młodzieży z AML, obejmującymi antracykliny, fludarabinę i cytarabinę z uwagi na ryzyko przedłużającej się normalizacji parametrów hematologicznych (na przykład przedłużającą się ciężką neutropenię i małopłytkowość) (patrz punkty 4.2 i 5.1 ChPL). Ciężkie zaburzenia czynności wątroby: Należy zachować ostrożność rozważając podanie midostauryny pacjentom z ciężkimi zaburzeniami czynności wątroby i starannie ich monitorować w kierunku działań toksycznych (patrz punkt 5.2 ChPL). Ciężkie zaburzenia czynności nerek: Należy zachować ostrożność rozważając podanie midostauryny pacjentom z ciężkimi zaburzeniami czynności nerek lub schyłkową niewydolnością nerek i należy uważnie monitorować pacjentów pod kątem działań toksycznych (patrz punkt 5.2 ChPL). Interakcje: Wymaga się zachowania ostrożności, gdy midostauryna jest przepisywana jednocześnie z produktami leczniczymi będącymi silnymi inhibitorami CYP3A4, takimi jak m. in. leki przeciwgrzybicze (np. ketokonazol), pewne leki antywirusowe (np. rytonawir), antybiotyki makrolidowe (np. klarytromycyna) i nefazodon, ponieważ mogą one zwiększać stężenie midostauryny w osoczu, zwłaszcza w przypadku (ponownego) rozpoczynania leczenia midostauryną (patrz punkt 4.5 ChPL). Należy rozważyć zastosowanie alternatywnych produktów leczniczych nieposiadających silnego działania hamującego na CYP3A4. W sytuacji braku zadowalającej alternatywy terapeutycznej należy uważnie monitorować pacjentów pod kątem działań toksycznych związanych z midostauryną. Substancje pomocnicze: Lek Rydapt zawiera hydroksystearynian makrogologlicerolu, który może powodować dyskomfort żołądkowy i biegunkę. Ten lek zawiera 666 mg alkoholu (etanolu) w każdej 200 mg dawce (maksymalnej dawce dobowej), co jest równoważne 14 % obj. bezwodnego etanolu. Ilość alkoholu w 200 mg dawce tego leku jest równoważna 16,9 ml piwa lub 7,0 ml wina. Mała ilość alkoholu w tym leku nie będzie powodowała zauważalnych skutków. Alkohol może być szkodliwy u pacjentów z problemami związanymi z alkoholem, padaczką lub chorobami wątroby bądź podczas ciąży lub karmienia piersią. Wpływ na płodność, ciążę i laktację: Kobiety w wieku rozrodczym: Kobiety w wieku rozrodczym należy poinformować, że badania na zwierzętach wykazują szkodliwy wpływ midostauryny na rozwijający się płód. Aktywnym seksualnie kobietom w wieku rozrodczym należy doradzić wykonanie testu ciążowego w ciągu 7 dni przed rozpoczęciem leczenia lekiem Rydapt oraz stosowanie skutecznej antykoncepcji (metod ze wskaźnikiem ciąż wynoszącym mniej niż 1%) podczas przyjmowania leku Rydapt i przez co najmniej 4 miesiące po zakończeniu leczenia lekiem Rydapt. Obecnie nie wiadomo, czy midostauryna może zmniejszać skuteczność hormonalnych środków antykoncepcyjnych i dlatego kobiety stosujące antykoncepcję hormonalną powinny dodatkowo używać barierowych metod antykoncepcyjnych. Ciąża: Midostauryna może powodować uszkodzenie płodu, gdy jest podawana kobietom w ciąży. Brak jest odpowiednich, dobrze kontrolowanych badań z udziałem kobiet ciężarnych. Badania wpływu na reprodukcję prowadzone na szczurach i królikach wykazały, że midostauryna wywoływała działanie toksyczne na płód (patrz punkt 5.3 ChPL). Lek Rydapt nie jest zalecany do stosowania w okresie ciąży lub u kobiet w wieku rozrodczym niestosujących skutecznej metody antykoncepcji. Należy poinformować kobiety w ciąży o potencjalnym ryzyku dla płodu. Karmienie piersią: Nie wiadomo, czy midostauryna lub jej czynne metabolity przenikają do mleka kobiecego. Dostępne dane pochodzące z badań na zwierzętach wykazały, że midostauryna i jej czynne metabolity przenikają do mleka karmiących szczurów. Karmienie piersią należy przerwać podczas leczenia lekiem Rydapt i przez co najmniej 4 miesiące po zakończeniu leczenia. Płodność: Brak jest danych dotyczących wpływu leku Rydapt na płodność ludzi. Badania na zwierzętach, którym podawano midostaurynę wykazały zaburzenia płodności (patrz punkt 5.3 ChPL). **Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: *AML:* Ocena bezpieczeństwa stosowania leku Rydapt (podawanego w dawce 50 mg dwa razy na dobę) u pacjentów z noworozpoznaną AML z mutacją FLT3 opiera się na wynikach randomizowanego, podwójnie zaślepionego badania III fazy kontrolowanego placebo z udziałem 717 pacjentów. Ogólna mediana czasu trwania ekspozycji wyniosła 42 dni (zakres od 2 do 576 dni) u pacjentów z grupy otrzymującej Rydapt w skojarzeniu ze standardową chemioterapią w porównaniu z 34 dniami (zakres od 1 do 465 dni) u pacjentów z grupy otrzymującej placebo w skojarzeniu ze standardową chemioterapią. Mediana czasu trwania ekspozycji na lek w fazie leczenia podtrzymującego wyniosła 11 miesięcy w obu grupach badania (16 do 520 dni u pacjentów otrzymujących Rydapt oraz 22 do 381 dni u pacjentów z grupy placebo) u 205 pacjentów (120 z grupy otrzymującej Rydapt i 85 z grupy otrzymującej placebo), którzy weszli do fazy leczenia podtrzymującego. Najczęstszymi działaniami niepożądanymi (ang. *adverse drug reaction* – ADR) w grupie otrzymującej Rydapt były: gorączka neutropeniczna (83,4%), nudności (83,4%), złuszczające zapalenie skóry (61,6%), wymioty (60,7%), ból głowy (45,9%), wybroczyny (35,8%) i gorączka (34,5%). Najczęstszymi ADR stopnia 3/4 były gorączką neutropeniczna (83,5%), limfopenia (20,0%), zakażenia związane z zastosowaniem aparatury medycznej (15,7%), złuszczające zapalenie skóry (13,6%), hiperglikemia (7,0%) i nudności (5,8%). Najczęstszymi odchyleniami w wynikach badań laboratoryjnych było zmniejszenie stężenia hemoglobiny (97,3%), zmniejszenie ANC (86,7%), zwiększenie aktywności AIAT (84,2%), zwiększenie aktywności AspAT (73,9%) i hipokaliemia (61,7%). Najczęstszymi odchyleniami w wynikach badań laboratoryjnych w 3/4 stopniu nasilenia było zmniejszenie ANC (85,8%), zmniejszenie stężenia hemoglobiny (78,5%), zwiększenie aktywności AIAT (19,4%) i hipokaliemia (13,9%). Poważne ADR wystąpiły z podobną częstością u pacjentów leczonych lekiem Rydapt, jak u pacjentów z grupy otrzymującej placebo. Najczęstszym poważnym ADR w obu grupach była gorączka neutropeniczna (16%). Zakończenie leczenia z powodu jakiegokolwiek działania niepożądanego miało miejsce u 3,1% pacjentów z grupy otrzymującej Rydapt w porównaniu z 1,3% pacjentów z grupy otrzymującej placebo. Najczęstszym działaniem niepożądanym stopnia 3/4 prowadzącym do zakończenia leczenia w grupie otrzymującej Rydapt było złuszczające zapalenie skóry (1,2%). *Profil bezpieczeństwa w fazie leczenia podtrzymującego:* W Tabeli 3 przedstawiono częstość występowania ADR w całym okresie badania, jednak po dokonaniu odrębnej oceny dla fazy leczenia podtrzymującego (monoterapia lekiem Rydapt lub placebo) stwierdzono różnicę dotyczącą rodzaju i nasilenia ADR. Całkowita częstość występowania ADR w fazie leczenia podtrzymującego była na ogół mniejsza niż w fazie leczenia indukcyjnego i konsolidacyjnego. Częstość występowania działań niepożądanych była jednak większa w grupie otrzymującej Rydapt niż w grupie placebo w fazie leczenia podtrzymującego. Do ADR występujących częściej w grupie midostauryny w porównaniu z grupą placebo w fazie leczenia podtrzymującego należały: nudności (46,4% w porównaniu z 17,9%), hiperglikemia (20,2% w porównaniu z 12,5%), wymioty (19% w porównaniu z 5,4%) i wydłużenie odstępu QT (11,9% w porównaniu z 5,4%). Większość zgłaszanych nieprawidłowości hematologicznych występowało w fazie indukcji i konsolidacji, gdy pacjenci otrzymywali Rydapt lub placebo w skojarzeniu z chemioterapią. Najczęstszymi zaburzeniami hematologicznymi stopnia 3/4 zgłaszanymi u pacjentów w fazie leczenia podtrzymującego lekiem Rydapt było zmniejszenie ANC (20,8% w por. z 18,8%) i leukopenia (7,5% w por. z 5,9%). ADR zgłaszane w fazie leczenia podtrzymującego były przyczyną przerwania leczenia u 1,2% pacjentów w grupie otrzymującej Rydapt i u żadnego pacjenta z grupy placebo. *ASM, SM-AHN i MCL:* Bezpieczeństwo stosowania leku Rydapt (100 mg dwa razy na dobę) podawanego w monoterapii pacjentom z ASM, SM-AHN i MCL było oceniane u 142 pacjentów w dwóch otwartych, wieloośrodkowych badaniach z jedną grupą leczenia. Mediana czasu trwania ekspozycji na lek Rydapt wyniosła 11,4 miesiąca (zakres: 0 do 81 miesięcy). Najczęstszymi ADR były nudności (82%), wymioty (68%), biegunka (51%), obrzęki obwodowe (35%) i uczucie zmęczenia (31%). Najczęstszymi ADR stopnia 3/4 były uczucie zmęczenia (8,5%), posocznica (7,7%), zapalenie płuc (7%), gorączka neutropeniczna (7%) i biegunka (6,3%). Najczęstszymi niehematologicznymi odchyleniami w wynikach badań laboratoryjnych były: hiperglikemia (93,7%), wzrost stężenia bilirubiny całkowitej (40,1%), wzrost aktywności lipazy (39,4%), wzrost aktywności aminotransferazy asparaginianowej (AST) (33,8%) i wzrost aktywności aminotransferazy alaninowej (ALT) (33,1%), natomiast do najczęstszych hematologicznych odchyleń w wynikach badań laboratoryjnych należało zmniejszenie bezwzględnej liczby limfocytów (73,2%) i zmniejszenie ANC (58,5%). Najczęstszymi odchyleniami w wynikach badań laboratoryjnych stopnia 3/4 było zmniejszenie bezwzględnej liczby limfocytów (45,8%), zmniejszenie ANC (26,8%), hiperglikemia (19%) i wzrost aktywności lipazy (17,6%). Modyfikacje dawkowania (przerwanie podawania leku lub dostosowanie dawki) z powodu ADR miały miejsce u 31% pacjentów. Najczęstszymi ADR powodującymi konieczność modyfikacji dawki (częstość występowania ≥5%) były nudności i wymioty. ADR, które doprowadziły do zakończenia leczenia wystąpiły u 9,2% pacjentów. Najczęstszymi (częstość występowania ≥1%) były: gorączka neutropeniczna, nudności, wymioty i wysięk opłucnowy. Wykaz działań niepożądanych: ADR wymieniono według klasyfikacji układów i narządów MedDRA. W każdej klasie układów i narządów ADR zostały przedstawione według częstości występowania, poczynając od najczęstszych według następującej konwencji (CIOMS III): bardzo często (≥1/10); często (≥1/100 do <1/10); niezbyt często (≥1/1000 do <1/100); rzadko (≥1/10 000 do <1/1000); bardzo rzadko (<1/10 000); nieznana (częstość nie może być określona na podstawie dostępnych danych). W obrębie każdej grupy działania niepożądane przedstawiono zgodnie ze zmniejszającym się nasileniem. *AML:* Działania niepożądane obserwowane w badaniu klinicznym III fazy u pacjentów z noworozpoznaną AML z mutacją FLT3. ***Bardzo często:*** zakażenie związane z zastosowaniem aparatury medycznej, gorączka neutropeniczna, wybroczyny, limfopenia, nadwrażliwość, bezsenność, ból głowy, hipotensja, krwawienie z nosa, ból krtani, duszność, nudności, wymioty, zapalenie jamy ustnej, ból w górnej części brzucha, guzki krwawnicze, złuszczające zapalenie skóry, nadmierne pocenie się, ból pleców, ból stawów, gorączka, zmniejszenie stężenia hemoglobiny*, zmniejszenie ANC*, zwiększenie aktywności AIAT*, zwiększenie aktywności AspAT*, hipokaliemia*, hiperglikemia, hipernatremia*, wydłużenie czasu kaolinowo-kefalinowego. ***Często:*** zakażenie górnych dróg oddechowych, hiperurykemia, omdlenie, drżenie, obrzęk powiek, częstoskurcz zatokowy, nadciśnienie, wysięk osierdziowy, wysięk opłucnowy, zapalenie nosogardła, zespół ostrej niewydolności oddechowej, dyskomfort w obrębie odbytnicy i odbytu, dyskomfort w jamie brzusznej, suchość skóry, zapalenie rogówki, ból kości, ból kończyny, ból szyi, zakrzepica związana z obecnością cewnika, hiperkalcemia*, zwiększenie masy ciała. ***Niezbyt często:*** posocznica neutropeniczna. *ASM, SM-AHN i MCL:* Działania niepożądane obserwowane w badaniach klinicznych z ASM, SM-AHN i MCL. ***Bardzo często:*** zakażenie układu moczowego, zakażenie górnych dróg oddechowych, ból głowy, zawroty głowy, duszność, kaszel, wysięk opłucnowy, krwawienie z nosa, nudności, wymioty, biegunka, zaparcie, obrzęk obwodowy, uczucie zmęczenia, gorączka, hiperglikemia (nie na czczo)*, zmniejszenie bezwzględnej liczby limfocytów*, zmniejszenie ANC*, zwiększenie stężenia bilirubiny całkowitej*, zwiększenie aktywności lipazy*, zwiększenie aktywności AspAT*, zwiększenie aktywności AIAT*, zwiększenie aktywności amylazy*. ***Często:*** zapalenie płuc, posocznica, zapalenie oskrzeli, opryszczka jamy ustnej, zapalenie pęcherza moczowego, zapalenie zatok, róża, półpasiec, gorączka neutropeniczna, nadwrażliwość, zaburzenia uwagi, drżenie, układowe zawroty głowy, hipotensja, krwiak, ból części ustnej gardła, niestrawność, krwotok żołądkowo-jelitowy, osłabienie, dreszcze, obrzęk, zwiększenie masy ciała, stłuczenia, upadki. Opis wybranych działań niepożądanych: *Zaburzenia żołądka i jelit:* U pacjentów z AML, ASM, SM-AHN i MCL obserwowano nudności, wymioty i biegunkę. U pacjentów z ASM, SM-AHN i MCL zdarzenia te prowadziły do dostosowania dawki lub przerwania leczenia u 26% oraz do zakończenia leczenia u 4,2% pacjentów. Większość z tych zdarzeń wystąpiła w ciągu pierwszych 6 miesięcy leczenia i reagowała na wspomagające produkty lecznicze o profilaktycznym działaniu. Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu leku do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania leku. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Aleje Jerozolimskie 181C, 02-222 Warszawa, tel.: +48 22 49 21 301, faks: +48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl. **Pozwolenia Komisji Europejskiej na dopuszczenie do obrotu nr:** EU/1/17/1218/001-002. **Kategoria dostępności:** Rpz – lek wydawany z przepisu lekarza do zastrzeżonego stosowania. **Podmiot odpowiedzialny:** Novartis Europharm Limited, Vista Building, Elm Park, Merrion Road, Dublin 4, Irlandia. **Uwaga:** Przed przepisaniem leku należy zapoznać się z pełną informacją o leku. **Pełna informacja o leku jest dostępna w:** Novartis Poland Sp. z o.o., 02-674 Warszawa, ul. Marynarska 15, tel. +48 22 375 4 888. **Opracowano:** 02/2021.

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* Częstość podana w oparciu o wyniki badań laboratoryjnych.

Zgodnie z aktualnym Obwieszczeniem Ministra Zdrowia w sprawie wykazu refundowanych leków, środków spożywczych specjalnego przeznaczenia żywieniowego oraz wyrobów medycznych, produkt leczniczy Rydapt (midostaurin) 25 mg, 56 kaps., EAN: 05909991353995; Rydapt (midostaurin) 25 mg, 112 kaps., EAN: 05909991341527 jest dostępny w ramach programu lekowego: • Załącznik B.114. Leczenie chorych na ostrą białaczkę szpikową (ICD-10 C92.0); • Załącznik B.115. Leczenie agresywnej mastocytozy układowej, mastocytozy układowej z współistniejącym nowotworem układu krwiotwórczego oraz białaczki mastocytarnej (ICD-10: C96.2, C94.3, D47.9). Rydapt 25 mg x 56 kaps.: Urzędowa cena zbytu: 28 095,24 PLN; Poziom odpłatności dla pacjenta: bezpłatnie. Rydapt 25 mg x 112 kaps.: Urzędowa cena zbytu: 56 190,48 PLN; Poziom odpłatności dla pacjenta: bezpłatnie.

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Refundacja w przewlekłej białaczce szpikowej (PBSz) oraz ostrej białaczce limfoblastycznej z obecnością chromosomu Filadelfia (OBL)[1]

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Zalecenia dotyczące terapii przewlekłej białaczki szpikowej w fazie przewlekłej (kategoria zaleceń: IA)[3]

Typ/faza choroby	
Leczenie III linii CML	
Nietolerancja lub niepowodzenie*	
Dazatynibu	Ponatynib 45 mg/d, nilotynib 2 × 400 mg/d lub bosutynib 500 mg/d
Nilotynibu	Ponatynib 45 mg/d, dazatynib 100 mg/d lub bosutynib 500 mg/d
Bosutynibu	Ponatynib 45 mg/d, dazatynib 100 mg/d lub nilotynib 2 × 400 mg/d
Niepowodzenie – wszyscy pacjenci	allo-HSCT**

* Jeżeli przyczyną niepowodzenia jest wykryta mutacja *ABL1*, to w wyborze leku należy wziąć pod uwagę jej wrażliwość. Potwierdzoną w badaniach klinicznych oporność na dazatynib wykazują mutacje *T315I/A*, *F317L* i *V299L*, na nilotynib oporne są mutacje *T315I*, *Y253H/F*, *E255V/K* oraz *F359V*, a na bosutynib – mutacje *T315I/A*, *V299L* i *E255V/K*. Jeżeli przyczyną niepowodzenia jest obniżenie poziomu transkryptu po pierwszych 3 miesiącach do ≥ 10%, to zmiany leczenia należy dokonać po potwierdzeniu tego wyniku, najpóźniej w 6. miesiącu terapii.
** U chorych niekwalifikujących się do przeszczepiania allogenicznych krwiotwórczych komórek macierzystych (allo-HSCT, *allogeneic hematopoietic stem cell transplant*) należy rozważyć leczenie ponatynibem. Ponatynib (*Iclusig®*, Incyte Biosciences Distribution B.V.) (TKI III generacji) może być skuteczny u chorych z mutacją *T315I*, a także u pacjentów bez tej mutacji, u których stwierdzono oporność lub nietolerancję leczenia dazatynibem albo nilotynibem i dla których kolejne leczenie imatynibem nie jest optymalną opcją leczenia.

Ponatynib (*Iclusig®*, Incyte Biosciences Distribution B.V.) (TKI III generacji) może być skuteczny u chorych z mutacją *T315I*, a także u pacjentów bez tej mutacji, u których stwierdzono oporność lub nietolerancję leczenia dazatynibem albo nilotynibem i dla których kolejne leczenie imatynibem nie jest optymalną opcją leczenia.

Refundacja w programie leczenia przewlekłej białaczki szpikowej B.14[1]

Refundacja w leczeniu ostrej białaczki limfoblastycznej z obecnością chromosomu Filadelfia B.65[1]

ICL/01/2021/1

1. Obwieszczenie Ministra Zdrowia z dnia 21 czerwca 2021 r.
2. Charakterystyka Produktu Leczniczego Iclusig®.
3. Sacha T. Wytyczne postępowania diagnostyczno-leczniczego w nowotworach złośliwych 2020. Onkol Prakt Klin Edu 2020; 6(Supl. A), str. 38-54.

ANGELINI PHARMA

NAZWA PRODUKTU LECZNICZEGO: Iclusig® 15 mg tabletki powlekane, Iclusig® 45 mg tabletki powlekane. **Postać:** Tabletki powlekane zawierające 15 mg lub 45 mg ponatynibu (w postaci chlorowodorku). **Wskazania:** Produkt Iclusig® jest wskazany do stosowania u dorosłych pacjentów z fazą przewlekłą, fazą akceleracji lub fazą przełomu blastycznego przewlekłej białaczki szpikowej (CML) z opornością lub nietolerancją leczenia dazatynibem lub nilotynibem i dla których kolejne leczenie imatynibem nie jest właściwe ze względów klinicznych lub u pacjentów z mutacją T315I. Produkt ten jest także wskazany w leczeniu dorosłych pacjentów z ostrą białaczką limfoblastyczną z obecnością chromosomu Filadelfia (Ph+ ALL) z opornością lub nietolerancją leczenia dazatynibem i dla których kolejne leczenie imatynibem nie jest właściwe ze względów klinicznych lub u pacjentów z mutacją T315I. **Dawkowanie i sposób podawania:** Leczenie powinno być rozpoczęte przez lekarza z doświadczeniem w rozpoznawaniu i leczeniu pacjentów z białaczką. Podczas leczenia można stosować wspomaganie układu krwiotwórczego przez przetaczanie płytek krwi i hematopoetycznych czynników wzrostu, jeśli będzie to wskazane klinicznie. Przed rozpoczęciem leczenia ponatynibem należy ocenić stan układu krążenia pacjenta, wraz z wywiadem i badaniem przedmiotowym, i podjąć czynne działania w celu ograniczenia czynników ryzyka sercowo-naczyniowego. Podczas leczenia ponatynibem należy w dalszym ciągu monitorować stan układu krążenia i odpowiednio dostosowywać leczenie zachowawcze i podtrzymujące w przypadku stanów zwiększających ryzyko sercowo-naczyniowe. Dawkowanie: Zalecana dawka początkowa wynosi 45 mg ponatynibu raz na dobę. Dostępne są tabletki powlekane 45 mg umożliwiające podawanie standardowej dawki 45 mg. Leczenie należy kontynuować do stwierdzenia u pacjenta oznak progresji choroby lub niedopuszczalnej toksyczności. Pacjenci powinni być obserwowani w celu wykrycia odpowiedzi na leczenie zgodnie ze standardowymi wytycznymi klinicznymi. Należy rozważyć przerwanie leczenia ponatynibem w razie nieosiągnięcia pełnej odpowiedzi hematologicznej w ciągu 3 miesięcy (90 dni). Ryzyko zdarzeń związanych z niedrożnością tętnic jest prawdopodobnie zależne od dawki. Należy rozważyć zmniejszenie dawki produktu leczniczego Iclusig® do 15 mg u pacjentów z fazą przewlekłą (CP) CML, którzy osiągnęli większą odpowiedź cytogenetyczną, biorąc pod uwagę następujące czynniki w indywidualnej ocenie pacjenta: ryzyko ze strony układu krążenia, działania niepożądane leczenia ponatynibem, czas do wystąpienia odpowiedzi cytogenetycznej oraz poziomu transkryptów BCR-ABL. W razie zmniejszania dawki zaleca się dokładne monitorowanie odpowiedzi. Postępowanie w razie działań toksycznych: W leczeniu hematologicznej i niehematologicznej toksyczności należy rozważyć modyfikację dawki lub przerwanie podawania produktu. W razie wystąpienia ciężkich działań niepożądanych należy wstrzymać leczenie. U pacjentów, u których działania niepożądane ustąpiły lub zmniejszyło się ich nasilenie, można rozważyć wznowienie podawania produktu Iclusig® i stopniowe zwiększenie dawki do dawki dobowej podawanej przed wystąpieniem danego działania niepożądanego, jeśli jest to uzasadnione klinicznie. Dostępne są tabletki powlekane 15 mg i 30 mg umożliwiające podawanie dawki 15 mg lub 30 mg. *Mielosupresja:* W Tabeli 1 przedstawione są modyfikacje dawki w przypadku neutropenii (ANC* $< 1{,}0 \times 10^9$/l) i małopłytkowości (liczba płytek $< 50 \times 10^9$/l), które nie są związane z białaczką.

Tabela 1. Modyfikacje dawki w przypadku mielosupresji

ANC* $< 1{,}0 \times 10^9$/l albo liczba płytek $< 50 \times 10^9$/l	Pierwsze wystąpienie: • Należy wstrzymać podawanie produktu Iclusig® i wznowić podawanie tej samej dawki po powrocie do ANC $\geq 1{,}5 \times 10^9$/l i liczby płytek $\geq 75 \times 10^9$/l
	Ponowne wystąpienie po dawce 45 mg: • Należy wstrzymać podawanie produktu Iclusig® i wznowić podawanie dawki 30 mg po powrocie do ANC $\geq 1{,}5 \times 10^9$/l i liczby płytek $\geq 75 \times 10^9$/l
	Ponowne wystąpienie po dawce 30 mg: • Należy wstrzymać podawanie produktu Iclusig® i wznowić podawanie dawki 15 mg po powrocie do ANC $\geq 1{,}5 \times 10^9$/l i liczby płytek $\geq 75 \times 10^9$/l

* ANC = całkowita liczba neutrofili

Niedrożność tętnic i żylna choroba zakrzepowo-zatorowa: W razie podejrzewania wystąpienia u pacjenta zdarzenia związanego z niedrożnością tętnic lub żylnej choroby zakrzepowo-zatorowej należy natychmiast przerwać leczenie produktem Iclusig®. Decyzja o wznowieniu leczenia produktem Iclusig® po ustąpieniu zdarzenia powinna być oparta na ocenie stosunku korzyści do ryzyka. Nadciśnienie może zwiększać ryzyko zdarzeń związanych z niedrożnością tętnic. Jeżeli nadciśnienia nie da się opanować leczeniem farmakologicznym, należy tymczasowo wstrzymać leczenie produktem Iclusig®. *Zapalenie trzustki:* W Tabeli 2 przedstawione są zalecane modyfikacje dawki w przypadku działań niepożądanych dotyczących trzustki.

Tabela 2. Modyfikacje dawki w przypadku zapalenia trzustki i zwiększenia aktywności lipazy/amylazy

Zapalenie trzustki stopnia 2. i (lub) bezobjawowe zwiększenie aktywności lipazy/amylazy	Należy kontynuować leczenie produktem Iclusig® w tej samej dawce
Tylko bezobjawowe zwiększenie aktywności lipazy/amylazy stopnia 3. lub 4. ($> 2{,}0 \times$ IGGN*)	Wystąpienie po dawce 45 mg: • Należy wstrzymać podawanie produktu Iclusig® i wznowić leczenie w dawce 30 mg po powrocie do ≤ stopnia 1. ($< 1{,}5 \times$ IGGN) Wystąpienie po dawce 30 mg: • Należy wstrzymać podawanie produktu Iclusig® i wznowić leczenie w dawce 15 mg po powrocie do ≤ stopnia 1. ($< 1{,}5 \times$ IGGN) Wystąpienie po dawce 15 mg: • Należy rozważyć przerwanie leczenia produktem Iclusig®
Zapalenie trzustki stopnia 3.	Wystąpienie po dawce 45 mg: • Należy wstrzymać podawanie produktu Iclusig® i wznowić leczenie w dawce 30 mg po powrocie do ≤ stopnia 2. Wystąpienie po dawce 30 mg: • Należy wstrzymać podawanie produktu Iclusig® i wznowić leczenie w dawce 15 mg po powrocie do ≤ stopnia 2. Wystąpienie po dawce 15 mg: • Należy rozważyć przerwanie leczenia produktem Iclusig®
Zapalenie trzustki stopnia 4.	Należy przerwać leczenie produktem Iclusig®

* IGGN = górna granica normy przyjęta w danej instytucji

Hepatotoksyczność: Może być konieczne przerwanie podawania lub odstawienie produktu zgodnie z opisem podanym w Tabeli 3.

Tabela 3. Zalecane modyfikacje dawki z powodu hepatotoksyczności

Zwiększenie aktywności aminotransferazy wątrobowej $> 3 \times$ GGN* Długotrwałe utrzymywanie się stopnia 2. (powyżej 7 dni) Stopień 3. lub wyższy	Występowanie po dawce 45 mg: • Należy przerwać podawanie produktu Iclusig® i monitorować czynność wątroby • Należy wznowić podawanie produktu Iclusig® w dawce 30 mg po powrocie do wartości ≤ stopień 1. ($< 3 \times$ GGN) lub do wartości sprzed leczenia Występowanie po dawce 30 mg: • Należy przerwać podawanie produktu Iclusig® i wznowić w dawce 15 mg po powrocie do wartości ≤ stopień 1. lub do wartości sprzed leczenia Występowanie po dawce 15 mg: • Należy przerwać leczenie produktem Iclusig®
Zwiększenie aktywności AspAT lub AlAT $\geq 3 \times$ GGN z towarzyszącym zwiększeniem stężenia bilirubiny $> 2 \times$ GGN i aktywności fosfatazy zasadowej $< 2 \times$ GGN	Należy przerwać leczenie produktem Iclusig®

* GGN = górna granica normy dla laboratorium

Pacjenci w podeszłym wieku: Wśród 449 pacjentów biorących udział w badaniu klinicznym produktu Iclusig® 155 (35%) miało ≥ 65 lat. W porównaniu do pacjentów w wieku < 65 lat u starszych pacjentów większe było prawdopodobieństwo wystąpienia działań niepożądanych. *Pacjenci z zaburzeniem czynności wątroby:* Pacjenci z zaburzeniem czynności wątroby mogą otrzymać zalecaną dawkę początkową. Zaleca się ostrożność przy podawaniu produktu Iclusig® pacjentom z zaburzeniem czynności wątroby. *Pacjenci z zaburzeniem czynności nerek:* Wydalanie przez nerki nie jest ważnym szlakiem eliminacji ponatynibu. Nie przeprowadzono badań produktu Iclusig® u pacjentów z zaburzeniem czynności nerek. Pacjenci z szacowanym klirensem kreatyniny ≥ 50 ml/min powinni być w stanie bezpiecznie przyjmować produkt Iclusig® bez dostosowywania dawki. Należy zachować ostrożność przy podawaniu produktu Iclusig® pacjentom z szacowanym klirensem kreatyniny < 50 ml/min lub w schyłkowej fazie niewydolności nerek. *Dzieci i młodzież:* Nie określono bezpieczeństwa stosowania i skuteczności produktu leczniczego Iclusig® u pacjentów w wieku poniżej 18 lat. Brak dostępnych danych. Sposób podawania: Produkt Iclusig® jest przeznaczony do podawania doustnego. Tabletki należy połykać w całości. Pacjenci nie powinni kruszyć ani rozpuszczać tabletek. Produkt Iclusig® może być przyjmowany jednocześnie z posiłkiem lub niezależnie od niego. Pacjentów należy poinformować, aby nie połykali pojemnika z pochłaniaczem wilgoci znajdującego się w butelce. **Przeciwwskazania:** Nadwrażliwość na ponatynib lub jakąkolwiek substancję pomocniczą. **Ostrzeżenia i środki ostrożności:** *Mielosupresja:* Stosowanie produktu Iclusig® wiąże się z ciężką małopłytkowością, neutropenią i niedokrwistością [stopień 3. lub 4. wg kryteriów NCI CTCAE (wspólnych kryteriów oceny zdarzeń niepożądanych Narodowego Instytutu Raka)]. U większości pacjentów z małopłytkowością, neutropenią lub niedokrwistością stopnia 3. lub 4. stwierdzono wystąpienie tych zdarzeń w ciągu pierwszych trzech miesięcy leczenia. Częstość występowania tych zdarzeń jest większa u pacjentów z fazą akceleracji CML (AP-CML) lub fazą przełomu blastycznego CML (BP-CML)/Ph+ ALL niż z fazą przewlekłą CML (CP-CML). Pełną morfologię krwi należy oznaczać co 2 tygodnie przez pierwsze 3 miesiące, a następnie co miesiąc lub zgodnie ze wskazaniami klinicznymi. Mielosupresja była generalnie odwracalna i zwykle ustępowała po okresowym odstawieniu produktu Iclusig® lub zmniejszeniu jego dawki. *Niedrożność tętnic:* U pacjentów leczonych produktem Iclusig® występowały przypadki niedrożności tętniczej, w tym przypadki zawału mięśnia sercowego zakończone zgonem, udaru, niedrożności tętnic siatkówki wiążące się niekiedy z trwałym uszkodzeniem wzroku lub jego utratą, stenozy dużych naczyń tętniczych w mózgu, ciężkiej choroby tętnic obwodowych, zwężenia tętnicy nerkowej (z towarzyszącym nasilającym się, niestabilnym lub opornym na leczenie nadciśnieniem tętniczym) i konieczność pilnych zabiegów rewaskularyzacyjnych. Zdarzenia takie występowały u pacjentów z czynnikami ryzyka sercowo-naczyniowego i bez takich czynników, w tym u pacjentów w wieku 50 lat i młodszych. Zdarzenia niepożądane związane z niedrożnością tętnic występowały częściej u starszych pacjentów oraz u pacjentów z niedokrwieniem, nadciśnieniem, cukrzycą lub hiperlipidemią w wywiadzie. Ryzyko zdarzeń związanych z niedrożnością tętnic jest prawdopodobnie zależne od dawki. W badaniu fazy II (z co najmniej 64-miesięcznym okresem obserwacji) działania niepożądane związane z niedrożnością naczyń tętniczych wystąpiły u 25% pacjentów (częstość zdarzeń występujących podczas leczenia). U części pacjentów wystąpiły zdarzenia więcej niż jednego typu. Działania niepożądane w postaci niedrożności tętniczych naczyń wieńcowych, naczyń mózgowych oraz naczyń obwodowych (częstość zdarzeń występujących podczas leczenia) wystąpiły u, odpowiednio, 13%, 9% i 11% pacjentów leczonych produktem Iclusig®. W badaniu fazy II poważne działania niepożądane związane z niedrożnością naczyń tętniczych wystąpiły u 20% pacjentów (częstość zdarzeń występujących podczas leczenia). Poważne działania niepożądane w postaci niedrożności tętniczych naczyń wieńcowych, naczyń mózgowych oraz naczyń obwodowych (częstość zdarzeń występujących podczas leczenia) wystąpiły u, odpowiednio, 10%, 7% i 9% pacjentów leczonych produktem Iclusig®. Mediana czasu do wystąpienia pierwszych zdarzeń związanych z niedrożnością tętniczych naczyń wieńcowych, naczyń mózgowych i naczyń obwodowych wynosiła, odpowiednio, 351, 611 i 605 dni. Nie należy stosować produktu Iclusig® u pacjentów z zawałem mięśnia sercowego, zabiegiem rewaskularyzacji lub udarem w wywiadzie, chyba że potencjalne korzyści z leczenia przeważają nad potencjalnym zagrożeniem. U pacjentów takich należy także rozważyć alternatywne możliwości leczenia przed rozpoczęciem leczenia ponatynibem. Przed rozpoczęciem leczenia ponatynibem należy ocenić stan układu krążenia pacjenta, wraz z wywiadem i badaniem przedmiotowym, i podjąć czynne działania w celu ograniczenia czynników ryzyka sercowo-naczyniowego. Podczas leczenia ponatynibem należy w dalszym ciągu monitorować stan układu krążenia i odpowiednio dostosowywać leczenie zachowawcze i podtrzymujące w przypadku stanów zwiększających ryzyko sercowo-naczyniowe. Pacjentów należy monitorować w celu wykrycia objawów niedrożności naczyń tętniczych, w przypadku pogorszenia wzroku lub wystąpienia nieostrego widzenia należy zaś wykonać badanie okulistyczne (w tym fundoskopię). W razie wystąpienia niedrożności naczyń tętniczych przerwać natychmiast leczenie produktem Iclusig®. Decyzja o wznowieniu leczenia produktem Iclusig® powinna być oparta na ocenie stosunku korzyści do ryzyka. *Żylna choroba zakrzepowo-zatorowa:* W badaniu fazy II (z co najmniej 64-miesięcznym okresem obserwacji) wystąpienie działań niepożądanych w postaci żylnej choroby zakrzepowo-zatorowej stwierdzono u 6% pacjentów (częstość występująca w trakcie leczenia). Ciężkie działania niepożądane w postaci żylnej choroby zakrzepowo-zatorowej stwierdzono u 5% pacjentów (częstość występująca w trakcie leczenia). Pacjentów należy monitorować w celu wykrycia objawów choroby zakrzepowo-zatorowej. W razie wystąpienia choroby zakrzepowo-zatorowej należy natychmiast przerwać leczenie produktem Iclusig®. Przed wznowieniem leczenia produktem Iclusig® należy dokonać oceny stosunku korzyści do ryzyka. U pacjentów leczonych produktem Iclusig® stwierdzono występowanie niedrożności naczyń żylnych siatkówki, która w niektórych przypadkach wiązała się z nieodwracalnym pogorszeniem wzroku lub utratą wzroku. W przypadku pogorszenia wzroku lub wystąpienia nieostrego widzenia należy wykonać badanie okulistyczne (w tym fundoskopię). *Nadciśnienie tętnicze:* Nadciśnienie może zwiększać ryzyko zdarzeń zakrzepowych w naczyniach tętniczych, w tym ryzyko zwężenia tętnicy nerkowej. Podczas leczenia produktem Iclusig® należy monitorować ciśnienie krwi pacjenta podczas każdej wizyty w przychodni; w razie nadciśnienia należy je leczyć do uzyskania wartości prawidłowych. Jeżeli nadciśnienie nie da się opanować leczeniem farmakologicznym, należy tymczasowo wstrzymać leczenie produktem Iclusig®. W przypadku wystąpienia istotnego, nasilającego się, niestabilnego lub opornego na leczenie nadciśnienia tętniczego należy przerwać stosowanie produktu leczniczego i rozważyć przeprowadzenie badań w celu wykluczenia zwężenia tętnicy nerkowej. U pacjentów leczonych produktem Iclusig® występowały podczas leczenia epizody nadciśnienia (w tym przełom nadciśnieniowy). Pacjenci mogą wymagać pilnej interwencji w razie nadciśnienia z towarzyszącym splątaniem, bólem głowy, bólem w klatce piersiowej lub dusznością. *Tętniak i rozwarstwienie tętnicy:* Stosowanie inhibitorów szlaku VEGF u pacjentów z nadciśnieniem lub bez nadciśnienia może sprzyjać tworzeniu tętniaka i (lub) rozwarstwieniu tętnicy. Przed rozpoczęciem stosowania leku Iclusig® należy starannie rozważyć to ryzyko, zwłaszcza u pacjentów z takimi czynnikami ryzyka, jak nadciśnienie lub tętniak w wywiadzie. *Zastoinowa niewydolność serca:* U pacjentów leczonych produktem Iclusig® występowały przypadki dysfunkcji lewej komory serca lub poważne i prowadzące do zgonu przypadki niewydolności serca, w tym zdarzenia związane z uprzednią niedrożnością naczyń. Należy monitorować pacjentów w celu wykrycia przedmiotowych i podmiotowych objawów wskazujących na niewydolność serca i wdrożyć odpowiednie leczenie w zależności od sytuacji klinicznej, w tym z przerwaniem podawania produktu Iclusig®. W przypadku pacjentów, u których wystąpi poważna niewydolność serca, należy rozważyć przerwanie leczenia ponatynibem. *Zapalenie trzustki i aktywność lipazy w surowicy:* Stosowanie produktu Iclusig® wiąże się z zapaleniem trzustki. Częstość występowania zapalenia trzustki jest większa w ciągu pierwszych 2 miesięcy leczenia. Należy oznaczać aktywność lipazy w surowicy co 2 tygodnie przez pierwsze 2 miesiące, a następnie okresowo. Konieczne może być przerwanie leczenia lub zmniejszenie dawki. Jeżeli zwiększeniu aktywności lipazy towarzyszą objawy w obrębie jamy brzusznej, przerwać stosowanie produktu Iclusig® i ocenić pacjenta pod kątem objawów zapalenia trzustki. Zaleca się ostrożność w przypadku pacjentów z przebytym zapaleniem trzustki lub nadużywaniem alkoholu w wywiadzie. U pacjentów z ciężką lub bardzo ciężką hipertriglicerydemią należy wdrożyć odpowiednie postępowanie, aby zmniejszyć ryzyko zapalenia trzustki. *Hepatotoksyczność:* Produkt Iclusig® może spowodować zwiększenie wyników oznaczenia AlAT, AspAT, bilirubiny i fosfatazy zasadowej. U większości pacjentów, u których stwierdzono hepatotoksyczność, pierwsze zdarzenie hepatotoksyczności zaobserwowano w pierwszym roku leczenia. Stwierdzono przypadki niewydolności wątroby (w tym zakończone zgonem). Testy wątrobowe należy przeprowadzić przed rozpoczęciem leczenia i powtarzać je okresowo, w odstępach zgodnych ze wskazaniami klinicznymi. *Krwotok:* U pacjentów leczonych produktem Iclusig® występowały ciężkie krwotoki, w tym przypadki zakończone zgonem. Częstość występowania ciężkiego krwawienia była większa u pacjentów z AP-CML, BP-CML i Ph+ ALL. Najczęściej zgłaszane epizody krwawienia stopnia 3. lub 4. obejmowały krwotok z przewodu pokarmowego i krwiak podtwardówkowy. Większość zdarzeń krwotocznych, choć nie wszystkie, wystąpiła u pacjentów z małopłytkowością stopnia 3./4. W razie wystąpienia poważnego lub ciężkiego krwotoku należy przerwać leczenie produktem Iclusig® i ocenić stan pacjenta. *Reaktywacja wirusowego zapalenia wątroby typu B:* U pacjentów będących przewlekłymi nosicielami wirusa zapalenia wątroby typu B dochodziło do reaktywacji zapalenia wątroby po otrzymaniu przez nich inhibitorów kinazy tyrozynowej BCR-ABL. Niektóre przypadki prowadziły do ostrej niewydolności wątroby lub piorunującego zapalenia wątroby, a w konsekwencji do przeszczepienia wątroby lub zgonu pacjenta. U pacjentów należy wykonać badania pod kątem zakażenia wirusem HBV przed rozpoczęciem leczenia produktem leczniczym Iclusig®. Przed rozpoczęciem leczenia u pacjentów z dodatnim wynikiem badania serologicznego w kierunku wirusowego zapalenia wątroby typu B (w tym u pacjentów z aktywną chorobą) i w przypadku pacjentów z dodatnim wynikiem badania w kierunku zakażenia wirusem HBV w trakcie leczenia należy skonsultować się z ekspertami ds. chorób wątroby i leczenia wirusowego zapalenia wątroby typu B. Nosiciele wirusa HBV, którzy wymagają leczenia produktem leczniczym Iclusig®, powinni być poddawani ścisłej obserwacji pod kątem objawów podmiotowych i przedmiotowych aktywnego zakażenia wirusem HBV w trakcie całego okresu leczenia i przez kilka miesięcy po jego zakończeniu. *Zespół odwracalnej tylnej encefalopatii:* U pacjentów leczonych produktem Iclusig® stwierdzono przypadki zespołu odwracalnej tylnej encefalopatii (ang. *posterior reversible encephalopathy syndrome*, PRES) po wprowadzeniu produktu do obrotu. PRES to schorzenie neurologiczne, które może się wiązać z takimi objawami przedmiotowymi i podmiotowymi, jak: napad drgawkowy, ból głowy, zmniejszenie czujności, zmienione funkcjonowanie psychiczne, utrata widzenia oraz inne zaburzenia wzrokowe i neurologiczne. W przypadku jego zdiagnozowania należy przerwać leczenie produktem Iclusig® i ponownie rozpocząć leczenie dopiero po ustąpieniu zdarzenia i jeśli korzyść z kontynuacji leczenia przewyższa ryzyko związane z PRES. *Zahamowanie czynności szpiku kostnego:* Stosowanie produktu leczniczego Iclusig® jest związane z ciężką małopłytkowością, neutropenią i niedokrwistością. Częstość występowania tych zdarzeń jest większa u pacjentów z AP-CML lub BP-CML/Ph+ ALL niż u pacjentów z CP-CML. Należy wykonywać pełne badanie morfologii krwi co 2 tygodnie w okresie pierwszych 3 miesięcy, a następnie co miesiąc lub z inną częstością, odpowiednią do wskazań klinicznych. *Zapalenie trzustki i stężenie lipazy w surowicy:* Stosowanie produktu leczniczego Iclusig® jest związane z zapaleniem trzustki. Częstość występowania zapalenia trzustki jest największa w okresie pierwszych 2 miesięcy stosowania tego produktu leczniczego. Należy oznaczać aktywność lipazy w surowicy co 2 tygodnie w okresie pierwszych 2 miesięcy, a następnie okresowo. Może być konieczne czasowe przerwanie leczenia lub zredukowanie dawki. W przypadku gdy podwyższeniu aktywności lipazy towarzyszą objawy ze strony jamy brzusznej, należy czasowo przerwać leczenie produktem Iclusig®, a u pacjenta należy przeprowadzić ocenę pod kątem oznak zapalenia trzustki. Zaleca się zachowanie ostrożności podczas leczenia pacjentów z zapaleniem trzustki lub nadużywaniem alkoholu w wywiadzie. U pacjentów z ciężką lub bardzo ciężką hipertriglicerydemią należy zastosować odpowiednie leczenie, aby zredukować ryzyko zapalenia trzustki. *Laktoza:* Produkt leczniczy Iclusig® zawiera laktozę jednowodną. Pacjenci z rzadko występującą dziedziczną nietolerancją galaktozy, niedoborem laktazy (typu Lapp) lub zespołem złego wchłaniania glukozy-galaktozy nie powinni przyjmować tego produktu. *Senność:* Należy zachować ostrożność podczas prowadzenia pojazdów lub obsługiwania maszyn. *Wydłużenie odstępu QT:* Nie przeprowadzono szczegółowego badania wpływu tego produktu leczniczego na odstęp QT; dlatego nie można wykluczyć klinicznie istotnego wpływu na odstęp QT. *Zaburzenia czynności wątroby:* Stosowanie produktu leczniczego Iclusig® może spowodować podwyższenie stężenia ALT, AST, bilirubiny i fosfatazy alkalicznej. Należy okresowo wykonywać badania czynnościowe wątroby, zależnie od wskazań klinicznych. *Niewydolność wątroby:* Zaleca się zachowanie ostrożności podczas stosowania produktu leczniczego Iclusig® u pacjentów z niewydolnością wątroby. *Niewydolność nerek:* Zaleca się zachowanie ostrożności podczas stosowania produktu leczniczego Iclusig® u pacjentów z szacunkowym klirensem kreatyniny < 50 ml/min lub schyłkową chorobą nerek. *Pacjenci w podeszłym wieku:* U pacjentów z tej grupy występuje podwyższone ryzyko działań niepożądanych. *Dzieci i młodzież:* Nie określono bezpieczeństwa ani skuteczności stosowania produktu leczniczego Iclusig® u pacjentów w wieku poniżej 18. roku życia. **Interakcje:** Substancje, które mogą zwiększyć stężenie ponatynibu w surowicy: *Inhibitory CYP3A:* Ponatynib jest metabolizowany przez CYP3A4. Jednoczesne podanie pojedynczej doustnej dawki 15 mg produktu Iclusig® w obecności ketokonazolu (400 mg na dobę), silnego inhibitora CYP3A, spowodowało pewne zwiększenie ogólnoustrojowej ekspozycji na ponatynib, gdzie wartości $AUC_{0-\infty}$ i C_{maks} ponatynibu były większe o, odpowiednio, 78% i 47% w porównaniu z podawaniem samego ponatynibu. Należy także zachować ostrożność i rozważyć zmniejszenie początkowej dawki produktu Iclusig® do 30 mg przy jednoczesnym stosowaniu silnych inhibitorów CYP3A, takich jak klarytromycyna, indynawir, itrakonazol, ketokonazol, nefazodon, nelfinawir, rytonawir, sakwinawir, telitromycyna, troleandomycyna, worykonazol oraz sok grejpfrutowy. Substancje, które mogą zmniejszyć stężenie ponatynibu w surowicy: *Induktory CYP3A:* Jednoczesne podanie 19 zdrowym ochotnikom pojedynczej dawki 45 mg produktu Iclusig® w trakcie leczenia ryfampicyną (600 mg na dobę), silnym induktorem CYP3A, zmniejszyło $AUC_{0-\infty}$ i C_{max} dla ponatynibu, odpowiednio, o 62% i 42% w porównaniu z podawaniem samego ponatynibu. Należy unikać jednoczesnego podawania silnych induktorów CYP3A4, takich jak karbamazepina, fenobarbital, fenytoina, ryfabutyna, ryfampicyna oraz ziele dziurawca z ponatynibem i poszukiwać preparatów alternatywnych dla induktorów CYP3A4, chyba że korzyści z ich stosowania przewyższają możliwe ryzyko zmniejszonej ekspozycji na ponatynib. Substancje, na których stężenie w surowicy może mieć wpływ ponatynib: *Substraty transporterów:* Ponatynib w warunkach *in vitro* jest inhibitorem P-gp i BCRP. W związku z tym ponatynib może teoretycznie zwiększać stężenie w osoczu podawanych jednocześnie substratów P-gp (np. digoksyny, dabigatranu, kolchicyny, prawastatyny) lub BCRP (np. metotreksatu, rozuwastatyny, sulfasalazyny), nasilając w ten sposób ich działanie lecznicze i działania niepożądane. Zaleca się ścisłą obserwację kliniczną w razie podawania ponatynibu z takimi produktami leczniczymi. Dzieci i młodzież: Badania dotyczące interakcji przeprowadzono wyłącznie u dorosłych. **Ciąża i karmienie piersią:** Kobiety zdolne do poczęcia/antykoncepcja u mężczyzn i kobiet: Kobietom zdolnym do poczęcia leczonym produktem Iclusig® należy odradzić zachodzenie w ciążę, a mężczyznom leczonym produktem Iclusig® należy odradzić płodzenie dziecka w okresie leczenia. W trakcie leczenia należy stosować skuteczną metodę antykoncepcji. Nie wiadomo, czy ponatynib wpływa na skuteczność ogólnoustrojowych hormonalnych środków antykoncepcyjnych. Należy stosować alternatywną lub dodatkową metodę antykoncepcji. Ciąża: Nie ma wystarczających danych dotyczących stosowania produktu Iclusig® u kobiet w ciąży. Badania na zwierzętach wykazały szkodliwy wpływ na reprodukcję. Potencjalne zagrożenie dla człowieka nie jest znane. Produkt Iclusig® można stosować w ciąży jedynie w przypadku zdecydowanej konieczności. Jeżeli produkt ten jest stosowany w okresie ciąży, należy poinformować pacjentkę o możliwym zagrożeniu dla płodu. Karmienie piersią: Nie wiadomo, czy produkt Iclusig® przenika do mleka karmiącej matki. Dostępne dane farmakodynamiczne i toksykologiczne nie wykluczają możliwości jego przenikania do mleka matki. Podczas leczenia produktem Iclusig® należy przerwać karmienie piersią. Płodność: Nie ma dostępnych danych dotyczących wpływu ponatynibu na płodność u ludzi. U szczurów leczenie ponatynibem wywierało wpływ na płodność samic, a płodność samców pozostawała niezmieniona. Znaczenie kliniczne tych wyników dla płodności człowieka jest nieznane. **Działania niepożądane:** Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Członkowie fachowego personelu medycznego powinni zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem krajowego systemu zgłaszania. Lekarze przepisujący ten produkt leczniczy powinni zapoznać się z ChPL, która zawiera pełne informacje na temat działań niepożądanych (ADR). Najczęstsze poważne działania niepożądane > 2% (częstość występowania podczas leczenia) obejmowały zapalenie płuc (7,3%), zapalenie trzustki (5,8%), ból w jamie brzusznej (4,7%), migotanie przedsionków (4,5%), gorączkę (4,5%), zawał mięśnia sercowego (4,0%), niedrożność tętniczych naczyń obwodowych (3,8%), niedokrwistość (3,8%), dusznicę bolesną (3,3%), zmniejszenie liczby płytek krwi (3,1%), gorączkę neutropeniczną (2,9%), nadciśnienie (2,9%), chorobę tętnic wieńcowych (2,7%), zastoinową niewydolność serca (2,4%), epizod naczyniowo-mózgowy (2,4%), sepsę (2,4%), zapalenie tkanki łącznej (2,2%), ostre uszkodzenie nerek (2,0%), zakażenie dróg moczowych (2,0%) i zwiększenie aktywności lipazy (2,0%). Poważne działania niepożądane w postaci niedrożności tętniczych naczyń wieńcowych, naczyń mózgowych oraz naczyń obwodowych (częstość zdarzeń występujących podczas leczenia) wystąpiły u, odpowiednio, 10%, 7% i 9% pacjentów leczonych produktem Iclusig®. Poważne działania obejmujące niedrożność naczyń żylnych (częstość zdarzeń występujących podczas leczenia) wystąpiły u 5% pacjentów. Działania niepożądane w postaci niedrożności tętniczych naczyń wieńcowych, naczyń mózgowych oraz naczyń obwodowych (częstość zdarzeń występujących podczas leczenia) wystąpiły u, odpowiednio, 13%, 9% i 11% pacjentów leczonych produktem Iclusig®. W sumie działania niepożądane w postaci niedrożności naczyń tętniczych i żylnych wystąpiły u 25% pacjentów leczonych produktem Iclusig® w badaniu fazy II, a poważne działania niepożądane wystąpiły u 20% pacjentów. U części pacjentów wystąpiły zdarzenia więcej niż jednego typu. Żylne powikłania zakrzepowo-zatorowe (częstość zdarzeń występujących podczas leczenia) wystąpiły u 6% pacjentów. Częstość występowania zdarzeń zakrzepowo-zatorowych jest większa u pacjentów z Ph+ ALL lub BP-CML niż u pacjentów z AP-CML lub CP-CML. Żadne zdarzenie związane z niedrożnością naczyń żylnych nie zakończyło się zgonem. Po okresie obserwacji trwającym minimum 64 miesiące częstość występowania działań niepożądanych prowadzących do przerwania leczenia wynosiła 20% u pacjentów z CP-CML, 11% u pacjentów z AP-CML, 15% u pacjentów z BP-CML oraz 9% u pacjentów z Ph+ ALL. *Bardzo częste działania niepożądane (≥ 1/10):* zakażenie górnych dróg oddechowych, niedokrwistość, zmniejszenie liczby płytek krwi, zmniejszenie liczby neutrofili, zmniejszenie apetytu, bezsenność, ból głowy, zawroty głowy, nadciśnienie tętnicze, duszność, kaszel, ból jamy brzusznej, biegunka, wymioty, zaparcie, nudności, zwiększenie stężenia lipazy, zwiększenie aktywności aminotransferazy alaninowej, zwiększenie aktywności aminotransferazy asparaginianowej, wysypka, suchość skóry, świąd, ból kości, ból stawów, ból mięśni, ból kończyn, ból pleców, kurcze mięśni, zmęczenie, astenia, obrzęk obwodowy, gorączka, ból. *Częste działania niepożądane (≥ 1/100 do <1/10):* zapalenie płuc, posocznica, zapalenie mieszków włosowych, zapalenie tkanki łącznej, pancytopenia, gorączka neutropeniczna, zmniejszenie liczby białych krwinek, zmniejszenie liczby limfocytów, niedoczynność tarczycy, odwodnienie, zatrzymanie płynów, hipokalcemia, hiperglikemia, hiperurykemia, hipofosfatemia, hipertriglicerydemia, hipokaliemia, zmniejszenie masy ciała, hiponatremia, zdarzenie naczyniowo-mózgowe, udar niedokrwienny mózgu, neuropatia obwodowa, ospałość, migrena, hiperestezja, hipestezja, parestezja, przemijający napad niedokrwienny, nieostre widzenie, suchość oka, obrzęk okołooczodołowy, obrzęk powiek, zapalenie spojówek, zaburzenia widzenia, niewydolność serca, zawał mięśnia sercowego, zastoinowa niewydolność serca, choroba niedokrwienna serca, dusznica bolesna, wysięk osierdziowy, migotanie przedsionków, zmniejszenie frakcji wyrzutowej, ostry zespół wieńcowy, trzepotanie przedsionków, choroba zarostowa tętnic obwodowych, niedokrwienie obwodowe, stenoza tętnic obwodowych, chromanie przestankowe, zakrzepica żył głębokich, uderzenia gorąca, niekiedy z zaczerwienieniem skóry, zatorowość płucna, wysięk opłucnowy, krwawienie z nosa, dysfonia, nadciśnienie płucne, zapalenie trzustki, zwiększenie stężenia amylazy we krwi, choroba refluksowa przełyku, zapalenie jamy ustnej, niestrawność, rozdęcie jamy brzusznej, dyskomfort w jamie brzusznej, suchość ust, krwotok żołądkowy, zwiększenie stężenia bilirubiny we krwi, zwiększenie aktywności fosfatazy alkalicznej, gamma- -glutamylotranspeptydazy, swędząca wysypka, złuszczająca wysypka, rumień, łysienie, łuszczenie się skóry, nocne poty, nadmierna potliwość, podbiegnięcia, wybroczyny, ból skóry, złuszczające zapalenie skóry, hiperkeratoza, hiperpigmentacja skóry, ból mięśniowo-szkieletowy, ból szyi, ból klatki piersiowej, zaburzenia wzwodu, dreszcze, choroba grypopodobna, ból w klatce piersiowej pochodzenia pozasercowego, powstanie wyczuwalnego guzka, obrzęk twarzy. *Niezbyt częste działania niepożądane (≥ 1/1000 do < 1/100):* zakrzepica żył siatkówki, niedrożność żył siatkówki, niedrożność tętnic siatkówki, zawał mięśnia sercowego, dyskomfort ze strony serca, kardiomiopatia niedokrwienna, skurcz tętnic wieńcowych, dysfunkcja lewej komory serca, słabe krążenie obwodowe, zawał śledziony, zatorowość żylna, zakrzepica żylna, przełom nadciśnieniowy, zwężenie tętnicy nerkowej, hepatotoksyczność, niewydolność wątroby, żółtaczka. *Nieznana częstość działań niepożądanych:* tętniak i rozwarstwienie tętnicy. **Rodzaj i zawartość opakowania:** Butelki z polietylenu o wysokiej gęstości (HDPE), z zakręcanym zamknięciem, zawierające 30 tabletek powlekanych zawierających dawkę 45 mg bądź 60 tabletek powlekanych zawierających dawkę 15 mg. **Podmiot odpowiedzialny:** Incyte Biosciences Distribution B.V., Paasheuvelweg 25, 1105 BP Amsterdam, Holandia. **Przedstawiciel podmiotu odpowiedzialnego:** Angelini Pharma Polska Sp. z o.o., ul. Podleśna 83, 05-552 Łazy, Polska, tel.: +48 22 70 28 200, faks: +48 22 70 28 202. **Numery pozwoleń na dopuszczenie do obrotu:** Tabletka 15 mg: 60 tabletek – EU/1/13/839/00; tabletka 45 mg: 30 tabletek – EU/1/13/839/003. **Kategoria dostępności:** Lek wydawany z przepisu lekarza – Rp. **Refundacja:** Lista B; Leki dostępne w ramach programu lekowego B.14. i B.65.; Obwieszczenie Ministra Zdrowia z 21 czerwca 2021 r. **Przed zastosowaniem należy zapoznać się z zatwierdzoną Charakterystyką Produktu Leczniczego.**

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Acta Haematologica Polonica

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Gazyvaro podawany w skojarzeniu z bendamustyną, a następnie w monoterapii w leczeniu podtrzymującym jest wskazany w leczeniu pacjentów z chłoniakiem grudkowym (FL), u których nie wystąpiła odpowiedź na leczenie lub u których podczas leczenia lub maksymalnie do 6 miesięcy po leczeniu rytuksymabem lub schematem zawierającym rytuksymab, wystąpiła progresja choroby. **Dawkowanie i sposób podawania:** Gazyvaro należy podawać pod ścisłym nadzorem doświadczonego lekarza, w miejscu, w którym natychmiast są dostępne środki do prowadzenia resuscytacji. Przewlekła białaczka limfocytowa cykl 1: zalecaną dawkę 1000 mg produktu leczniczego Gazyvaro, podaje się w 1.- 2. dniu, 8. dniu i 15. dniu pierwszego 28-dniowego cyklu leczenia. Do infuzji w 1.- 2. dniu cyklu należy przygotować dwie torebki infuzyjne (100 mg na dzień 1. i 900 mg na dzień 2.). Jeżeli podczas podawania pierwszej torebki nie było przerw ani konieczności modyfikacji prędkości podawania, drugą torebkę można podać tego samego dnia (bez konieczności opóźnienia podania) i bez powtórzenia premedykacji, pod warunkiem zapewnienia właściwych warunków, odpowiedniego czasu i nadzoru personelu medycznego podczas trwania infuzji. W przypadku jakichkolwiek zmian w prędkości infuzji lub wystąpienia przerw podczas podawania pierwszych 100 mg, drugą torebkę z produktem leczniczym należy podać następnego dnia. Cykle 2 do 6: zalecana dawka 1000 mg Gazyvaro podawana w dniu 1. cyklu. Chłoniak grudkowy wcześniej nieleczony: cykl 1: zalecaną dawkę 1000 mg produktu leczniczego Gazyvaro w skojarzeniu z CHOP, CVP lub bendamustyną podaje się w 1. dniu, 8. dniu i 15. dniu pierwszego cyklu leczenia. Cykle 2-6 (lub 2-8): dawkę 1000 mg produktu leczniczego Gazyvaro w skojarzeniu z CHOP, CVP lub bendamustyną podaje się w 1. dniu każdego cyklu leczenia. Gazyvaro należy podawać z chemioterapią według schematu: sześć 28-dniowych cykli w skojarzeniu z bendamustyną lub sześć 21-dniowych cykli w skojarzeniu z CHOP, a następnie 2 dodatkowe cykle leczenia Gazyvaro w monoterapii lub osiem 21-dniowych cykli w skojarzeniu z CVP. Leczenie podtrzymujące: pacjenci, u których uzyskano całkowitą lub częściową odpowiedź na leczenie indukcyjne Gazyvaro w skojarzeniu z chemioterapią (CHOP lub CVP lub bendamustyna) powinni kontynuować przyjmowanie dawki 1000 mg Gazyvaro w monoterapii raz na 2 miesiące przez 2 lata lub do wystąpienia progresji choroby. Chłoniak grudkowy u chorych, u których nie wystąpiła odpowiedź na leczenie lub u których podczas leczenia lub maksymalnie do 6 miesięcy po leczeniu rytuksymabem lub schematem zawierającym rytuksymab, wystąpiła progresja choroby: cykl 1: dawkę 1000 mg Gazyvaro w skojarzeniu z bendamustyną podaje się w 1., 8. i 15. dniu pierwszego 28-dniowego cyklu leczenia. Cykle 2-6: Zalecaną dawkę 1000 mg Gazyvaro w skojarzeniu z bendamustyną podaje się w 1. dniu każdego 28-dniowego cyklu leczenia. Leczenie podtrzymujące: pacjenci, u których wystąpi reakcja na leczenie indukcyjne (tj. pierwszych 6 cykli leczenia) Gazyvaro w skojarzeniu z bendamustyną lub pacjenci, u których choroba jest stabilna, powinni kontynuować przyjmowanie dawki 1000 mg Gazyvaro w monoterapii raz na 2 miesiące przez dwa lata lub do progresji choroby. Profilaktyka zespołu rozpadu guza (ZRS): u pacjentów z dużą liczbą limfocytów (> 25 x 109/l), w celu zmniejszenia ryzyka zespołu rozpadu guza jest zalecana profilaktyka, polegająca na odpowiednim nawodnieniu i podawaniu leków hamujących wytwarzanie kwasu moczowego (np. allopurinol) na 12-24 godziny przed rozpoczęciem terapii. Profilatyka i premedykacja: przed rozpoczęciem wlewu należy zastosować premedykację (kortykosteroid dożylny, doustny lek przeciwbólowy/przeciwgorączkowy, lek przeciwhistaminowy). Premedykacja kortykosteroidami jest zalecana u pacjentów z FL i obowiązkowa u pacjentów z PBL w pierwszym cyklu. Podczas dożylnego podawania Gazyvaro może wystąpić niedociśnienie tętnicze jako reakcja związana z wlewem, dlatego należy rozważyć przerwanie stosowania leków obniżających ciśnienie na 12 godzin przed rozpoczęciem terapii i podczas każdego wlewu leku Gazyvaro, oraz w pierwszej godzinie po jego podaniu. Sposób podawania: lek Gazyvaro należy podawać we wlewie dożylnym po uprzednim rozcieńczeniu, przez przeznaczoną do tego celu linię infuzyjną. Nie podawać produktu w dożylnym wstrzyknięciu lub bolusie. Przewlekła białaczka limfocytowa cykl 1 dz.1 (100 mg): 25 mg/godz. przez 4 godziny. Nie zwiększać prędkości wlewu. Cykl 1 dz.2 lub kontynuacja dz. 1 (900 mg): 50 mg/godz. Jeśli podczas poprzedniego wlewu wystąpiła IRR, rozpocząć podawanie z prędkością 25 mg/godz. Prędkość wlewu może być stopniowo zwiększana o 50 mg/godz. co kolejne 30 minut do maksymalnej prędkości 400 mg/godz. Cykl 1 dz.8, 15 i cykl 2 – 6: jeżeli podczas poprzedniego wlewu nie wystąpiła IRR przy prędkości wynoszącej 100 mg/godz lub większej to podawać z prędkością początkową 100 mg/godz. i można stopniowo zwiększać o 100 mg/godz. co kolejne 30 min. do maksymalnej prędkości 400 mg/godz. Jeśli podczas poprzedniego wlewu wystąpiła IRR, rozpocząć podawanie leku z prędkością 50 mg/godz., którą można stopniowo zwiększać o 50 mg/godz. co 30 minut do maksymalnej 400 mg/godz. Postępowanie w przypadku wystąpienia reakcji związanych z wlewami może wymagać czasowego przerwania podawania, zmniejszenia prędkości wlewu lub zakończenia leczenia. Chłoniak grudkowy cykl 1 dz.1 (1000 mg): 50 mg/godz prędkość wlewu można stopniowo zwiększać o 50 mg/godzinę co kolejne 30 minut do maksymalnej prędkości 400 mg/godz. Cykl 1 dz.8, 15, cykl 2 – 6 lub 2 – 8 i leczenie podtrzymujące co 2 miesiące przez 2 lata lub do progresji choroby: prędkość początkowa wlewu 100 mg/godz. stopniowo zwiększana o 100 mg/godz. co kolejne 30 min. do maksymalnej prędkości 400 mg/godz. Jeśli podczas poprzedniego wlewu wystąpiła IRR st. 2 lub większego, rozpocząć podawanie leku z prędkością 50 mg/godz. Prędkość wlewu można stopniowo zwiększać o 50 mg/godz co 30 minut do maksymalnej 400 mg/godz. Postępowanie w przypadku wystąpienia reakcji związanych z wlewami może wymagać czasowego przerwania podawania, zmniejszenia prędkości wlewu lub zakończenia leczenia. Czas trwania leczenia: Przewlekła białaczka limfocytowa 6 cykli leczenia, z których każdy trwa 28 dni. Chłoniak grudkowy: 6 cykli leczenia, z których każdy trwa 28 dni, w przypadku leczenia skojarzonego z bendamustyną lub 8 cykli leczenia, z których każdy trwa 21 dni, w przypadku leczenia skojarzonego z CHOP lub CVP, a następnie dawka podtrzymująca co 2 miesiące przez 2 lata lub do progresji choroby. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą. **Specjalne ostrzeżenia i środki ostrożności**: Reakcje związane z wlewem należą do najczęściej obserwowanych działań niepożądanych i występują głównie podczas wlewu pierwszych 1000 mg produktu Gazyvaro, a następnie ich nasilenie i częstotliwość znacząco spadają. Reakcje związane z wlewem mogą mieć związek z zespołem uwalniania cytokin, który obserwowano u pacjentów leczonych Gazyvaro. Należy stosować działania zmniejszające reakcje związane z wlewem. Pacjenci z dużą masą guza, mogą być szczególnie narażeni na wystąpienie ciężkich reakcji związanych z wlewem. U pacjentów z zaburzeniami czynności nerek istnieje zwiększone ryzyko reakcji związanych z wlewem, w tym ciężkich. Zgłoszono przypadki wystąpienia zespołu uwalniania cytokin. W przypadku reakcji st. 3. - tymczasowo wstrzymać wlew i zastosować leczenie objawowe, dla st. 1-2. zmniejszyć prędkość wlewu i zastosować leczenie objawowe. Wlew można wznowić po ustąpieniu objawów z prędkością zmniejszoną o 50%, którą można stopniowo zwiększać w zależności od dawki i występowania objawów. Należy bezwzględnie zakończyć stosowanie produktu Gazyvaro zawsze w przypadkach: jednorazowego wystąpienia reakcji związanej z wlewem st. 4 lub powtórnego wystąpienia reakcji związanych z wlewem st. 3 oraz wystąpienia ostrych, zagrażających życiu objawów ze strony układu oddechowego. Reakcje nadwrażliwości, w tym anafilaktyczne mogą być trudne do odróżnienia od reakcji związanych z wlewem. Jeżeli podczas wlewu podejrzewa się wystąpienie reakcji nadwrażliwości wlew musi być przerwany i leczenie zakończone. Nie wolno podawać Gazyvaro pacjentom z nadwrażliwością na obinutuzumab zależną od IgE w wywiadzie. Zespół rozpadu guza (ZRG): pacjenci ze zwiększonym ryzykiem ZRG powinni otrzymać odpowiednie leczenie zapobiegające lizie guza i powinni być odpowiednio nawodnieni na 12-24 godziny przed rozpoczęciem wlewu produktu Gazyvaro a objawy ZRG powinny być odpowiednio leczone. Neutropenia: Pacjenci, u których wystąpiła neutropenia powinni być ściśle monitorowani i odpowiednio leczeni aż do ustąpienia objawów. Należy rozważyć opóźnienie podania kolejnej dawki produktu leczniczego Gazyvaro w przypadku wystąpienia ciężkiej, zagrażającej życiu neutropenii. U pacjentów z ciężką i długo trwającą neutropenią zaleca się zastosowanie profilaktyki przeciwbakteryjnej oraz rozważenie profilaktyki przeciwwirusowej i przeciwgrzybiczej. Małopłytkowość: Podczas leczenia Gazyvaro zgłaszano przypadki ciężkiej i zagrażającej życiu małopłytkowości, w tym ostrej. Zgłaszano również przypadki krwotoków zakończonych zgonem w trakcie 1. cyklu leczenia. Należy uważnie monitorować pacjentów pod kątem wystąpienia małopłytkowości, zwłaszcza w trakcie pierwszego cyklu leczenia; należy regularnie wykonywać badania laboratoryjne, aż do czasu ustąpienia małopłytkowości, a w przypadku małopłytkowości ciężkiej lub zagrażającej życiu należy rozważyć opóźnienie podania dawki Gazyvaro. Pogorszenie przebiegu współistniejących chorób serca: u pacjentów z chorobami serca, arytmią występowały dusznica bolesna, ostry zespół wieńcowy, zawał mięśnia sercowego i niewydolność serca. Mogą one wystąpić jako reakcje związane z wlewem i spowodować zgon. Zaleca się ścisłe monitorowanie pacjentów z chorobami serca. Zakażenia: Gazyvaro nie należy podawać w przypadku czynnego zakażenia oraz zachować ostrożność u pacjentów z przewlekłymi lub nawracającymi zakażeniami w wywiadzie. Reaktywacja zakażenia wirusem zapalenia wątroby typu B: u pacjentów leczonych Gazyvaro, może dojść do reaktywacji zakażenia wirusem zapalenia wątroby typu B i prowadzić do piorunującego zapalenia wątroby, niewydolności wątroby i zgonu. Przed rozpoczęciem leczenia Gazyvaro należy przeprowadzić badania przesiewowe w kierunku wirusowego zapalenia wątroby typu B (HBcAb i HBsAg). Nie należy stosować Gazyvaro u pacjentów z czynnym zakażeniem HBV. Postępująca wieloogniskowa leukoencefalopatia (PML): U pacjentów leczonych Gazyvaro zgłaszano przypadki wystąpienia postępującej PML. Leczenie Gazyvaro należy wstrzymać podczas diagnostyki PML i bezwzględnie zakończyć w przypadku rozpoznania PML. Immunizacja: ze względu na brak danych nie zaleca się szczepienia szczepionkami z żywymi wirusami podczas terapii Gazyvaro i u pacjentów ze zmniejszoną liczbą limfocytów B (w tym noworodków z wewnątrzmaciczną ekspozycją na obinutuzumab). **Działania niepożądane:** Zakażenie górnych dróg oddechowych, zapalenie zatok, zakażenia układu moczowego, zapalenie jamy nosowej i gardła, opryszczka jamy ustnej, zapalenie błony śluzowej nosa, zakażenie płuc, zapalenie płuc, półpasiec, grypa, opryszczka jamy ustnej, rak kolczystokomórkowy skóry, rak podstawnokomórkowy, neutropenia, małopłytkowość, niedokrwistość, leukopenia, gorączka neutropeniczna, zespół rozpadu guza, zwiększone stężenie kwasu moczowego we krwi, hipokalemia, depresja, migotanie przedsionków, tachyarytmia, dusznica bolesna, ostry zespół wieńcowy, zawał mięśnia sercowego, nadciśnienie tętnicze, kaszel, niedrożność nosa, ból jamy ustnej i gardła, katar, biegunka, zaparcia, niestrawność, guzki krwawnicze, tysienie, świąd, wyprysk, ból stawów, ból pleców, ból mięśniowo-szkieletowy klatki piersiowej, ból kończyn, ból kości, bolesne oddawanie moczu, nietrzymanie moczu, dyzuria, gorączka, astenia, zmęczenie, ból w klatce piersiowej, zmniejszona liczba białych krwinek, zmniejszona liczba krwinek białych obojętnochłonnych, zwiększenie masy ciała, zmęczenie, zapalenie oskrzeli, bezsenność, lęk, gorączka neutropeniczna, posocznica, PML, reaktywacja WZW typu B, perforacja żołądkowo-jelitowa, reakcje związane z wlewem: nudności, wymioty, biegunka, ból głowy, zawroty głowy, zmęczenie, dreszcze, gorączka, niedociśnienie tętnicze, nagłe zaczerwienienie twarzy, nadciśnienie tętnicze, tachykardia, duszność, uczucie dyskomfortu w obrębie klatki piersiowej, skurcz oskrzeli, podrażnienie gardła i krtani, sapanie, obrzęk krtani, migotanie przedsionków. **Numer pozwolenia na dopuszczenie do obrotu:** EU/1/14/937/001 nadany przez Komisję Europejską. **Podmiot odpowiedzialny:** Roche Registration GmbH; Emil-Barell-Strasse 1; 79639 Grenzach-Wyhlen; Niemcy. **Przedstawiciel podmiotu odpowiedzialnego:** Roche Polska Sp. z o.o., Domaniewska 39 B, 02 672 Warszawa. **Kategoria dostępności:** Produkt leczniczy wydawany z przepisu lekarza, do zastrzeżonego stosowania. 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EDITORIAL

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Scientific life is changing: virtual and real-world experience

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It had been arranged that we would meet each other at the XXIX Meeting of the Polish Society of Hematology and Transfusion Medicine in September 2021. The coronavirus disease 2019 (COVID-19) pandemic has caused this Meeting to be cancelled. However, more than 70 years of tradition, and the prestigious status of the Society, requires that we demonstrate the progress that has been made in hematology and blood transfusion. The virtual XXIX Meeting will present highlights of 2021. We plan to meet, hopefully face-to-face, next year.

The pandemic has already changed our scientific lives. Unusually in a world where activity in most areas has been severely curtailed, scientists have been pushed to much higher levels of activity, much of it via virtual meetings. Our scientific life has expanded to encompass teleconferences and webinars. Some of these practices will undoubtedly remain. Most one-day events, scientific, organizational and work-related, are being replaced by virtual meetings. Educational courses are also being moved to the web. But we all miss our participation in large conferences with personal meetings, both at the national and international levels.

Scientific activity during the pandemic can be easily measured by an increase in manuscript publications and more submissions and presentations of abstracts at leading conferences. Recently, there have been released impact factor values that have shown increasing values for most hematological journals. Obviously, "Acta Haematologica Polonica" cannot compete with these journals. But papers published in "Acta Haematologica Polonica" can be cited everywhere, and must be cited, if we want to maintain our status within the scientific community. This is the continuous task for all of us [1–4]!

To help achieve this goal, this 'meeting' issue of "Acta Haematologica Polonica" contains a number of leading papers on topics including hematopoietic cell transplantation, myeloproliferative and lymphoproliferative disorders, as well as on non-malignant hematology, hemostasis, and blood transfusion. The Autoimmune Disease Working Party of the European Society for Blood and Marrow Transplantation (EBMT) presents a report on their activity, calling for cooperation with Polish centers [5]. The Hemostasis Group presents their interdisciplinary impact and cooperation with cardiology, gynecology and obstetrics [6–8]. We see how targeted therapy including immunotherapy is becoming standard practice nowadays [9, 10]. Hematology is indeed becoming an interdisciplinary value in the early 2020s: e.g. hemato-cardiology, hemato-nephrology, and hemato-immunology.

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JS — sole author.

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Ethics

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REVIEW ARTICLE

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Hematopoietic stem cell transplantation in autoimmune diseases: update from the EBMT Autoimmune Diseases Working Party with special reference to Poland

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Abstract

Hematopoietic stem cell transplantation (HSCT) is now evolving into a standard treatment in some autoimmune diseases (AD) alongside other modern therapy. The main indications are multiple sclerosis and systemic sclerosis for which HSCT has become an integral and standard-of-care part of treatment algorithms. From 1994 to the beginning of 2021, data from the (European Society for Blood and Marrow Transplantation) EBMT Registry indicates that 3,442 patients (60% females, 40% males; 91% adults, 9% pediatric) received 3,514 transplant procedures for autoimmune diseases, with over 90% receiving autologous transplant. Autoimmune diseases are currently the fastest growing indication for autologous HSCT in EBMT, whilst allogeneic HSCT for ADs is mainly restricted to pediatrics, especially diseases with a genetic component. Patient selection plays a key role in providing the best risk/benefit ratio of the procedure. Intensity of conditioning regimen and center experience are also important. Ultimately, the future of HSCT for ADs depends on the standard of care therapy, which influences uptake within national/international disease specialist communities. Further studies are necessary in order to establish relative benefit over current/future standard of care therapy, to establish the best HSCT regimen for each disease, to define mechanisms, develop clinical biomarkers to help select and monitor patients, and to define health economic benefits and public health delivery. We present a current perspective summarizing activity across EBMT, including centers in Poland.

Key words: autoimmune diseases, stem cells, transplantation, autologous, allogeneic

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Introduction

Autoimmune diseases (AD) are relatively common, affecting 5–8% of the population. MS occurs in about 1 in 1,000 people, while acute myeloid leukemia (AML) occurs in approximately 1 in 100,000. Not all ADs are severe, but some are very disabling and life-threatening. Cures remain elusive and almost all patients with severe ADs require long-term therapy. The impacts of both the disease and its treatment are severe, since patients require steroids or other immunosuppressive drugs. The consequences of treatment can be as damaging as the disease itself, in terms of the combined short-term and chronic effects of both ADs and treatments. Additionally, the costs of the disease are high in terms of drugs, personal costs (disability) and societal impact. For a long time, there has been a desire for a one-off intensive means of long-term disease control, to achieve disease eradication, rather than chronic suppression.

Autoimmune diseases (ADs) are the fastest growing area of autologous hematopoietic stem cell transplantation (HSCT) worldwide, yet they still comprise only 2% of all transplants. Even with the significant advances made in transplants in patients with multiple sclerosis (MS) and systemic sclerosis (SSc), this is still a very specialized area and will never be considered as a treatment for anything other than the most severely affected poor-prognosis patients with autoimmune diseases where the risk:benefit ratio can be justified comfortably.

The objective of this paper is to present an overview of HSCT as an exciting and evolving therapeutic avenue in severe ADs.

Concept of HSCT in autoimmune diseases

The concept of HSCT in ADs is already four decades old. It started in the 1980s in animal models and in 1995 the first patients were treated with autologous HSCT specifically for AD. In 1996–1997, the Autoimmune Diseases Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT) was formed, and this was followed by developing the EBMT database and guidelines [1, 2]. Activity in ADs predominantly involves autologous HSCT (auto-HSCT), while allogeneic HSCT (allo-HSCT) procedures are rare in ADs, particularly outside of pediatrics, because children can tolerate this treatment better than adults. On the other hand, allogeneic HSCT can provide complete 'immune replacement'. The outcome of allogeneic HSCT has dramatically improved over the past decade. A recent retrospective EBMT study assessed the use and long-term outcomes of allogeneic HSCT in 128 patients with various hematological and non-hematological severe ADs within the registry between 1997 and 2014 [3]. In multivariate analysis, age <18 years, male gender, and more recent transplant were found to be significantly associated with improved outcomes.

Table I. Hematopoietic stem cell transplantations (HSCTs) for autoimmune diseases (ADs) in European Society for Blood and Marrow Transplantation (EBMT) registry (1994–2021*)

	Autologous HSCT (n =3,277)	Allogeneic HSCT (n =237)
First	3,245	197
Second	39	34
Third	2	6
Median age at first HSCT	38 years (range 3–76)	11 years (range <1–65)

*As at February 2021

For auto-HSCT, the procedure is relatively straightforward in well-selected patients. The standard pathway of transplant procedure includes granulocyte-macrophage colony-stimulating factor (G-CSF)-mobilization of peripheral blood stem cells, which are then frozen until the patient receives conditioning, followed by thawing and re-infusion of cells into the patient, who is then supported through the period of pancytopenia until hematopoietic recovery. Immune reconstitution is associated with an 'immune reboot', which, in some diseases leads to long-term drug-free remission, and in others re-sets disease activity to controllable levels [4].

Transplant activity in autoimmune diseases

Data from the EBMT Registry of ADs indicates that 3,442 patients (60% females, 40% males; 91% adults, 9% pediatric) have received 3,514 transplants for autoimmune disease, with over 90% getting autologous transplant (Table I). There are a smaller number of allogeneic transplants, mainly in pediatric patients. Some patients have received a second transplant. Overall, transplants for ADs have been performed in 310 EBMT centers in 44 countries.

Indications for transplant in autoimmune diseases

ADs have been the fastest growing indication for autologous HSCT in the EBMT Registry in recent years. The main indications since 1994 have been MS and SSc. The evolution of HSCT has coincided with a period when many biological therapies for autoimmune diseases have competed. However, not all patients respond to biologics, so in recent years the number of transplantations has grown in diseases where biological therapies have a limited effect. The growth has been highest for MS, followed by SSc.

Many countries are active in this field. Ranked according to the number of auto-HSCTs in AD, the 10 most active countries are Italy (n =520), United Kingdom (n =494), Germany (n =352), Sweden (n =321), the Netherlands

Table II. Indications for transplant in autoimmune diseases according to European Society for Blood and Marrow Transplantation (EBMT) Registry (1994–2021)

Indications	Number	Indications	Number
Multiple sclerosis	1738	**Hematological**	139
		ITP	37
Connective tissue	886	AIHA	33
SSc	702	Evans syndrome	26
SLE	121	Other	43
PM-DM	18		
Sjögren syndrome	6	**Vasculitis**	64
Antiphospholipid syndrome	6	Wegener's	14
Other/unknown	33	Behçet's	14
		Takayasu	3
Arthritis	196	Polyarteritis	4
Rheumatoid arthritis	83	Churg-Strauss	2
Juvenile chronic arthritis:		Other/Unknown	27
• systemic JIA	66		
• other JIA	19	**Other neurological**	134
• polyarticular JIA	17	NMO	26
Psoriatic arthritis	3	CIDP	62
Other	8	Myasthenia gravis	9
		Other/unknown	37
Inflammatory bowel	258		
Crohn's disease	212	Insulin-dependent diabetes	20
Ulcerative colitis	4	Other	79
Other	42		

SS — systemic sclerosis; SLE — systemic lupus erythematosus; PM-DM — polymyositis and dermatomyositis; JIA — juvenile idiopathic arthritis; ITP — immune thrombocytopenic purpura; AIHA — autoimmune hemolytic anemia; NMO — neuromyelitis optica; CIDP — chronic inflammatory demyelinating polyradiculoneuropathy

(n =229), Spain (n =226), France (n =195), Poland (n =189), Australia (n =162), Russia (n =99) and Belgium (n =93). Some countries are more active in neurological, some in rheumatological, and some in gastroenterological and other diseases.

In EBMT indications guidelines, MS and SSc are recommended as a standard care indication for transplant, and supported by randomized control trials [5]. Most patients have been treated for MS (Table II). The other indications include connective tissue diseases such as SSc and systemic lupus erythematosus. Arthritis is no longer a frequent indication, as conservative treatment of arthritis has been established since the early 2000s.

Among inflammatory bowel diseases, Crohn's disease has been a long-time indication for auto-HSCT. There have been a number of rare conditions, such as insulin-dependent diabetes in its 'honeymoon' phase, which have proceeded to auto-HSCT to produce remission in some patients.

Multiple sclerosis (MS)

MS is the most frequent autoimmune disease for which HSCT has been used, accounting for 1,738 patients reported in the EBMT registry. The countries most active in auto-HSCTs for MS are Italy (n =306), United Kingdom (n =293), Sweden (n =245), Poland (n =139), and Spain (n=98). These figures support the strong evidence in treatment algorithms.

In most patients, MS is a two-phase disease: an inflammation (relapsing-remitting) phase and a progressive (destructive) phase [6]. First line treatments include: steroids, plasmapheresis, glatiramer-acetate, interferon-beta, fingolimod, fumaric acid, and teriflunomide. Second-line treatments include: natalizumab, alemtuzumab, ocrelizumab and cladribine. Auxiliary treatments include regular physical activity, sun exposure, and vitamin D. MS may be assessed by Expanded Disability Status Scale (EDSS) Disability Score, ranging from 0 to 10, as well as a "no evidence

of disease activity" (NEDA3) assessment, which requires neurologists to assess patients clinically (relapse and disability progression) and radiologically with magnetic resonance imaging (MRI).

In most patients, there is an inflammatory phase, relapsing/remitting MS, which is followed by a secondary, progressive phase. It is during this inflammatory relapsing/remitting phase when a significant therapeutic effect with either drugs or autologous transplant is possible. In the inflammatory phase, 'enhancing' lesions with reactive areas of inflammation damage the brain and the spinal cord. Periods of disability often partly recover, but inflammatory lesions that continually reoccur cause permanent damage and lead to progressive disease.

If auto-HSCT is performed in the inflammatory phase, the inflammatory lesions disappear for long periods, potentially permanently. In the progressive phase, there is ultimately no inflammation and a different disease process for which there is a limited or no response to transplant, even with the most intensive forms of transplant conditioning. Therefore, it is important to be in the right phase of disease, and patient selection plays a key role in providing the best risk/benefit ratio of the procedure [7].

Accumulating evidence and follow up in the literature indicate the potential of auto-HSCT to induce a disability improvement in patients transplanted in the relapsing-remitting phase. Improvement is usually sustained and free of immunosuppression for several years, and potentially permanently. Patients considered for transplant should be clinically severe enough and have resisted at least first line treatment. Guidelines prepared by ADWP support this process and also summarize transplant technology [8]. Early transplantation, which demonstrates the potential to stop disease progression and to prevent disability formation in up to 92% of patients, appears to be the most promising treatment strategy in MS. The favorable factors for HSCT in MS are: early transplantation (EDSS <4.0), age <30 years, disease duration <5 years, and relapsing-remitting type.

In contrast, secondary progressive MS patients with rapid disability accrual, low inflammatory activity, and severe spinal cord involvement are at high risk of treatment failure and should be extremely carefully selected because some inflammatory activity may be suppressed [9].

Since the drawing up of the EBMT ADWP guidelines, which summarize the evidence base [8], there have been a few remarkable publications. In a recent retrospective analysis of the Italian database on long-term clinical outcomes of HSCT in MS, 210 patients were included (58% in RR). With median baseline EDSS score 6 (range, 1–9), in RR-MS patients, the use of the BEAM+ATG (74%) conditioning protocol was independently associated with a reduced risk of NEDA3 failure [hazard ratio (HR) =0.27; 95% confidency interval (CI): 0.14–0.50, p <0.001] [10]. There has been a non-significant trend of a correlation between treatment intensity and quality of outcome in the results of recent studies [11–13].

In addition, 20 patients with 'aggressive' MS received auto-HSCT as a first-line DMT in five European and North American centers. Median interval between diagnosis and auto-HSCT was 5 months (range 1–20). Conditioning regimens used Bu–Cy–ATG, BEAM–ATG or Cy–ATG. After a median follow-up of 30 months (range 12–118), the median EDSS score improved to 2.0 (range 0–6.5), p <0.0001. Following auto-HSCT, no patient had clinical relapse or confirmed disability progression. When MRIs were re-baselined at 6 months, the cumulative NEDA rate was 100%. There was no TRM [14].

At least eight prospective phase II and III studies are ongoing across the world in MS patients in order to establish the treatment as a standard of care [8, 15]. Further studies are needed to assess the optimal intensity and transplant technique, including mobilization and conditioning regimens, as well as graft manipulation.

As with all transplantation decisions, the benefits need to be justified alongside the risks. A number of published clinical trials and other studies have reported no or very low level TRM, which, when pitched against high rates of success in preventing relapse and/or progressive disability, justify a role for auto-HSCT in carefully selected patients. Overall, recent retrospective EBMT data [2] reported 100-day TRM of 1.1%, 3-year TRM of 1.5%, relapse incidence of 34.4%, progression-free survival of 64%, and an overall survival of 95.5%. A tailored approach, with close working between transplant hematologists and neurologists, should optimize the risk/benefit ratio.

Systemic sclerosis (SSc)

SSc is a rare disease, associated with inflammation of the skin (scleroderma), lungs (fibrosis), and heart (pulmonary hypertension). It affects the kidneys and is associated with fibrosis and scarring. In severe cases, it carries a poor life expectancy, even in the biologics era. The European League Against Rheumatism (EULAR) now recommends transplant to treat skin and lung disease in systemic sclerosis.

SSc is a standard indication in EBMT guidelines, with increases over the last decade. Up to 2021, the number of reported auto-HSCTs for SSc was 702. The countries with the highest number of transplants were Germany (n =149), the Netherlands (n =123), France (n =92), Italy (n =78), Australia (n =58), United Kingdom (n =26), Spain (n =24), Poland (n =20), Norway (n =18) and Switzerland (n =17).

Durable responses to auto-HSCT in the skin have been observed, so transplant is effective in reducing skin inflammation and fibrosis. It is also successful in inflammation and fibrosis in the lungs, which is a feature associated with a poor prognosis. Three randomized controlled trials have

been performed for SSc [16–19], resulting in a grade 1 recommendation for auto-HSCT in patients with SSc. These trials support improved overall and progression-free survival with HSCT.

Systemic sclerosis, apart from skin, can cause pulmonary, cardiac (valvular disease of endocardium, microvascular disease of myocardium with myocarditis and fibrosis, and pericardial effusion), gastrointestinal, renal, musculoskeletal and exocrine complications, as well as digital ulceration and macrovascular disease [20, 21]. In a prospective non-interventional study of ADWP-EBMT on auto-HSCT with progressive systemic sclerosis, OS was 90%, PFS 81.8%, and TRM was 6.25% [22].

Inclusion and exclusion criteria, as well as principles of the necessary multidisciplinary approach were updated recently [23]. Toxicity of HSCT has been predominantly attributed to SSc-related cardiac dysfunction, especially related to pulmonary arterial hypertension, and drug-induced cardiotoxicity, which should be part of routine pre-transplant screening [24]. For patients with poor cardiac function, a cardiac 'safe' HSCT regimen was reported in a pilot study that showed safety using fludarabine-based conditioning [25].

Crohn's disease

Crohn's disease is the most frequent indication for transplantation in the gastroenterology field. Between 1995 and 2021, 212 patients were auto-transplanted due to Crohn's disease, with three countries performing more than 20 auto-HSCTs for Crohn's disease overall (Spain, United Kingdom, and Italy).

Consideration of auto-HSCT in Crohn's Disease include: established diagnosis of CD, objective evidence of inflammatory activity, severe course of the disease over time, inadequate response to available medical therapies, and surgery considered an unsuitable option [26, 27].

In an EBMT retrospective study [28], 82 patients were transplanted between 1996 and 2015 due to previous failure of a median six medical therapies, including surgery in 74% of cases. Transplants have been performed in 19 centers from eight countries. Overall, 68% remission or significant improvement was observed, and no re-treatment was required in 27% of cases. In 24 out of 42 patients (57%), remission or significant improvement was observed after re-treatment.

Auto-HSCT provides a therapeutic alternative to Crohn's disease patients with severe and refractory disease, although it is not curative or permanently effective in most patients, at least not without re-introduction of salvage or maintenance treatment, where there appears to be some evidence for re-setting and better disease control. In addition to implementation of extraordinary supportive measures before, during, and after transplant to improve safety, a number of studies are ongoing to evaluate different mobilization and conditioning regimens. Allo-HSCT might be an option in highly selected patients [29, 30], but further studies are warranted.

Communication with patients

Communication with patients is essential, particularly as HSCT procedures are very different to most other immunosuppressive treatments. Each patient should be managed individually, with appropriate specialist and nursing support. Written information should be provided to patients and carers. Education is crucial, not only for specialist AD clinicians, but also for non-specialists (GPs and other clinical staff), and also for broader HSCT team members who look after patients during their inpatient stay. The EBMT ADWP works closely with the EBMT Patient Advocacy Committee and EBMT Nurses Group to produce guidelines for patients, and also non-specialists [31]. Rehabilitation after transplantation e.g. in MS, is an essential component of recovery [32].

Perspectives: the Polish experience

Overall, 189 transplant procedures have been performed for AD in Poland since 1994. Nine Polish centers are active in HSCT for ADs (Table III): Katowice, Warsaw (Central Clinical Hospital), Poznan, Lodz, Gliwice, Krakow, Warsaw (Military Medical Academy), Lublin (Children's University Hospital) and Lublin. The main indication was MS (n =141), mainly treated in the Department of Hematology and Bone Marrow Transplantation in Katowice, followed by SSc (n =20) and type 1 diabetes (n =20). Increasing interest in transplanting patients with ADs is expected in Poland. We encourage local centers to register all treated patients, and to report on follow-up at designated intervals in order to improve the quality of the EBMT registry.

Conclusions

HSCT for severe ADs reflects the gradual transition from basic science to evidence-based therapy. Autologous HSCT is evolving into a standard treatment in some autoimmune diseases, to be considered alongside modern therapy. HSCT for AD will continue to increase at variable rates between the different types of ADs. As more centers undertake this work, it is important to recognize that HSCT for AD presents significant challenges that may be unfamiliar even to experienced HSCT teams. Allogeneic HSCT is potentially curative through 'immune replacement', but rarely used in the treatment of ADs. Improved outcomes have been reported in recent years. Further clinical studies are warranted to evaluate this therapeutic option, especially in pediatric ADs with a strong genetic component.

Table III. Hematopoietic stem cell transplantation (HSCT) activity according to European Society for Blood and Marrow Transplantation (EBMT) Center and autoimmune diseases (AD) indication in Poland

Centre	Multiple sclerosis	Systemic sclerosis	Type I diabetes	Systemic lupus	Juvenile idiopathic arthritis (Stills)	Autoimmune neutropenia	Other AD	Total
Katowice	134	19		2				155
Warsaw (WUM)	1		20					21
Poznan	3							3
Lodz	2							2
Gliwice		1				1		2
Kraków					2			2
Warsaw (WIM)				2				2
Lublin (peds)							1	1
Lublin	1							1
Total	141	20	20	4	2	1	1	189

WUM (*Warszawski Uniwersytet Medyczny*) – Warsaw Medical University; WIM (*Wojskowy Instytut Medyczny*) – Military Medical Institute

The future of HSCT for ADs depends on the dynamic with modern and future 'standard of care' therapy, and acceptance within national/international specialist communities, which is the goal of specialty society guidelines. Multi-professional and inter-disciplinary team working is vital. Further studies are necessary in order to establish relative benefit over current/future 'standard of care' therapy, to establish the best HSCT regimen for each disease, to define mechanisms and develop clinical biomarkers to select and monitor patients, and to define health economic benefits and public health delivery.

Finally, the impact of coronavirus disease 2019 (COVID-19) has yet to be fully understood. Recently, the ADWP reviewed the impact of the pandemic on specific groups of patients with neurological, rheumatological and gastroenterological indications, along with the challenges of delivering HSCT as a specific treatment in these patient populations during the pandemic. The EBMT has provided consensus-based guidelines and recommendations to support multidisciplinary teams delivering HSCT in ADs [33].

Author's contributions

JAS had primary responsibility for this review. JAS and RG provided data from the EBMT registry. All authors contributed to manuscript writing and critical revision.

Conflict of interest

All authors declare no conflict of interest related to this review.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

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Autologous stem cell transplantation in lymphomas: current indications

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Abstract

Hematopoietic stem cell transplantation is an established curative treatment for a number of conditions including malignant hematologic diseases and non-malignant congenital and acquired disorders involving the hematopoietic system and some types of solid tumors, e.g. germ cell tumors and soft tissue sarcomas. Hodgkin's disease and non-Hodgkin lymphomas can be treated and, in a large number of cases cured, by first-line chemotherapy or radiotherapy. Unlike many other malignancies, relapse is not uniformly fatal but the treatment is usually markedly myelotoxic with the high doses of chemotherapy (HDC) used in relapse. Hematopoietic reconstitution with either autologous marrow or peripheral stem cells post-chemotherapy has made HDC relatively safe, with mortality rates as low as 2% in some centers. The choice of conditiong regimen has traditionally been based on institutional experience, and several regimens are considered standard and routinely used for patients with all histologies of lymphoma. Each HDC regimen is associated with its own unique toxicities, based on the individual agents or modalities used. Novel targeted and immunotherapy approaches, including chimeric antigen receptor T-cell therapy, are currently being studied in clinical trials with promising early results, so the role of autologous stem cell transplantation in the treatment of lymphomas could be changed. The current clinical indications for HDC followed by autologous hematopoietic stem cell transplantation in lymphomas management for patients with a bad prognosis (as a consolidation therapy) or relapsed/refractory disease are reviewed in this paper.

Key words: lymphoma, high-dose chemotherapy, autologous hematopoietic stem cell transplantation

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Introduction

The World Health Organization (WHO) has categorized more than 30 unique histopathologic types of lymphomas. Approximately 88% are B-cell lymphomas. The current indications for autologous hematopoietic stem cell transplantation in some types of lymphomas [including Hodgkin lymphoma (HL)] are presented here.

Follicular lymphoma

Follicular lymphoma (FL) is a heterogeneous disease with a varying prognosis owing to differences in clinical, laboratory, and disease parameters. Although generally considered incurable, prognosis for early- and advanced-stage disease has improved because of therapeutic advances, several of which have resulted from elucidation of the biological and molecular basis of the disease. The choice of treatment for FL is highly dependent on patient and disease characteristics. Several tools are available for risk stratification, although limitations in their routine clinical use exist [1–7].

Investigators explored the role of autologous hematopoietic stem cell transplantation (ASCT) as a consolidation strategy following first-line therapy. Promising initial studies culminated in the development of several large randomized

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studies where ASCT was compared to either no further therapy or interferon alpha. While some of these studies demonstrated an improvement in disease control, no overall survival (OS) benefit could be demonstrated.

These observations, combined with a growing realisation of the acute and long-term toxicities of autologous hematopietic stem cell transplantation (auto-HSCT), have led to the abandonment of ASCT as a first-line consolidation procedure [8, 9].

Recent data suggests the strongest predictor of long-term FL outcomes is length of first remission after front-line therapy. Patients with progression of disease within 24 months of completing induction chemoimmunotherapy (POD24), which made up 19% of patients in this data set, had poorer outcomes compared to those with longer remission durations (5-year OS: 50% vs. 90%, respectively), even after adjustment for Follicular Lymphoma International Prognostic Index (FLIPI) score. The m7-FLIPI, a clinicogenetic risk score derived from a combination of the mutation status of seven candidate genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP, CARD11*) together with clinical parameters [FLIPI score and Eastern Cooperative Oncology Group (ECOG) performance status], stratifies patients into a low-risk group (78% of patients) with a 5-year failure-free survival of 68% versus 25% in a high-risk group (22% of patients). m7-FLIPI was used to identify patients at risk of early relapse (POD24) using data from the German Low-Grade Lymphoma Study Group trial and the British Columbia Cancer Agency population-based registry. They confirmed that m7-FLIPI had a higher accuracy in predicting POD24 compared to FLIPI. Currently, no single treatment option exists for patients with POD24, and therapeutic approaches are generally intensification with standard agents or use of agents with novel mechanisms of action compared to front-line therapy [2–7]. Type of induction chemotherapy may influence survival of patients experiencing POD24. In the GALLIUM study, obinutuzumab-based chemotherapy was associated with a 34% reduction in the number of POD24 events. However, postprogression survival was similar in all treatment groups. Other recent analyses of POD24 after bendamustine-based induction also suggested a decreased risk of POD24 events (9–12%), with similarly poor outcomes. These findings suggest that early disease-related events after chemomimmunotherapy occur regularly and reproducibly in FL. So, the patients with POD24 are biologically distinct, possessing tumor- and/or host-related factors contributing to chemotherapy resistance, and require novel therapeutic approaches to improve poor outcomes. For fit patients aged up to 70 without an appropriate clinical trial option, aggressive treatment involving salvage chemoimmunotherapy and consolidative ASCT should be considered. This strategy can induce prolonged remissions in FL. The observation of a plateau in PFS curves suggests cure in a subset of patients, differentiating transplantation from other treatment modalities [5].

Retrospective data suggests that patients with POD24 benefit from ASCT (i.e. increased progression-free survival (PFS) and OS compared to those not receiving transplant). A recent study compared ASCT to either matched-sibling donor (MSD) or unrelated matched donor (UMD) allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with POD24. Findings suggest that outcomes are similar with either autologous or allogeneic transplant with MSD, whereas outcomes with UMD transplant were inferior, largely due to higher transplant-related mortality [1–7].

The bone marrow is infiltrated in approximately 75% of FL patients at diagnosis, and consequently a number of investigators have studied the role of marrow purging in ASCT [10]. However, no clear benefit for purging could be demonstrated in prospective studies [11, 12], and there was some evidence that purging resulted in significant additional immunosuppression. Consequently, purging remains an experimental procedure in ASCT for FL.

There is a wide variety of different conditioning regimens that may be employed for ASCT in FL but a paucity of randomized trials comparing the efficacy and toxicity of these different regimens. The BEAM regimen had become the most widely used prior to ASCT in malignant lymphoma, and has been adopted in many countries. A number of investigators have changed conditioning schemes to improve results of BEAM by including rituximab and dexamethasone, substituting BCNU with bendamustine [13] or incorporating bortezomib, mitoxantrone or fotemustine. Several groups have also incorporated radioimmunotherapy (RIT) into the conditioning regimen prior to auto-HSCT in NHL [14].

Disease relapse following high doses of chemotherapy (HDC) and ASCT remains the principal cause of mortality in patients with relapsed or refractory lymphomas. In an effort to prevent post-ASCT relapse, a number of studies have evaluated the role of maintenance therapy, with varying success. In a randomized phase III study of FL, 280 rituximab-naive patients with chemosensitive, relapsed FL were randomized to pre-transplant rituximab purging or observation. Following transplant, patients were randomized to observation or maintenance with rituximab (MR) (375 mg/m^2 every two months for a total of four infusions). Post-ASCT MR therapy was associated with significantly higher 10-year PFS (54% vs. 37%, p =0.012), and no difference in OS was seen between the arms (73.1% vs. 67.8%, p =NS). In addition, MR therapy was associated with a nonstatistically significant increase in neutropenia in the first year of therapy. Based on the lack of benefit in OS seen in this large phase III study, MR has not been widely adopted following ASCT in FL [12, 15].

Conclusions [16]:

1) Autologous stem cell transplantation should not be used in first remission.
2) Autologous stem cell transplantation should be considered in patients with relapsed disease responding to reinduction therapy.
3) Autologous stem cell transplantation leads to a 5-year PFS of approximately 50% cases and may be curative in a significant minority of patients.
4) There is no proven role for purging strategies.
5) Maintenance rituximab for four infusions should be considered post ASCT.

Waldenström's macroglobulinemia

Small retrospective studies and a large registry analysis suggested that ASCT might improve the outcome of Waldenström's macroglobulinemia (WM) when applied as first-line consolidation. With the advent of more effective agents such as rituximab, purine analogs and bortezomib, this approach is increasingly questionable and should not be used outside clinical trials. In contrast, auto-HSCT is an option for salvage therapy in selected patients with chemosensitive disease who have not been exposed to numerous treatment lines [17].

Other indolent lymphomas

Despite improvements over the past decade in the OS of patients with indolent NHLs, these neoplsms remain largely incurable with standard therapies. Immunochemotherapy with rituximab-based regimens has become a well-established standard of care in primary and relapsed disease settings. Autologous stem cell transplantation offers a safe treatment platform, but relapse remains a significant issue. The role of transplantation in the current treatment landscape of immunochemotherapy has not been conclusively proven, and randomized trials are lacking. It is widely accepted that ASCT should no longer be performed routinely as consolidation of primary treatment, given the excellent results seen with primary immunochemotherapy. For relapsed or refractory disease, ASCT is likely to be the clinician's preferred choice, given the low non-relapse mortality (NRM) of the procedure [18].

Hodgkin lymphoma

In patients with advanced HL with poor prognostic features, the role of high-dose chemotherapy with autologous stem cell transplantation has been evaluated as part of initial therapy. Patients with advanced unfavorable HL achieving a complete or partial remission after four courses of doxorubicin-containing regimens were found to have a favorable outcome with conventional chemotherapy, and no benefit from an early intensification with HDC and ASCT was shown [19, 20].

Although the majority of patients with HL are cured with initial therapy, 10–15% of patients with early stage disease and 15–30% of patients with advanced disease have primary refractory or relapsed lymphoma [19–21]. So, despite the approval of novel therapies including brentuximab, nivolumab, and pembrolizumab, consolidation with high-dose chemotherapy and ASCT in patients responding to second line or subsequent therapy remains the standard of care in the majority of patients. Initial phase II studies suggested that HDC followed by ASCT may produce a better long-term disease-free survival than conventional chemotherapy in 30–65% of patients. Two subsequent randomized studies confirmed an improved outcome in patients with relapsed HL treated with HDC, followed by ASCT as compared to conventional salvage chemotherapeutic regimens.

In both studies, event-free survival (EFS) after three years of patients treated with HDC was over 50%. Elderly patients treated with an ASCT have increased treatment-related mortality, and commonly have an inferior EFS compared to younger patients. Some patients have relentlessly progressive disease and have been treated with tandem ASCT or allo-HSCT. Preliminary results have suggested that these therapies are feasible, but toxicity and relapses have been common [21].

Given the activity of brentuximab vedotin (BV) in patients with relapsed or refractory HL with an overall response rate (ORR) of 75% with approximately a third of patients achieving complete response (CR), the AETHERA study investigated the role of maintenance BV following ASCT. Patients with high-risk disease with primary refractory HL or relapse within 12 months of completion of frontline therapy or extranodal involvement at relapse were randomized to up to 12 months of brentuximab given every three weeks versus placebo. At 5-year follow-up, 59% of patients who received BV were progression free compared to 41% in the control arm.

The benefit was most prominent in patients with two or more of the following risk factors: relapse within 12 months or refractoriness to frontline therapy, partial response or stable disease after most recent salvage therapy, extranodal disease at relapse, B symptoms at relapse, and more than two prior salvage therapies. Common toxicities in the BV arm included peripheral neuropathy, which was reversible in the vast majority of patients, and neutropenia. This confirmed a benefit for BV therapy post-transplant in high-risk patients [22]. A much smaller study of 30 patients evaluated the use of pembrolizumab given for eight doses post-transplant in a similar cohort of patients. The primary endpoint was that pembrolizumab would improve PFS at 18 months after ASCT, from 60% to 80%. PFS at 18 months for the 28 evaluable patients was 82%, meeting the primary endpoint. However, the benefit of immune checkpoint blockade post-ASCT will need to be confirmed in a randomized trial [21]. Based on studies suggesting that

anti-programmed cell death protein 1 (anti-PD-1) monoclonal antibodies (mAb) can sensitize patients to subsequent chemotherapy, Merryman et al. [23] hypothesized that anti-PD-1 therapy before ASCT would result in acceptable outcomes among high-risk patients who progressed on, or responded insufficiently to, ≥1 salvage regimen, including chemorefractory patients who are traditionally considered poor HSCT candidates. They retrospectively identified 78 HL patients who underwent HSCT after receiving an anti-PD-1 mAb (alone or in combination) as third-line or later therapy across 22 centers. Chemorefractory disease was common in this group of patients. After a median post-ASCT follow-up of 19.6 months, 18-month PFS and OS were 81% and 96%, respectively. Favorable outcomes were observed for patients who were refractory to two consecutive therapies immediately before PD-1 blockade (18-month PFS, 78%), had a positive pre-ASCT positron emission tomography (PET) (18-month PFS, 75%), or received ≥4 systemic therapies before HSCT (18-month PFS, 73%), while PD-1 nonresponders had inferior outcomes (18-month PFS, 51%). In this high-risk cohort, ASCT after anti-PD-1 therapy was associated with excellent outcomes, even among heavily pretreated, previously chemorefractory, patients [23].

Peripheral T-cell lymphomas

Peripheral T cell lymphomas (PTCLs) are a heterogeneous group of diseases and represent approximately 10–15% of all NHLs. There are over 27 different subtypes of PTCLs and we are now beginning to understand the differences between the various subtypes beyond histologic variations. Multiagent chemotherapy with a CHOP-like regimen is the current standard of care in the frontline setting, but outcomes for PTCL patients generally remain poor. Strategies used to improve survival and reduce the risk of relapse in PTCL patients include autologous and allo-HCT. Due to the relative rarity of these diseases, the evidence supporting the use of auto-HCT and allo-HCT is based on retrospective and single-arm prospective studies. Novel targeted therapies are now being incorporated into the treatment of PTCL, and they may play important roles in improving upon current standards of care. Given recent improvements in OS and PFS in CD30+ PTCL using the drug-antibody conjugate BV, new questions arise regarding the impact of BV on consolidative ASCT, and its role as a maintenance therapy. Multiple histone deacetylase inhibitors have been approved for the treatment of relapsed/refractory PTCL, and these agents are being incorporated into HCT approaches, both in frontline and maintenance settings. Early data incorporating these agents into novel conditioning regimens has been reported, and emerging evidence suggests that chimeric antigen therapy (CAR) T cell therapies may prove effective in relapsed/refractory PTCL. The recommended treatment strategy in non-anaplastic large cell lymphoma (ALK)+ PTCL remains induction with a CHOP-like regimen followed by consolidative auto-HCT in first remission. In the relapsed/refractory setting, salvage chemotherapy followed by HCT (auto-HCT or allo-HCT depending on histologic subtype and HCT history) offers the only potential for cure or long-term remission.

Results from prospective studies suggest a substantial effect of up-front ASCT on the outcome of patients with PTCL, which should be further evaluated in randomized trials. The global conclusion of reported trials is that pre-transplantation treatment must be improved to increase the transplantation success and that one of the major challenges is knowing which patients with PTCL in first remission to select for consolidative ASCT, as patients with low International Prognostic Index (IPI), ALK+ anaplastic large cell lymphoma (ALCL) disease in remission do not need consolidation transplant. For patients with ALK+ALCL with high IPI score and poor outcomes, alternative strategies, including ASCT, should be considered. High-dose chemotherapy followed by ASCT may improve the outcome in PTCL, but the available data comes from non-randomized studies, meaning definitive recommendations cannot be made. The achievement of a first complete remission before ASCT has proven to be a strong predictor of improved outcome. Thus, any potential benefit from consolidative auto-HCT will be conferred only on those with chemo-sensitive disease. Secondly, rates of relapse after auto-HCT are significant and range from 18% to 55%. This suggests the presence of residual disease despite achievement of CR by conventional detection methods (e.g. PET). Finally, there are limited studies utilizing novel therapeutics such as BV; thus, it remains to be determined how the incorporation of novel agents may affect outcomes with HCT.

Despite these limitations, the preponderance of data demonstrates that there is an important role for autoHCT as consolidation in CR1 for patients with PTCL.

It is recommmended that all fit patients with non-ALK+ ALCL proceed with auto-HCT in CR1 upon completion of six cycles of induction CHOP-based chemotherapy. Relapses in patients with PTCL tend to be very aggressive, with poor survival and low response rates outside of ALCL; the best chance to cure patients with PTCL is in CR1 [25–27].

Outcomes for relapsed/refractory non-ALK+ PTCL are generally poor with median OS of 9.1 months. Available data suggests that patients who respond to salvage chemotherapy (i.e. those with chemo-sensitive disease) are most likely to derive benefit from ASCT. Clinical studies found that ASCT performed in earlier states of remission (i.e. CR1 ±PR1) was associated with significantly longer PFS, and that patients with refractory disease had particularly poor outcomes. Additionally, these studies suggest that a significant minority of patients with chemo-sensitive relapsed disease (i.e. CR2+/PR2+) may derive durable benefit from auto-HCT. These and other retrospective studies

indicate that prognostic scores such as the IPI/age-adjusted International Prognostic Index (aaIPI) may be useful in predicting which relapsed patients with chemosensitive disease are most likely to benefit from ASCT [25–27].

Conclusions [25–27]:

1) The recommended treatment strategy in non-ALK+ PTCL remains induction with a CHOP-like regimen followed by consolidative auto-HCT in first remission.
2) For patients with relapsed/refractory PTCL, the only potentially curative therapy is hematopoietic stem cell transplantation.
3) For patients with chemosensitive disease who attain a rapid CR to salvage therapy, particularly ALCL, ASCT in CR2 may provide curative therapy for a subset of patients (approx. 50% for ALCL, 35–40% for non-ALCL in select cases).
4) For patients with primary refractory PTCL, or PTCL that relapsed after ASCT or multiple prior lines of therapy, allo-HCT provides the only potential curative therapy with long-term survival rates of 40–50%. Due to the high risk of NRM, particularly with myeloablative conditioning in patients who have recently received ASCT or who have received extensive salvage chemotherapy, reduced-intensity regimens are preferred due to lower NRM.
5) Additional prospective trials and novel therapeutic approaches, including cellular therapy techniques, are desperately needed for this population.

Mantle cell lymphoma

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma which is characterized by the chromosomal translocation t(11;14)(q13;q32) and overexpression of cyclin D1 in the vast majority of cases. Most patients present with advanced stage disease, often with extra-nodal dissemination, and have an unfavorable clinical course. Treatment with conventional chemotherapy resulted in unsatisfactory outcomes and median survival is less than three years after diagnosis of MCL [28].

The use of ASCT consolidation in first remission is supported by data published by the European and Nordic groups who noted significantly prolonged PFS with ASCT, with the European group randomizing patients to interferon versus ASCT. However, this data was attained before the widespread use of cytarabine induction regimens, maintenance rituximab in first remission, and the discovery of Bruton tyrosine kinase (BTK) inhibitors. Thus, randomized data confirming the efficacy of ASCT is greatly needed because of the number of novel strategies recently developed in MCL. The European MCL Network phase III TRIANGLE study is currently randomizing patients to an induction regimen containing the BTK inhibitor ibrutinib while also assessing whether an ibrutinib-containing induction regimen with maintenance can replace ASCT. This will be the first phase III trial to incorporate a targeted molecular therapy into the MCL induction regimen and also the first randomized study to test the efficacy of ASCT in the cytarabine and rituximab era. Post-ASCT bortezomib, although associated with an improvement in PFS, leads to significant toxicity including peripheral neuropathy and cytopenias, and therefore this approach is seldom utilized. Mature follow-up from ongoing clinical studies, along with further randomized prospective data, will help further assess toxicity and the impact of maintenance therapy post ASCT on OS [12, 29, 30]. In a phase III trial, 240 patients were randomly assigned to receive rituximab maintenance therapy or to undergo observation after autologous stem-cell transplantation. The primary endpoint was EFS (with an event defined as disease progression, relapse, death, allergy to rituximab, or severe infection) after transplantation among patients who underwent randomization. The median follow-up from randomization after transplantation was 50.2 months (range, 46.4–54.2). Starting from randomization, the rate of EFS at 4 years was 79% in the rituximab group versus 61% in the observation group (p =0.001). The rate of PFS at 4 years was 83% in the rituximab group versus 64% in the observation group (p <0.001). The rate of OS was 89% in the rituximab group versus 80% in the observation group (p =0.04). According to a Cox regression unadjusted analysis, the rate of OS at 4 years was higher in the rituximab group than in the observation group (p =0.04). Rituximab maintenance therapy after transplantation prolonged EFS, PFS, and OS among patients with mantle-cell lymphoma who were 65 years or younger at diagnosis [30].

Conclusions [28]:

1) In the ibrutynib era, autologous stem cell transplantation and rituximab maintenance still should be recommended as the standard treatment for transplant-eligible patients with MCL.
2) A second autologous stem cell transplantation does not appear to be a promising option in patients with MCL failing a first auto-HSCT. For these patients, allo-HSCT should be considered.

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphomas (DLBCL) is the most common subtype of nHL, accounting for 30–40% of all cases. There are several types of DLBCL, with most people being diagnosed with the subtype known as DLBCL or 'not otherwise specified'. First-line treatment of patients with DLBCL generally consists of rituximab (R) at standard dose (375 mg/sqm) in combination with CHOP or one of its variants such as ACVBP, CHOEP, or DA-EPOCH chemotherapy. Six cycles of R-CHOP are generally used. However, this can be reduced to four without jeopardizing treatment outcomes in patients with IPI 0 [31, 32].

Several studies have evaluated the role of consolidative high-dose therapy followed by auto-HSCT in the R era.

French [33], Italian [34], and German [35] studies failed to demonstrate an advantage of auto-HSCT over conventional chemotherapy. The only American study [36] reported an advantage of auto-HSCT in younger patients with high-risk disease (aaIPI 3); however, this study included patients treated with CHOP only and patients with T-cell lymphoma, and as a consequence was underpowered in order to show a significant advantage of auto-HSCT over R-CHOP [37]. In young patients who remain PET positive after two cycles of chemoimmunotherapy, auto-HSCT is performed in a few countries.

Autologous HSCT is still considered to be the standard treatment for patients with refractory or relapsed (R/R) DLBCL. However, in the rituximab era, the results of salvage therapy followed by auto-HSCT are less convincing than before, and the benefit of auto-HSCT, even for those patients achieving PR or CR with salvage chemotherapy and RTX, is limited [38]. In particular, patients with refractory disease or early relapse pretreated with rituximab as part of first-line therapy rarely achieve long-term remission after auto-HSCT. In the CORAL study, 3-year PFS for such patients was only 23%, although those proceeding to auto-HSCT showed 3-year PFS of 39%. Adverse prognostic factors for auto-HSCT identified in prospective studies include early relapse within 12 months of induction therapy, prior exposure to R, secondary aaIPI, poor performance status, and involvement of two or more extranodal sites at relapse [31, 38].

In the phase III CORAL study, patients with relapsed or refractory DLBCL were allocated to one of two salvage chemotherapy regimens. Those responding to therapy subsequently underwent high-dose chemotherapy followed by ASCT. Following transplant, patients were again randomized to either maintenance therapy with rituximab (MR) (375 mg/m^2 every two months for six doses) or observation. This study failed to demonstrate an improvement in 4-year EFS (52% vs. 53%) or OS (61% vs. 65%) in the MR arm compared to observation. In addition to its lack of benefit, MR was associated with increased toxicity compared to observation (30% vs. 17%), with more serious adverse events noted [12, 39].

Shortly after ASCT, there are increased circulating populations of PD-1 expressing cells, including CD45RO+ effector/memory T cells, natural killer cells and monocytes, which are integral in immune reconstitution [12, 40, 41]. It has been hypothesized that PD-1 blockade in this setting would limit tumor driven lymphocyte exhaustion *via* the PD-1 pathway and potentially lead to improvement in outcomes through eradication of residual disease. In a prospective phase II study, the anti-PD-1 monoclonal antibody pidilizumab was administered every 42 days for three cycles following ASCT in patients with R/R DLBCL, primary mediastinal B-cell lymphoma or transformed indolent B-cell lymphoma. The 16-month PFS and OS from the start of first treatment was 72% and 85%, respectively. Of particular note, in the subgroup of patients with measurable disease post ASCT, pidilizumab was associated with an ORR of 51% with 34% achieving a CR by computed tomography (CT) criteria. Overall, the therapy was well tolerated without report of significant autoimmune toxicity and no infusion reactions or treatment-related mortality [42]. Although these findings have yet to be confirmed in larger randomized studies, therapy with checkpoint inhibitors such as pidilizumab shows promise in the post-ASCT setting in DLBCL, and is the subject of active clinical studies. Although a promising option, currently MR post ASCT is not a standard of care, and should only be considered in the context of a clinical trial. Although larger confirmatory studies are needed, immune manipulation with checkpoint blockade appears to be a rational target with encouraging preliminary data [12, 31].

Conclusions [31]:

1) In the rituximab era, autologous stem cell transplantation is generally not recommended as part of first-line therapy in DLBCL, although recent data on PET-guided auto-HSCT is promising [43].
2) Auto-HSCT is still the standard of care for those DLBCL patients with chemosensitive first relapse.
3) There is no recommendation for tandem transplantation in DLBCL.

Burkitt's lymphoma

Burkitt's lymphoma (BL) accounts for around 2% of all adult NHL, with a higher incidence in patients with immunodeficiency and in patients who are human immunodeficiency virus (HIV)-positive. BL is a highly aggressive tumor with a Ki67 expression of nearly 100% requiring prompt multiagent chemotherapeutic programs. Several studies have identified risk factors for poor outcomes. Besides older age, advanced stage, and comorbidities, such risk factors are: an elevated serum lactate dehydrogenase (LDH), failure to achieve CR, anemia, central nervous system (CNS) involvement, and bone morrow (BM) infiltration. Several studies have explored the role of ASCT in first remission. In a prospective trial, the HOVON group treated 27 patients with two cycles of intensive induction followed by BEAM-conditioned ASCT for those patients achieving at least a partial remission. The 5-year EFS and OS was 73% and 81%, respectively. In a retrospective analysis of 117 patients receiving auto-HSCT as part of first-line therapy between 1984 and 1994, patients in CR at time of transplant had a 3-year OS of 72%. In the relapse situation, patients who were chemotherapy-sensitive had a 3-year OS of 37% following auto-HSCT compared to just 7% for those who were chemotherapy resistant.

In summary, auto-HSCT in BL is feasible, but there is no documented advantage compared to standard combination

chemotherapy for patients responding sufficiently to first-line treatment. Auto-HSCT may be used to optimize remission in patients with insufficient response or as bridging to allo-HSCT. In the relapse setting, given the intensive regimens usually used as first-line treatment, the difficulty lies in achieving a response good enough to proceed to auto-HSCT and to collect autologous hematopoietic stem cells; hence, auto-HSCT is rarely used in BL [44].

Lymphoblastic lymphoma

Lymphoblastic lymphoma (LBL) is an aggressive neoplasm of precursor B cells (B-LBL) or T cells (T-LBL) with features of acute leukemia. It accounts for approximately 2% of all NHL. In adults, around 90% of all LBL are T-LBL. There are only very few studies evaluating the role of auto-HSCT in LBL. In CR1, the use of auto-HSCT as a consolidation may improve relapse-free survival but has no effect on OS. In another study in 128 patients with LBL receiving auto-HSCT, response rate (RR) at 5 years was 56%. No documented role in more advanced disease >CR1 has been reported either. In conclusion, data for auto-HSCT in LBL is too sparse to reach firm conclusions [44].

Primary central nervous system lymphoma

Primary central nervous system lymphoma (PCNSL) is an extranodal NHL, which is classified as a discrete entity in the classification of the WHO. It is an aggressive malignancy that involves the brain parenchyma, spinal cord, eyes, cranial nerves and meninges. The PCNSLs constitute about 1% of all NHLs, 4% of intracranial lymphomas and 4–6% of extranodal lymphomas. The great majority (90%) of the PCNSLs are DLBCL. PCNSL is a rare disease of increasing incidence mainly affecting the elderly. The major risk factor for PCNSL is immunodeficiency, especially the HIV infection. The standard approach to PCNSL, that is high-dose methotrexate (HDMTX)-based chemotherapy followed by whole brain radiotherapy (WBRT), is associated with disappointing outcomes. Moreover, this strategy is associated with increased risk of disabling neurotoxicity, especially in elderly patients. Several drugs and strategies have been investigated to improve results and neurotolerability. Some investigators use ASCT as consolidation after primary chemotherapy and in patients with relapsed/refractory PCNSL. Current therapeutic knowledge in PCNSL management results from a limited number of single-arm phase II trials, meta-analyses and large retrospective series. Thus, several questions such as the optimal primary chemotherapy, the identification of new active drugs and the role of intrathecal chemotherapy, consolidation radiotherapy and ASCT remain unanswered. The latter is an important issue, since preliminary evidence seems to suggest a central role for this strategy in PCNSL. Some investigators showed that in refractory/relapse disease, complete remission rate (CRR) after ASCT was 40%, with 20% treatment-related mortality (TRM). Estimated 2-year RFS and OS rates were 37% and 40%, respectively. Although OS seem to be increased with ASCT in relapsed PCNSL, the difference was not significant (p =0.21). So, even with the application of ASCT in relapsed disease, the prognosis of patients with PCNSL is far from what could be hoped for [45, 46].

Conditioning regimens

There is limited data to guide the choice of HDC regimen prior to ASCT for patients with HL and NHL. 4,917 patients were studied (NHL n =3,905; HL n =1,012) who underwent ASCT from 1995 to 2008 using the most common high-dose chemotherapy schemes: BEAM (n =1,730), CBV (n =1,853), BuCy (n =789), and totalbody irradiation (TBI)-containing (n =545). CBV was divided into CBVhigh and CBVlow based on BCNU dose. One analyzed the impact of regimen on development of idiopathic pulmonary syndrome (IPS), TRM, PFS and OS). The 1-year incidence of IPS was 3–6%, and was highest in recipients of CBVhigh and TBI compared to BEAM. 1-year TRM was 4–8% and was similar between regimens. Among patients with NHL, there was a significant interaction between histology, HDT regimen, and outcome. Compared to BEAM, CBVlow was associated with lower mortality in follicular lymphoma (p <0.001), and CBVhigh with higher mortality in diffuse large B-cell lymphoma (p =0.001). For patients with HL, CBVhigh, CBVlow, BuCy and TBI were associated with higher mortality compared to BEAM (p <0.001). The impact of specific HDC regimen on post transplant survival is different depending on histology; therefore, further studies are required to define the best regimen for specific diseases [47].

Author's contributions

PR contributed all this work.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Autologous hematopoietic stem cell transplantation (auto-HSCT) in children in Poland: 2021 indications and practice

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Abstract

Unlike in adults, the number of pediatric autologous hematopoietic stem cell transplants (HSCT) has decreased in last years. This is because of changing indications for this type of treatment and new therapies available in recent years. Polish pediatric HSCT centers have followed the published recommendations of the Polish Pediatric Hematopoietic Stem Cell Transplantation Group, which are generally based on European Society for Blood and Marrow Transplantation indications. Differences are observed in obtaining autologous hematopoietic stem cells from children compared to adults in terms of the timing of the scheduled harvest and technical aspects of the harvesting procedure. As a result, stem cell harvesting in pediatric populations involves more medical professionals, and requires more time and more financial resources compared to adults. Pediatric autologous stem cell transplantation in neuroblastoma and Ewing's sarcoma has confirmed efficacy. Autologous cell harvesting in young children established in autologous transplant procedure is now increasingly used in apheresis of lymphocytes for approved CAR-T cell therapies in relapsed/resistant leukemia and lymphoma. Recent studies suggest that cell-based immunotherapy is a potential treatment for refractory or relapsed pediatric solid tumors.

Key words: autologous stem cell transplantation, children, stem cell harvest

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Specificity of auto-HSCT in children

Since 1990, the European Blood and Marrow Transplantation Society (EBMT) has collected data about hematopoietic stem cell transplantations performed in Europe. Currently, of about 700 accredited and annually reporting centers, only 122 are dedicated solely to children, and 128 declare that they treat children and adults. Out of 28,714 autologous hematopoietic stem cell transplants (HSCTs) performed in 2019 (59% of all procedures), pediatric patients comprise only 1,199 reported cases. Unlike in adults, the number of pediatric autologous hematopoietic stem cell transplants has decreased in recent years [1]. This is because of changes in indications for this type of treatment and new therapies becoming available.

Indications and qualifications

Polish pediatric HSCT centers follow the recommendations of the Polish Pediatric Hematopoietic Stem Cell Transplantation Group (PPGTKK, *Polska Pediatryczna Grupa ds. Transplantacji Komórek Krwiotwórczych*), which are generally based on EBMT indications. Current indications for auto-HSCT in children according to PPGTKK [2] and EBMT [3] are set out in Table I.

Course of procedure

Important differences are observed in obtaining autologous hematopoietic stem cells in children compared to adults in terms of the time of scheduled harvest. Current treatment protocols of neuroblastoma and Ewing's sarcoma indicate a time of apheresis early in treatment course i.e.

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Table I. Indications for autologous hematopoietic stem cell transplants (auto-HSCT) in children

Indication	PPGTKK	EBMT
Leukemia, myelodysplastic syndrome, myeloproliferative disorders	Not recommended	Not superior to chemotherapy
Hodgkin lymphoma	Progression during first line treatment and relapse in intermediate and high-risk group	Primary refractory disease and chemosensitive first relapse
Non-Hodgkin lymphoma	Pre-B and pre-T lymphomas in isolated relapse (except CNS) >12 months after diagnosis Primary mediastinal large B-cell lymphoma with residual tumor with live tumor cells after resection	Pediatric indications not specified
Neuroblastoma	In 1st CR — HR patients, >1 CR — all children not transplanted in first CR	First-line high-risk NBL >18 months at diagnosis, metastatic disease or any age with MYCN-amplified tumors
Ewing's sarcoma	In 1st CR — poor histological response for chemotherapy, large tumor volume or metastatic disease, all chemosensitive relapses not transplanted in first CR	In 1st CR — poor histological response for chemotherapy, large tumor or metastatic disease, all chemosensitive relapses not transplanted in first CR
Other solid tumors	As clinical studies or individual consult with national coordinator	High risk medulloblastoma, recurrent germ cell tumor in biological remission
Autoimmune disease	Juvenile rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, systemic sclerosis or Crohn's disease not responding to conventional, biological and low doses cytostatic drug therapies	Multiple sclerosis, systemic sclerosis or Crohn's disease not responding to conventional and biological drug therapies

PPGTKK (*Polska Pediatryczna Grupa ds. Transplantacji Komórek Krwiotwórczych*) — Polish Pediatric Hematopoietic Stem Cell Transplantation Group; EBMT — European Blood and Marrow Transplantation Society; CNS — central nervous system; CR — complete remission; HR — high-risk; NBL — neuroblastoma

2–3 months after diagnosis, and several months before auto-HSCT.

The common practice is implantation, for apheresis purpose, of a temporary double lumen catheter in the vast majority of pediatric patients. Peripheral veins as well as permanent catheters, pre-implanted for chemotherapy, are believed to have a flow rate not sufficient for harvest. However, a recently published report from three German pediatric centers demonstrates comparable efficacy and safety of apheresis using permanent catheters. This is leading to reconsideration of this common practice [4].

The second important technical difference is related to the low body weight of children: in patients weighing below 10–12 kg, priming with red blood cells and continuous infusion of calcium is necessary to avoid short term complications [5]. For these reasons, stem cell harvesting in pediatric populations involves more medical professionals, and requires more time and more financial resources compared to adults.

Megachemotherapy protocols in pediatric patients are based on busulfan, treosulfan, melphalan or thiotepa at maximum tolerated doses in main indications. Due to the low rate of co-morbidities in children, procedure-related deaths and life-threatening complications are rare, even in highly pretreated patients. Most treatment failures are related to disease relapse or progression, and therefore the remission status and optimal timing of auto-HSCT in children continues to be the most important prognostic factor for outcomes [6, 7].

Auto-HSCT in children — practice in Poland

Hematopoietic stem cell transplantation in dedicated pediatric centers in Poland began in 1989, but the first auto-HSCT was not performed until 1994. Until 2016, autologous procedures comprised 31% of all reported transplants in five Polish pediatric centers, very close to the 33% rate performed in European centers dedicated for children [8].

The current practice in auto-HSCT in Poland in the last two years compared to previous data is presented in Table II. Neuroblastoma and Ewing's sarcoma continues to be the leading indication for autologous HSCT, and these procedures are no longer performed in children for acute leukemia or non-Hodgkin lymphoma.

Auto-HSCT prospects in the near future

Pediatric autologous stem cell transplantation in neuroblastoma and Ewing's sarcoma has confirmed efficacy. A combination of this method with specific immunotherapy, differentiating agents or meta-iodobenzyl guanidine therapy (MIBG) can improve the outcome of patients in the future [10, 11].

Table II. Changes in practice of autologous stem cell transplantation (auto-HSCT) in children in Poland (data from Prof Jacek Wachowiak, personal communication)

Cause of auto-HSCT	1993–2016 [9] N =788	2018–2019 N =96
Leukemia (ALL or AML)	96 (12%)	0
Hodgkin lymphoma	42 (5%)	4 (4%)
Non-Hodgkin lymphoma	100 (13%)	0
Neuroblastoma	326 (42%)	46 (48%)
Ewing's sarcoma	111 (14.5%)	17 (18%)
CNS tumors	26 (3%)	5 (5%)
Other solid tumors	82 (10.5%)	14 (15%)

ALL — acute lymphocytic leukemia; AML — acute myeloid leukemia; CNS — central nervous system

Autologous cell harvesting in young children established in transplant procedure is now increasingly used in apheresis of lymphocytes for approved CAR-T cell therapies in relapsed/resistant leukemia and lymphoma [12]. EBMT data confirms that this type of cellular therapy is increasingly used in EBMT centers (adult and pediatric): from 151 procedures in 2017 to 1,111 (824 in non-Hodgkin lymphoma/ /Hodgkin lymphoma, 232 in acute lymphocytic leukemia, and 55 in other malignancy) in 2019 [13]. Recent studies suggest that CAR-T cell-based immunotherapy has potential also for the treatment of refractory or relapsed pediatric solid tumors [14].

Author'scontributions

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Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Optimal timing and conditioning regimen in allogeneic stem cell transplantation for AML

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Abstract

In all stages of the disease, allogeneic stem cell transplantation (allo-SCT) plays an important role in the treatment of acute myeloid leukemia. It is an ongoing challenge to find the right balance between the chance of a cure and the risk of dying from side effects of the procedure. With respect to the conditioning, the large number of available protocols, ranging from non-myeloablative to a classical high-dose regimen, offers the opportunity to individualize the treatment, considering both the clinical situation and patient-specific factors such as age and co-morbidities.

As a consequence, allo-SCT has become available to a larger percentage of patients, and the question as to whether or not to undergo a transplantation needs to be answered more frequently. The factors to be considered vary widely among patients in remission, those with relapsed disease, and those who never responded to conventional therapy. This review addresses this discussion, focusing on how to define an individualized and weighted treatment concept for each patient.

Key words: AML, allogeneic transplantation, timing, conditioning

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Introduction

Allogeneic stem cell transplantation (allo-SCT) is the treatment modality with the highest potential to cure acute myeloid leukemia (AML). However, antileukemic efficacy is often counterbalanced by a unique treatment-relapse mortality (TRM). Hence, the decision to undergo allo-SCT needs to be thoroughly weighed in each individual patient, considering both the risk of the leukemia and the individual risk factors for TRM. This is of particular importance in the early stages of the disease, when it is crucial to identify those patients who might not require an allo-SCT to achieve long-term disease control, and should therefore not be exposed to the risk of treatment-related toxicity and mortality at that stage.

At the other end of the spectrum, in patients with relapsed or refractory AML, allo-SCT definitely represents the only chance for long term remission, making every patient at this stage a potential transplant candidate. However, both relapse incidence and TRM are high in this patient population, which is why deciding between a high-risk transplant approach and palliative treatment is a challenge. The indication for allo-SCT requires careful consideration on the transplanter's side, as well as extensive discussion with the patient and his or her family.

This review was aimed to discuss factors that might play a role in the decision-making around allo-SCT in the different stages of AML. Particular attention has been paid to the conditioning regimen to be used in each stage, given the fact that both antileukemic control and TRM,

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among other factors, are highly influenced by the preparative protocol.

General remarks on conditioning for allogeneic transplantation in AML

Historically, conditioning for allo-SCT has been applied for three major reasons:

- eradication of leukemia;
- immune suppression to allow engraftment and prevent graft-versus-host disease (GvHD);
- providing space in recipient's bone marrow for transplanted donor hematopoiesis.

During the development of allo-SCT, this third purpose has been questioned by animal studies showing engraftment without conditioning after the transfusion of mega doses of stem cells and repeated administrations, and by the development of non-myeloablative, purely immunosuppressive, conditioning regimens, whose therapeutic efficacy is based on the allogeneic graft-versus-leukemia (GvL) effect alone.

Over time, a large number of conditioning regimens has been developed, and only a minority have been prospectively validated or compared in randomized trials. At the upper end of the spectrum, a traditional myeloablative conditioning (MAC) regimen incorporating high-dose cyclophosphamide (usually 120 mg/kg), plus either total body irradiation (TBI) at a dose of 12 Gy, or busulfan 16 mg/kg per os/12.8 mg/kg intravenous are used. At the other end of the range, a non-myeloablative regimen (NMA) comprising fludarabine and 2 Gy TBI has been shown to allow engraftment.

In between, a wide range of more or less reduced intensity protocols have been published as reduced toxicity (RCT) regimens. As shown in several retrospective comparative studies, TRM was significantly reduced by RCT/NMA. However, this advantage came at the cost of increased relapse incidence, resulting in overall comparable outcomes. In prospective trials comparing RIC to MAC, results were identical in both groups in one trial using two TBI based regimen, whereas in another trial mainly using a chemotherapy-based regimen, MAC was of advantage [1, 2]. Nevertheless, the introduction of RIC/NMA definitely has opened up the opportunity of allo-SCT for elderly patients up to the age of 75, and to patients with co-morbidities who are unable to tolerate a MAC regimen. As a further variant initially designed for high-risk myeloid malignancies, in 2005 the sequential regimen approach was introduced, aiming to combine increased antileukemic efficacy and reduced non-relapse mortality (NRM) [3]. Initial results of this regimen, called FLAMSA-RIC, were promising, in particular in high-risk and advanced disease [4]. However, no advantage could be shown in a recent prospective trial comparing a variant of the original protocol to a fludarabin/busulfan regimen in patients transplanted in complete remission (CR) [5].Further variants of the sequential regimen approach have been developed [6].

In general, no single regimen has been identified as being definitely superior to any other. Hence, no clear standard has been established. To classify the increasing number of protocols, the definitions for MA and NMA protocols have been published on the EBMT website [7]. A more detailed classification was proposed in 2009, using strict definitions for MA protocols (causing irreversible pancytopenia and requiring mandatory stem cell (SC) support) and for NMA regimen (causing minimal cytopenia only and allowing engraftment without SC transfusion). All regimens not fulfilling either definition are categorized as reduced intensity conditioning (RIC) [8]. Most recently, the Acute Leukemia Working Party (ALWP) of the EBMT finally developed a new classification based on intensity weight scores for frequently used conditioning regimen components, using their sum to define the transplant conditioning intensity (TCI) score [9].

Timing of allo-SCT in AML

Aspects for the optimal timing and execution of allo-SCT in AML are different among clinical stages at which the procedure is considered.

Allo-SCT in primary refractory AML (PREF AML)

Primary refractory AML is defined by either morphologically persisting leukemia or by hematological CR with incomplete reconstitution of hematopoesis (CRi) after at least two courses of induction therapy, usually including at least one course that contains high-dose cytosine arabinoside (Ara-C) [10]. In some studies, patients with persisting minimal residual disease (MRD), or patients with >15% blasts or a <50% proportional reduction of blasts after the first course of induction, had a similar prognosis [11]. Risk factors for refractoriness primarily include older age and adverse genetic aberrations. Independently of the exact definition, the overall survival (OS) of patients with PREF AML after conventional chemotherapy is below 10%. Hence, there is broad consent that allo-SCT is the treatment of choice for these patients, who represent about 10–40% of all adults with AML [12].

Following allo-SCT, long-term disease control can be achieved in 25–35%. In fact, it is not completely clear which patients with PREF AML will benefit most from allo SCT, although those proceeding to transplant as soon as possible after a diagnosis of PREF AML without repeated courses of chemotherapy [13] and those transplanted with a lower tumor burden, seem to achieve the best outcome. Hence, starting the search for a donor immediately after initial diagnosis is mandatory among patients with a high risk of developing PREF AML who are able to undergo allo-SCT.

The optimal conditioning for patients with PREF AML remains to be defined. MAC should probably be preferred in patients <50 years, whereas a reduced regimen has led to equivalent or even superior results in older patients. Sequential protocols such as the FLAMSA regimen represent an attractive option up to the age of 65 [4]. Definitely, maintenance therapy after allo-SCT is recommended in patients with PREF AML, either using DLI or pharmacological treatment [14].

Allo-SCT in first complete remission (CR1)

Patient selection and timing of allo-SCT in CR1 is probably the most hotly debated question in the field of AML therapy. In general, for each individual patient, the task is to define the specific balance between the reduction of the risk of relapse and leukemia-associated death by allo-SCT compared to conventional treatment, against the risk of TRM.

Determinants of relapse risk

Besides increasing age, two major determinants for the risk of relapse have been identified: Firstly, adverse genetics at diagnosis as defined by the European LeukemiaNet (ELN) [10] clearly define the biological risk for adverse outcomes. Secondly, the detection of MRD before allo-SCT either by molecular genetics including next generation sequencing (NGS) [15, 16] or by flow cytometry [17], has been shown to be a highly predictive variable for increased relapse incidence and inferior survival post-transplant. Unfortunately, MRD measurement is difficult to standardize both among AML subtypes and among different laboratories, which is why it is hard to define clear cutoff values generally indicating a clinically relevant risk modification. Moreover, in contrast to other markers, detection of mutations in several epigenetic regulators (e.g. *ASXL1, DNMT3A* and *TET2*) did not influence the risk of relapse and these are therefore difficult to be considered for the indication for allo-SCT [15]. Hence, the inclusion of MRD in general into risk estimates remains challenging. Beyond genetics and MRD, some study groups include variables such as leukocyte counts at diagnosis or the quality of response to the first course of induction therapy into the decision for allo-SCT [18].

The risk of TRM

To assess the risk of TRM, several scores have been proposed: Sorror et al. were among the first to adapt a validated comorbidity index for the setting of allo-SCT. More recently, patient age was included into a refined version of this score, allowing for a clear separation of patient cohorts with different risks of TRM based on their comorbidities [19].Using the data from >50,000 transplants reported to the EBMT registry, Gratwohl et al. have established another risk model, comprising patient, disease, and donor variables [20] that was validated for AML in CR1 [18]. More recently, the Acute Leukemia Working Party has proposed a combination of both scores adapted for reduced intensity conditioning [21].

Risk-adapted decision

In general, using genetic risk assessments, MRD and estimators of risk for TRM, the published guidelines [12,18] recommend allo-SCT as consolidation of choice for AML in CR1, if:

- the risk of relapse without allo-SCT is expected to be >35–50%, and
- the chance of achieving long-term leukemia-free survival after allo-SCT is increased by >10%, considering the patient's individual risk for TRM.

In order to further refine the risk-adapted approach, modern techniques such as knowledge bank approaches have been introduced into this field, using multistage models to simulate survival of a given patient in different treatment scenarios [22]. Refined guidance for allo-SCT in CR1 based on this strategy has been proposed [23] However, continuous integration of newly detected variables such as NGS-based genetic risk constellations on one side, and achievements in the management of transplant-associated complications on the other side, is warranted to improve these kinds of scores. Additionally, a prospective validation is mandatory.

Modifying the risk

The increasing amount of data on the prognostic importance of MRD pre-transplant offers the possibility of improving overall results by approaches to improve the quality of remission before the start of conditioning. Novel agents such as the liposomal cytarabine–daunorubicine formulation CPX-351 seem to improve outcomes post-transplant by inducing deeper levels of response [24]. More recently, initial findings were reported on the elimination of MRD pre-transplant by innovative treatments without adding intolerable toxicities [25], opening a window of opportunity to modify an important risk factor for post-transplant relapse.

Conditioning

With respect to conditioning for AML in CR1, there is no one-size-fits-all recommendation, and the ideal regimen is not yet defined. As mentioned above, a MAC regimen is usually preferred for patients below the age of 50nd without significant comorbidities, given its superior antileukemic activity [2]. In older patients, a regimen with reduced toxicity might lead to an overall improvement of survival, given the increasing importance of TRM for outcomes within this patient subgroup [26, 27]. The presence of MRD might be another reason to prefer a MAC regimen, since levels of MRD do alter outcomes after RIC, but not MAC, transplants [28]. However, intensification of the conditioning using a sequential regimen does not modify the role of MRD [5].

Maintenance

Finally, as discussed above in the section on PREF AML, there is increasing interest in the administration of pharmacological or cellular maintenance treatment in order to reduce the risk of post-transplant relapse in high-risk disease. Among others, Flt3 inhibitors such as sorafenib or midostaurin, hypomethylating agents, or HDAC inhibitors such as panobinostat have been successfully used [29–31], and synergistic effects with the graft-versus-leukemia effect [32] have been shown. Unmodified DLI, as well as specifically modified donor immune effector cells, have also been shown to be promising [33], although their prospective validation is still awaited.

Allo-SCT in beyond CR1

Once AML has reached a stage beyond CR1, the indication for allo-SCT is indisputable for all patients who can tolerate the procedure. Scoring systems have been developed to estimate the prognosis of these patients [34]. Long-term survival rates of 30–50% have been reported after allo-SCT in second CR (CR2), whereas outcomes were inferior in patients with untreated or refractory relapse. If morphological CR2 has been achieved, the patient should proceed to allo-SCT as soon as possible [12]. However, since a considerable percentage of relapsed patients will not achieve CR2 due to refractory leukemia or TRM under salvage therapy, the role of re-induction in a patient with an available donor has been questioned. Also, the number of chemotherapy courses has been a risk factor for response and survival in several studies of relapsed and refractory AML [4, 13]. This problem is currently being addressed in the prospective, randomized ETAL 3 trial in Germany (NCT02461537).

Summary

Allo-SCT is an important part of the therapeutic armoury for all stages of AML. Prognostic estimates, the introduction of new methods for disease characterization and monitoring, as well as the use of novel drugs and cellular treatments, will all improve patient selection and clinical outcomes. Increasingly, both the indication for allo-SCT, as well as the way in which the procedure is performed, have become an individualized process into which all available evidence should be included.

However, beyond data from well-designed prospective trials and retrospective studies, neither should the physician's clinical judgement nor the patient's individual preferences be neglected in the decision-making process.

Author's contributions

CS — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Tolerance and efficacy of total body irradiation and cladribine prior to allogeneic hematopoietic cells transplantation in patients with acute myeloid leukemia and myelodysplastic syndromes — synopsis of clinical study

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Abstract

Introduction: Allogeneic hematopoietic cell transplantation (allo-HCT) is a standard of care for patients with acute myeloid leukemia (AML) and patients with intermediate and high-risk myelodysplastic syndrome (MDS). Despite many years of experience there is still no standard for conditioning regimen. The aim of this study is to analyse the efficacy and safety of a conditioning treatment with cladribine in combination with total body irradiation (TBI).

Material and methods: A group of 40 adult patients referred for allo-HCT due to AML and MDS are to be enrolled in the study. The inclusion criteria are: informed consent, chemo-sensitivity for cladribine treatment regimens (when used in induction therapy), age 18–60, and performance status 0–2 according to World Health Organization. The conditioning regimen consists of cladribine and TBI at a total dose of 12 Gy in three fractions given over three consecutive days. The goal of the study is to assess the tolerability and efficacy of the regimen.

Results: Our results may stimulate further investigation in this field i.e. phase III trials to compare this regimen to others.

Conslusion: A myeloablative conditioning regimen consisting of total body irradiation in combination with cladribine may contribute to improved outcomes after allo-HCT for AML and MDS patients.

Key words: allo-HCT, myeloablative regimen, transplantation, cladribine, total body irradiation, TBI

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Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a standard of care for patients with acute myeloid leukemia (AML) and patients with intermediate and high-risk myelodysplastic syndrome (MDS). Most patients up to 60 years old qualify for transplantation with a myeloablative regimen which traditionally involves the use of high doses of alkylating agents alone, or in combination with total body irradiation (TBI). An advantage of one over the other has not been proven. A German group showed that TBI can be used with the purine analog fludarabine instead of the previously used cyclophosphamide. This contributes to reduced toxicity while maintaining high efficacy [1, 2].

In AML and MDS, the evidence from clinical trials, including those conducted by the Polish Adult Leukemia

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Group (PALG), indicates that cladribine is more effective than fludarabine. Using it in the conditioning regimen may therefore contribute to increasing the effectiveness of the allo-HCT procedure, i.e. to reducing the risk of disease relapse. The results of studies comparing the efficacy of cladribine to fludarabine in the first-line treatment of AML patients indicate a similar tolerability of both drugs. It is therefore assumed that the conditioning regimen with cladribine will not increase the toxicity [3–5].

Current research

Our project assumes that the use of TBI in combination with another purine analog, cladribine, could be even more favorable. Cladribine is produced in Poland based on an original synthesis process. This assumption is based on the results of the PALG studies. It was proven that the addition of cladribine to daunorubicin and cytarabine during induction treatment increases the chance of achieving complete remission and the probability of overall survival in patients with AML, including AML preceded by MDS. This effect with fludarabine was not seen. Both drugs increase Ara-CTP levels in leukemia blasts. However, only cladribine showed direct cytotoxic and hypomethylating activity, which justifies the observed differences in efficacy [5]. The initial experience of our clinical practice indicates good tolerance of TBI and cladribine as part of the allo-HCT preparation protocol.

This study is led by the Department of Bone Marrow Transplantation and Oncohematology in Maria Sklodowska-Curie National Research Institute of Oncology (MSCNRIO), Gliwice Branch, Poland. The partner of the study is PALG.

Material and methods

The study population consists of 40 patients undergoing allo-HCT due to high-risk MDS and AML. The criteria for patient inclusion in the study are: diagnosis of AML in the first with complete remission from the group of intermediate or high cytogenetic risk, AML in the second or subsequent complete remission, or high-risk MDS. Patients have to be 18 to 60, with clinical condition of a 0–2 level according to the World Health Organization (WHO) scale. All type of donors are allowed: human leukocyte antigen (HLA)-compliant family donor, matched or mismatch unrelated donor, or haploidentical donor. The patient signed informed consent to participate in the study.

The conditioning regimen consists of cladribine in a dose of 5 mg/m^2 of patient per day for five consecutive days. After that, TBI is performed in a total dose of 12 Gy in three fractions of 4 Gy each for three consecutive days. The source of hematopoietic cells is bone marrow or peripheral blood. Immunosuppressive therapy depends on HLA matching. In transplantation from matched donors, a combination of cyclosporin A and methotrexate is used. In this type of donor, ATG is also used. The dose depended on the type of donor (sibling vs unrelated). In the case of HLA, mismatch and haploidentical donors post-transplant cyclophosphamide and tacrolimus with mycophenolate mofetil are used.

Patients are referred for transplant eligibility from PALG partner centers. These centers are responsible for the proper preparation of patients. The final qualification of the patient for allo-HCT as well as for a clinical trial takes place within the transplant center, i.e. the study lead.

The main goal of the study is to assess the tolerability and efficacy of the regimen. The probability of progression-free survival (PFS) after 24 months was the primary study end-point. Secondary endpoints include: rates of adverse events, the probability of overall survival (OS) at 24 months, relapse incidence (RI) at 24 months, non-relapse mortality (NRM) at 24 months, the incidence of acute and chronic graft-versus-host disease, and the time of neutrophil and platelet engraftment.

The duration of the study is 56 months from the date of signing the contract for the implementation of the clinical trial. During its duration, 24 months are set aside for recruiting patients, with a 24-month observation period.

Discussion

Research by the PALG has confirmed the improved effectiveness of induction therapy by adding cladribine to daunorubicin and cytosine arabinoside. A randomized trial comparing the DA, DAF, and DAC regimens has further demonstrated a benefit in OS and PFS in patients treated with a DAC induction regimen. This scheme has been included as a standard of care in the recommendations of the National Comprehensive Cancer Network (NCCN) [5, 6].

TBI-based protocols formed the conditioning regimen in patients prior to allo-HCT. The combination of cyclophosphamide with TBI (referred to as the Cy-TBI regimen) was used in pioneering transplants by Thomas in 1971. Currently, 12 Gy is the most common myeloablative dose of TBI. Recent years have brought about the combination of irradiation with less toxic chemotherapeutic agents such as fludarabine.

Cladribine alone in conditioning treatment has already been described in small groups of patients, mainly in reduced intensity and nonmyeloablative conditioning [7–9]. To date, no clinical trial has been conducted describing its therapeutic effect at this stage of treatment, especially in treatment with myeloablative potential. Also, the combination of cladribine with radiotherapy has not yet been analyzed.

The myeloablative conditioning regimen consisting of TBI in combination with cladribine may contribute to improved outcomes after allo-HCT for AML and MDS patients. Results may stimulate further investigation in this field, i.e. phase III trials to compare this regimen to others.

Conclusions

The good experience of the PALG group in the use of cladribine in the treatment of AML and MDS, and of the German group in using the combination of TBI with fludarabine in conditioning treatment, raise hopes that the study conditioning regimen will be more effective.

Authors' contributions

MSK, SG — design of study, manuscript writing, revision and approval.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of The Word Medical Association (Declaration of Helsinki) for experiments involving humans: EU Directive 2010/63/EU for animal experiments: Uniform requirements for manuscripts submitted in biomedical journals.

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VIA MEDICA

REVIEW ARTICLE

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Allogeneic hematopoietic stem cell transplantation in elderly patients with acute myeloid leukemia

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Abstract

The incidence of acute myeloid leukemia (AML) significantly increases with age. Most AML patients are elderly and rarely receive curative treatment. Even those who eventually achieve complete remission have a grim prognosis due to the high risk of relapse. In elderly patients, allogeneic hematopoietic stem cell transplantation (allo-HSCT) increases the probability of prolonged survival compared to standard treatment. The decision as to whether to refer a patient for transplantation must be preceded by a careful risk assessment based on the patient's remission status, comorbidities, and type of available donor. Although allo-HSCTs are routinely performed in the seventh decade of life, they are not common in those aged over 70. In recent years, the results of allo-HSCT in the elderly have improved, mainly thanks to refined conditioning regimen techniques and better supportive care. It can be anticipated that with growing data on allogeneic transplants in older AML patients, the proportion of this population among transplant recipients will continue to rise.

Key words: acute myeloid leukemia, elderly patients, allogeneic hematopoietic stem cell transplantation

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Introduction

Current life expectancy at birth in the European Union is 81 years according to Eurostat data [1]. It is better for women with 83.7 years than for men with 78.2 years. The steady increase of predicted life duration over the last 20 years raises the question as to how to define elderly and old populations. The recent World Health Organization definition describes persons over the age of 65 as old [2]. The process of ageing or senescence in an individual starts anywhere between 45 and 65 years of age and proceeds at different paces depending on genetic, ethnic and biological factors as well as on socio-economic circumstances. Thus, an exact age definition of an elderly patient is lacking. Usually those above 60 years are considered to be elderly. Epidemiology data shows that the median age of patients diagnosed with AML is between 64 and 70 [3–5]. Therefore, the majority of newly diagnosed AML patients fall into the elderly population category.

Diagnosis and risk factors in acute myeloid leukemia

According to the 2017 European LeukemiaNet (ELN) guidelines, AML is diagnosed based on leukemic blasts in the bone marrow in excess of 20% with the exception for AML with recurrent genetic abnormalities t(15;17), t(8;21), inv(16) or t(16;16) [6]. Patients who are diagnosed with AML are stratified into low-, intermediate- or high-risk groups according to genetic abnormalities in line with ELN recommendations. However, the prognosis in AML strongly depends also on other factors such as age, performance status, sex, comorbidities, pre-existing hematological conditions, previous cancer treatment, and response of the disease to therapy [7].

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AML in the elderly

The prognosis in elderly patients with AML is dismal. Five-year disease-free survival in the elderly is c. 5%, while in young patients it is c.40% [8, 9]. Poor outcomes in older patients are both disease- and patient-related. Among these patients, there is a distinct high-risk subgroup with therapy-related AML after previous exposure to radiotherapy or chemotherapy as well as secondary AML after antecedent myeloproliferative neoplasm or myelodysplastic syndrome (MDS) [10]. Adverse cytogenetic and molecular abnormalities affect 50–60% of older patients compared to 30% of those younger than 60 [11, 12]. Older age is frequently associated with comorbidities and frailties which preclude intensive (or even any) anti-leukemic treatment. Only about 55% of patients aged 65+ receive specific therapy for AML, and the proportion of treated patients is decreasing with age. This translates into median survival of 2 months in untreated vs. 6 months in treated patients [13]. Published data indicates that allogeneic hematopoietic stem transplantation (allo-HSCT) as post-remission therapy yields the best results in terms of survival benefit in elderly AML patients [13, 14].

Eligibility of elderly AML patients for allogeneic stem cell transplantation

The achievement of complete remission of AML is the prerequisite for successful allo-HSCT. Treatment options in the elderly include intensive chemotherapy, demethylating agents, low dose chemotherapy, and palliative care. The choice of treatment depends on age, performance and comorbidities. Both the Charleston Comorbidity Index (CCI) and Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) have been proved useful in predicting outcomes of chemotherapy in the elderly. They are frequently employed to determine the optimal treatment modality in individual patients [15–17]. Complete remission rates are higher in those who receive intensive treatment compared to patients treated with azacitidine, decytabine or decitabine. According to different studies, patients receiving more intensive therapy tend to be younger with lower CCI or HCT-CI [18, 19]. Retrospective analyses show that expected CR rates with intensive chemotherapy in those patients can reach 50–60% [20, 21].

The recently published results of a study combining decitabine or azacitidine with venetoclax as first line treatment in elderly AML patients show promising outcomes, with CR rates of 65% and treatment-related mortality of 8% [22]. Despite relatively high CR rates among older patients receiving remission-aimed treatment, real-life data shows that only a fraction of newly diagnosed patients ever even enter the allo-HSCT procedure. A single-center French analysis showed that less than 20% of older patients actually receive allo-HSCT [23]. A large Center for International Blood and Marrow Transplant Research registry-based analysis indicates that only 6% of those aged 60–75 are offered transplantation [24]. Most elderly patients are deemed ineligible for the procedure by their treating physicians, decide themselves against this treatment option, or lack a suitable donor even if they achieve complete remission.

Allogeneic stem cell transplantation in older AML patients

Pre-transplant considerations

European Society for Blood and Marrow Transplantation (EBMT) annual reports consistently show AML as the main indication for allo-HSCT. The fraction of patients aged 65+ receiving allo-HSCT rose from <1% in 2000 to 6.7% in 2014 [25]. It is predicted that the proportion of older patients will increase in coming years in spite of emerging novel treatments for AML. Accepting older patients for allo-HSCT requires a specific approach apart from routine pre-transplant assessments including AML remission status or HCT-CI. Careful evaluation of performance status, as well as modified EBMT and pretransplant assessment of mortality (PAM) scores, are recommended [26, 27]. Functional geriatric tests or patient-reported functional history should be evaluated by qualified personnel.

Standard evaluation elements may include gait speed, grip strength, 6-minute walk test, independence in everyday life, psychosocial or cognitive tests as well as nutritional status and pharmacological treatment requirements. The Geriatric Assessment in Hematology (GAH) scale may be useful to determine a patient's eligibility for transplant [28]. Several studies have proved that Karnofsky Performance Score <80% and higher HCT-CI negatively influence transplantation outcomes [29, 30]. Cognitive impairment in elderly patients must be considered as an independent risk factor for increased non-relapse mortality and decreased survival after allo-HSCT [31]. A multidisciplinary team geriatric assessment before allo-HSCT may optimize patient selection for transplantation, and mitigate post-transplant morbidity and mortality [32].

Remission status before allo-HSCT

One of the most important factors determining overall and disease-free survival in patients transplanted for AML is complete remission of the disease before allo-HSCT. Not only the presence of overt leukemia, but even detection of minimal residual disease (MRD) before transplantation, negatively impact prognosis in AML. Regardless of the method used for the detection of MRD, those patients who are MRD positive have a significantly increased risk of relapse post-transplant [33]. The probability of obtaining CR in elderly patients is lower even with intensive treatment.

Thus, if medically fit, older patients may proceed to transplant with a partial response only. In a German study, AML patients aged 60 to 77 who received allo-HSCT in 1st CR had 3-year OS probability of 49%. But even those with active disease at transplantation had 3-year OS probability of 30% [34]. Nevertheless, elderly patients with AML who are MRD negative before transplant fare significantly better. In a study including 185 patients aged 65+, there was a substantial difference in 2-year overall survival (OS) and incidence of relapse between MRD negative patients and those with detectable leukemia. The results for OS were 76% vs. 8% in MRD negative and positive patients [35].

Donors

Most allo-HSCTs in older AML patients are performed from unrelated donors. Even if patients have HLA-matched siblings, they usually are of a similar age and have chronic medical conditions which preclude stem cell donation. In patients older than 65, only 28% of donors are siblings [36]. Particularly in older AML patients, there is an additional issue of whether an unrelated younger donor would be better over an older but matched sibling. This issue was resolved by a retrospective study form the EBMT published a few years ago which included AML patients whose median age was over 61.

This study found similar outcomes in terms of relapse, non-relapse mortality, leukemia-free survival (LFS), and OS of transplants from younger unrelated and older sibling donors [37]. According to the National Marrow Donor Program, there is a 74% chance of finding a fully human leukocyte antigen (HLA) matched unrelated donor (MUD) for a recipient of Caucasian European descent. Patients of Middle Eastern, African or Native American descent have a decreasing likelihood of finding a complete match. For AML patients in need of an allogeneic transplant, there is a possibility of finding an alternative donor: unrelated donor with acceptable HLA mismatch (mMUD), haploidentical family donor (Haplo) or cord blood (CB). Results of a retrospective EBMT analysis indicate that transplants from mMUD yield worse results than transplants from MUD in AML patients [38]. In a recently published large study from Japan in 1,577 AML patients aged 60+, the probability of OS at 3 years after single unit CB transplant was 31% [39]. Haploidentical transplants have recently generated great interest as an attractive option for patients who lack an HLA compatible sibling or an unrelated donor. Indeed, earlier papers indicated comparable results in AML for transplants from Haplo, MUD and matched sibling donors. Yet an evaluation from the Center for International Blood and Marrow Transplant Research (CIBMTR) and the EBMT revealed higher non-relapse mortality, and overall mortality, after transplants from haploidentical compared to matched related donors in acute leukemia patients aged 55 to 76 [40]. Analysis from the CIBMTR comparing the results of transplants from young (18–40) MUD and Haplo donors in AML patients aged 50–76, showed probability of 5-year OS after young MUD and Haplo transplants of 42% and 32%, respectively [41].

Conditioning

Commonly, older patients with AML receive reduced intensity conditioning (RIC) that is composed of lower doses of alkylating agents or irradiation frequently combined with a purine analog replacing classical cyclophosphamide for immunosuppression. This is aimed at reducing toxicity and ultimately non-relapse mortality (NRM) while preserving anti-leukemic and immunosuppressive effects. Some regimens are non-myeloablative (NMA), based entirely on the immunosuppressive effect to ensure engraftment of donor cells but allow autologous hematopoietic recovery without transplantation.

A large registry-based study by the EBMT compared outcomes of allo-HSCT from sibling donors in 1,423 AML patients aged 50+ after myeloablative conditioning (MAC) and RIC. In a long-term 10-year follow-up, probabilities of OS and LFS were comparable in patients older and younger than 55. Results were also comparable with regard to intensity of the conditioning. Ten-year LFS was 31% and 32% after MAC and RIC. An advantage was observed with RIC compared to MAC in patients older than 55 who had 28% vs. 20% LFS probability respectively. Ten-year OS was also comparable, with 33% and 35% after MAC and RIC [42]. This study, along with other papers, has demonstrated lower risks of treatment-related mortality and graft-versus-host disease (GvHD) in patients receiving allo-HSCT after RIC, although the relapse incidence (RI) was higher [43]. Even though elderly AML patients are nearly universally transplanted after various RIC regimens, there is little data from prospective investigations. One such study from the Cancer and Leukemia Group B included patients aged 60–74 who received RIC consisting of 6.4 mg/kg total dose busulfan, fludarabine with or without anti-thymocyte globulin. Two-year probabilities of LFS and OS in the entire group were 42% and 48% respectively [44]. Similar efficacy was reported with other RIC regimens combining melphalan at doses of 100 mg/m^2, 140 mg/m^2 or 180 mg/m^2 with fludarabine or cladribine. In patients with high-risk AML or MDS whose median age was 55, such conditioning was efficacious even in those who entered transplants without CR. Two-year OS was achieved in 40% and 23% of patients with active disease and circulating blasts, respectively [45].

In another study, 36 patients at median age 57 received similar conditioning while in CR. Long-term follow-up of the entire cohort revealed 71% and 68% probabilities of OS and LFS, respectively [46]. A meta-analysis of seven studies with a total of 1,861 patients compared RIC regimens combining fludarabine with either 6.4 mg/kg busulfan (BuFlu) or 140 mg/m^2 melphalan (FluMel). The results

in AML and MDS significantly favored FluMel over BuFlu in terms of OS [hazard ratio (HR) 0.83]. The risk of clinically significant acute GvHD was lower after BuFlu (HR 0.71) as was the risk of NRM, even though the difference was not statistically significant in AML patients (HR 0.86). The risk of chronic GvHD was similar after the two regimens [47]. Among alkylating agents, treosulfan is recognized as myeloablative with a favorable toxicity profile, hence its use for reduced intensity conditioning in older patients. A meta-analysis determined long-term outcomes of busulfan vs. treosulfan based conditioning in AML and MDS patients. No significant differences were found for relapse, NRM, LFS and chronic GvHD. Treosulfan conditioning resulted in significantly decreased incidence of acute GvHD (HR 0.7) and improved OS (HR 0.8) [48]. Strictly non-myeloablative conditioning is frequently based on low-dose 2 gray total body irradiation combined with fludarabine (FluTBI). This modality was retrospectively compared to RIC BuFlu regimen in 1,088 AML patients in first CR aged 60+ reported to the EBMT. The results in this large group of elderly patients were nearly identical in terms of OS, LFS, NRM and risk of relapse. Patients who received FluTBI had a significantly higher risk of developing chronic GvHD, particularly when transplanted from unrelated donors [49].

Transplant versus non-transplant approach

The decision as to whether to refer a patient to allo-HSCT is based on meticulous assessment of the risk and benefit ratio. ELN guidelines recommend transplantation in high-risk AML patients or whenever the risk of relapse estimated on factors present at diagnosis exceeds 30% or 40% [50]. Additionally, in those who never achieve CR on treatment, or who have detectable MRD, allo-HSCT is the only potentially curative option, providing that patients are fit enough to withstand the procedure.

There is no doubt that fit elderly patients with AML who respond to therapy should be considered as potential candidates for allo-HSCT. Several retrospective studies have compared outcomes of allo-HSCT with chemotherapy as post-remission treatment. A registry-based study from the CIBMTR compared allo-HSCT in 190 patients aged 60 to 70 to those who received chemotherapy only. At 3 years, LFS was significantly improved in the transplant group (32% vs. 15%), and less relapse was noted (32% vs. 81%), at a cost of increased NRM (36% vs. 4%) with a trend towards increased OS (37% vs. 27%) [51]. A similar single center study from Japan compared 152 patients older than 50 (range 50–70) who received allo-HSCT to 880 patients in the same age range who received chemotherapy. Again, at 3 years RI was lower after transplantation (22% vs. 62%) with higher TRM (21% vs. 3%) but with statistically better both LFS (56% vs. 29%) and OS (62% vs. 51%) [52]. A multicenter Dutch-Belgian-Swiss study consortium (HOVON-SAKK) conducted a prospective trial in 640 AML patients aged 60+ who achieved remission after induction treatment. Ninety-seven patients proceeded to allo-HSCT with RIC. A time-dependent analysis was performed in which transplantation was compared to other post-remission treatment modalities. The results showed that patients after allo-HSCT had significantly higher probability of OS at five years than those after chemotherapy (35% vs. 26%), especially in intermediate and adverse risk groups [53]. A nationwide study from Denmark studied 1,031 AML patients who achieved CR with chemotherapy. Of those, 196 received allo-HSCT in first remission. Allo-HSCT was studied as a time-dependent co-variate and was associated with significantly superior OS compared to chemotherapy in cytogenetically intermediate- and high-risk patients. The positive effect of allo-HSCT was especially pronounced in patients aged 60+ (HR 0.42) [54].

Finally, a recent single-center study from the Netherlands confirmed survival benefit with allo-HSCT in elderly patients. Three-hundred and fifty-five individuals aged 60+ were included in the analysis. Of those, 68 proceeded to transplantation. Median OS for transplanted patients was 68 months compared to eight months for those who did not proceed. In patients who achieved CR with either intensive chemotherapy or demethylating agents, median OS after allo-HSCT was not reached vs. 25 months in those CR patients who were not consolidated with transplant. Of interest, the type of therapy that led to CR had no influence on survival post-allo-HSCT [55].

Maintenance therapy

Relapse of the original disease remains the major cause of allo-HSCT failure. The greatest risk for disease recurrence is observed in the first year after transplantation. Much interest is currently paid to possible prophylactic therapy in patients after transplant who have a significant risk of relapse. The idea of effective maintenance is particularly appealing after reduced intensity conditioning and in elderly patients. Today, only two therapies in acute leukemia after allo-HSCT are nearly universally recognized. One is dasatinib in BCR-ABL-positive acute lymphoblastic leukemia, and the other is multi kinase inhibitor sorafenib in FLT3-ITD-positive AML [56, 57]. No other tested maintenance therapy has yet emerged as standard. Initial interest focused on the hypomethylating agents azacitidine and decitabine. Small studies indicated a possibly beneficial effect of such maintenance, but a large randomized phase III trial showed contradictory results [58, 59]. Recently, the outcomes of a prospective trial with azacitidine administered as MRD guided preemptive treatment were published. In patients aged 52–69 who became MRD positive after allo-HSCT, azacitidine therapy resulted in 46% relapse-free survival at 12 months [60]. In a small phase I/II trial with oral azacitidine in high-risk AML or MDS patients, RI at 12 months was 21% with acceptable toxicity of maintenance

[61]. Apart from sorafenib, other FLT3 inhibitors are under investigation in clinical trials. In a phase III randomized trial, midostaurin provided 89% RFS at 18 months vs. 76% in non-maintenance FLT3-ITD+ patients (p =0.27) [62]. Also, a gilteritinib vs. placebo trial in FLT3-ITD+ patients is underway, although accrual is slow due to common usage of sorafenib for maintenance [63].

Of other drugs, venetoclax alone or in combination with azacitidine has been evaluated in high-risk AML and MDS patients. Preliminary results of a small single center study in elderly patients with median age 65 indicate an 87% probability of 6-month survival after allo-HSCT on venetoclax maintenance [64]. Early phase clinical trials with other targeted compounds such as isocitrate dehydrogenase, histone deacetylase and hedgehog inhibitors are being conducted, but so far very little information on their progress or results is available.

Summary and conclusions

Allogeneic stem cell transplantation in elderly AML patients has become the standard of care with improving outcomes over the years. To take full advantage of the curative potential of the procedure, careful individual estimation of the risk-benefit ratio is necessary.

Apart from routine pre-transplant evaluation, elderly patients require a specific geriatric assessment to avoid excessive non-relapse mortality. Treatment in transplant candidates should aim at achieving complete remission to maximize survival probability, but in some patients with a partial response survival benefit with allo-HSCT can be achieved. In most cases, patients aged 55+ will benefit from reduced intensity or reduced toxicity conditioning before transplantation, although in carefully selected patients a standard myeloablative conditioning may be considered. The best donors for elderly patients are either siblings or well-matched unrelated donors, because transplantation from alternative donors yields significantly inferior results.

Various post-transplant maintenance modalities are under investigation, and at present one of them, namely sorafenib, has become the standard of care for FLT3-ITD+ AML. There is growing evidence that allogeneic transplantations are feasible in older patients, with improving results over the last decade. Several studies confirm that in elderly patients, allo-HSCT as post-remission treatment prolongs survival compared to standard therapy. However, we must keep in mind that most of these studies are retrospective with a selection bias and that the majority of newly diagnosed elderly AML patients are still, at best, being offered palliative treatment.

Author's contributions

KH — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Allogeneic hematopoietic stem cell transplantation for paroxysmal nocturnal hemoglobinuria in the era of complement inhibition

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Abstract

The only potentially curative treatment for paroxysmal nocturnal hemoglobinuria (PNH) is allogeneic hematopoietic stem cell transplantation (allo-HSCT).

However, its use has been largely abandoned following the introduction of efficient symptomatic treatment with complement inhibition. Nevertheless, the population of PNH patients is diverse, and some of them might still gain advantage from allo-HSCT, while anti-complement treatment would be the first choice for others. Both treatment modalities may be also sequentially applied in the same patient when needed.

This review aimed to present the current status of allo-HSCT in the treatment of patients with PNH, with special reference to Poland where the previous unavailability of anti-complement therapy enabled the acquisition of extensive experience in performing allo-HSCT for PNH, a treatment option currently restricted only to selected patients who are not candidates for eculizumab.

Key words: paroxysmal nocturnal hemoglobinuria, allogeneic hematopoietic stem cell transplantation, eculizumab

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Paroxysmal nocturnal hemoglobinuria (PNH) originates from acquired *PIGA* gene somatic mutation in hematopoietic stem cells, leading to deficiency in membrane proteins requiring glycosyl phosphatidyl inositol anchor [1]. The absence of these proteins, most importantly including natural complement inhibitors CD55 and CD59, is responsible for complement-mediated intravascular hemolysis, leading in classical hemolytic PNH to a wide spectrum of clinical symptoms mainly related to the presence of free hemoglobin, nitric oxide scavenging, and the increased occurrence of thrombosis. Patients with classical PNH nowadays are treated with a terminal complement protein C5 inhibitor, the monoclonal antibody eculizumab, which has been proved to be highly effective in reducing PNH-related morbidity (hemolytic anemia and thrombosis) and mortality [2]. Progress in anti-complement treatment has been achieved by substantial prolongation of half-life of a newer anti-C5 monoclonal antibody, ravulizumab, which instead of twice a month is given once every two months. However, access to this drug is restricted [3, 4]. Further progress in complement inhibition aims to overcome C5 polymorphisms responsible for resistance to eculizumab, and to reduce breakthrough episodes of hemolysis and extravascular hemolysis resulting from complement C3 opsonization. Moreover, other new monoclonal antibodies and complement inhibitors are currently in development, e.g. C1 esterase inhibitor [5], C3 inhibitors [6], and factor D inhibitors [7]. It is highly likely that they will improve response rates as well as quality of life. However, they inhibit the immune system which makes the organism more prone

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to infectious complications [8]. Also, once started, they cannot be withdrawn and patients require life-long treatment with these drugs, which may negatively influence their quality of life. There are also reports indicating that only a minority of patients obtain complete, or at least major, hematological response [9, 10]. The main limitation of C5 inhibitors treatment however is that they are not curative, and they do not correct the underlying stem cell defect [11].

Therefore, despite the success of complement inhibitors in hemolytic PNH, there is still a continued need for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in bone marrow failure-associated PNH, in which the presence of PNH clone overlaps with aplastic anemia, myelodysplastic syndrome, or both. PNH clone in this subtype is usually smaller than in classical PNH, however over the last decade progress has been achieved also in PNH diagnosis [12] through the introduction of high sensitivity flow cytometry which enables the detection of even very small PNH clones [13].

Allo-HSCT is indicated for PNH patients with very severe or severe aplastic anemia, high- or higherintermediate-risk myelodysplastic syndrome, or patients with severe hemolysis or thrombosis unresponsive to eculizumab [14], or those without access to it.

Allo-HSCT can cure the disease thanks to cytotoxicity of conditioning treatment and immunoreactivity of donor T-cells, leading to eradication of the PNH clone [15]. Allo-HSCT can be proposed after complement blocker therapy in the absence of alternative treatment and after careful assessment of the risk-benefit ratio, especially in transfused patients. Eculizumab did not change the risk of HSCT complications in PNH patients who sequentially received both treatment options. The optimal timing for the last eculizumab infusion before transplantation seems to be during the conditioning regimen [16]. Bridging therapy with eculizumab prior to allo-HSCT is safe, and does not negatively influence the engraftment of hematopoietic stem cells [17].

Identifying patients with PNH who may benefit from allo-HSCT is challenging. The low incidence of PNH, and the treatment of most patients with eculizumab, makes it practically impossible to conduct a randomized prospective trial. Thus, outcomes of allo-HSCT in PNH are generally obtained from observational studies and retrospective activity reports. Most of these have been based on low numbers of patients, except for a few registry or multicenter group studies.

The first large study was reported in 1999 from the International Bone Marrow Transplant Registry, which presented the results of 57 consecutive allo-HSCTs performed between 1978 and 1995. The two-year overall survival rate was 56%. The most common causes of treatment failure were graft failure and infection. Acute and chronic graft-versus-host disease (GvHD) occurred in 34% and 33%, respectively. Sustained engraftment was observed in 77% of patients [18].

Another long-term study of allo-HSCT in PNH was reported in 2010 by an Italian group. This included 26 patients transplanted between 1988 and 2006. Fifteen patients received myeloablative, and 11 were given reduced intensity, conditioning. Graft failure was 8%, and transplant-related mortality was 42% (26% and 63% following myeloablative or reduced intensity conditioning, respectively). The 10-year probability of disease-free survival was 57% for all patients, with better results after transplants from an identical donor (65%, 23 patients) and with myeloablative conditioning (73%, 15 patients) [19].

The largest study was reported in 2012 from European Society for Blood and Marrow Transplantation (EBMT): a retrospective analysis of 212 patients with PNH transplanted in 83 EBMT centers from 1978 to 2007 who were compared to 402 non-transplanted patients diagnosed during 55 years in French centers, and who were not treated with eculizumab. The overall survival at 5 years was 68% for the entire transplanted group. Overall mortality reached 30%, with an unacceptably higher risk of mortality in patients with a pre-transplant thrombosis history. Worse survival with allo-HSCT was reported in patients with thromboembolism (OS =54%, hazard ratio =10.0; p =0.007), but not in patients with aplastic anemia or with recurrent hemolytic anemia without thromboembolism (OS =69% and 86%, respectively) [20].

The obtained results have improved significantly in newer reports, which was recently confirmed by the Polish PALG group in a retrospective analysis of 78 patients, 27 with classical PNH and 51 with bone-marrow-failure-associated PNH (BMF/PNH), who underwent allo-HSCT in 11 Polish centers between 2002 and 2016, when eculizumab was not yet reimbursed in Poland. Treosulfan-based reduced toxicity conditioning was used in 66% of patients, classic myeloablative conditioning in 6%, and reduced intensity conditioning in 28%. Sustained engraftment was observed in 96% of patients. The 3-year overall survival for cPNH and BMF/PNH was 88.9% and 85.1%, respectively, and was highest in subgroups of patients with cPNH without thrombosis (92%) or with BMF/PNH with hemolysis (93.9%). Rate of acute GvHD II–IV was 23%; cumulative 1-year incidence of extensive chronic GvHD was 10.8% in BMF/PNH and 3.7% in cPNH [21].

In a retrospective analysis of 28 PNH patients, median age 28 (range 6–54) years, who received haplo-HSCT between 2010 and 2018 in China, despite one early failure due to septicemia, all evaluable patients achieved myeloid engraftment and complete chimerism. One secondary graft failure occurred, platelet recovery was delayed in three, and failed in one patient. Rate of acute GvHD II–IV was 14.82% and the cumulative incidence of moderate-severe chronic GvHD was 11.73%. The transplantation-related mortality

rate at 1 year was 15.25%, and the probability of 3-year overall survival was 84.8 ±7.1%. Haplo-HSCT has been recognized as a valuable option for PNH patients who lack HLA-matched donors [22].

The results of the presented registry or group studies, as well as of numerous single center reports not cited here, indicate that in patients with PNH who cannot be effectively treated with eculizumab for different reasons, allo-HSCT constitutes a valid therapeutic option with satisfactory overall survival and acceptable toxicity. These studies confirm that most patients with PNH can be definitively cured with allo-HSCT. The trends in allo-HSCT for PNH include the use of reduced toxicity conditioning to attain the graft versus PNH effect or the use of haploidentical donors. An interesting, although seldom implemented, approach involves the use of eculizumab immediately post-transplant as prophylaxis of thrombosis and hemolysis. This treatment was feasible and neither delayed engraftment nor increased infections [23]. Nevertheless, thrombosis or hemolysis were not reported as problematic post-transplant complications in the majority of other previously reported studies.

In summary, the indications for allo-HSCT in PNH have changed since the introduction of anti-complement therapy. Firstly, the risk of transplant-related mortality prevents the use of allo-HSCT as initial therapy in most patients with classical PNH, who can benefit more from complement inhibition, with exceptions in countries where the availability of eculizumab is a limiting factor. Allo-HSCT is a reasonable option for patients with classical PNH who do not respond well enough to eculizumab therapy.

Secondly, in patients with bone marrow failure (aplastic anemia or myelodysplastic syndrome)-associated PNH, allo-HSCT continues to be the preferred, and the only potentially curative, therapy.

Author's contributions

MM — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Autoimmune cytopenias complicating hematopoietic cell transplantation

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Abstract

Immune cytopenias after allogeneic hematopoietic cell transplantation are rare, albeit increasingly recognized, complications. Autoimmune diseases are serious complications of HCT and include immune-mediated cytopenias i.e. autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP) and autoimmune neutropenia. Severe cytopenia is usually defined by decreases of hemoglobin concentration below 7 g/dL, of platelet count below 20 G/L, or of absolute granulocyte count below 0.5 G/L, and it is mediated by the presence of auto-antibodies. ITP occurring in combination with AIHA is known as Evans Syndrome. Immune dysregulation is caused by impaired immune reconstitution and/or loss of self-tolerance. Primary risk factors of autoimmune cytopenias include: peripheral blood or cord blood as a stem cell source, unrelated HCT, non-malignant disease, use of alemtuzumab, acute/chronic graft-versus-host disease (GvHD), cytomegalovirus reactivation, infections, and, in pediatric settings, conditioning omitting total body irradiation. Diagnosis of autoimmune cytopenia is challenging due to a broad differential diagnosis: primary or secondary graft failure, infections, GvHD, disease relapse, drug-induced side effects, transplant-associated thrombotic microangiopathy, ABO-incompatibility, or disseminated intravascular coagulation. Treatment should be tailored to the individual patient, and ranges from watchful waiting to aggressive management in life-threatening situations. Apart from specific treatment adjusted for specific cytopenia, supportive care should include transfusions of leukocyte-reduced and irradiated red blood cell concentrates or pathogen-reduced platelet concentrates; treatment of infections and GvHD; modification of immunosuppression; and supplementation with microelements. Autoimmune cytopenias are usually highly resistant to standard therapy and are associated with increased risks of high morbidity and mortality, particularly when coexisting with other post-transplant complications.

Key words: autoimmune hemolytic anemia, AIHA, immune thrombocytopenia, ITP, autoimmune neutropenia, AIN

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Introduction

Definition

Post-transplant autoimmune cytopenia can occur as a single lineage disorder or in combination with other cell lines [1]. Autoimmune diseases are serious complications occurring after hematopoietic cell transplantation (HCT), including immune-mediated cytopenias: autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP) and autoimmune neutropenia (AIN) [2–5]. Severe cytopenia is usually defined by a decrease of hemoglobin concentration below 7 g/dL, of platelet count below 20 G/L, or of absolute granulocyte count below 0.5 G/L, and is mediated by the presence of auto-antibodies.

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ITP occurring in combination with AIHA is known as Evans syndrome.

Pathophysiology

These cytopenias largely develop due to immune dysregulation due to impaired immune reconstitution [6–8], loss of self-tolerance, inability of functional regulatory T cells (Tregs) to suppress auto-reactive T-cells and auto-reactive B-cells [9–12], and transfer of autoantibodies or autoreactive T-cells. It can be associated with graft-versus-host disease (GvHD) or infections as well as the use of drugs used in prophylaxis and/or treatment of these complications [4, 9, 10, 13].

Risk factors

Due to the heterogenous patient populations analyzed in various studies, risk factors of post-transplant autoimmune cytopenias are not yet fully established. Primary risk factors include: peripheral blood stem cells or cord blood as a stem cell source, unrelated HCT, non-malignant disease, use of alemtuzumab, acute and chronic GvHD, cytomegalovirus (CMV) reactivation, infections, and in pediatric settings, conditioning omitting total body irradiation (TBI) [9, 10, 13–18].

Principles of diagnosis

Initial clinical symptoms and signs are typical for developing cytopenia, including anemia or thrombocytopenia or neutropenia. Diagnostics is usually difficult and challenging. In differential diagnosis, cytopenia needs to be distinguished from primary or secondary graft failure, infections, GvHD, disease relapse, drug-induced side effects, transplant-associated thrombotic microangiopathy (TA-TMA), ABO-incompatibility, and disseminated intravascular coagulation (DIC) [1].

General principles of management

Autoimmune cytopenias are usually highly resistant to standard therapy and are associated with increased risks of high morbidity and mortality, particularly when coexisting with other post-transplant complications such as infections or relapse [5]. Nevertheless, the outcome of immune cytopenias is slowly improving over the calendar years. Supportive care includes transfusions of leukocyte-reduced and irradiated red blood cell (RBC) concentrates or pathogen-reduced platelet concentrates. Successful treatment of infections and GvHD might be helpful in maintaining cytopenia. Modification of immunosuppression has to be balanced against an increased risk of GvHD or relapse. In cases of deficiency, supplementation with vitamin B12, folate or iron is necessary. Prophylactic anticoagulation due to increased risk of thromboembolic events should be taken into account [19–21].

Autoimmune hemolytic anemia (AIHA)

The most frequent causes of hemolysis after allogeneic-hematopoietic cell transplantation (allo-HCT) are donor/recipient (D/R) ABO incompatibility, autoimmune hemolytic anemia (AIHA) and TA-TMA. Some diseases can increase the risk of hemolysis i.e. non-Hodgkin lymphoma, paroxysmal nocturnal hemogobinuria, and sickle-cell disease. Drugs used in conditioning (fludarabine), or in treatment of infections or GvHD, can induce hemolytic anemia [22]. AIHA after allo-HCT occurs in 1–5% of patients a median 5–10 months on from the day of transplant, and can be presented as warm (wAIHA) or cold AIHA (cAIHA) [22].

Diagnosis of AIHA

Symptoms and signs include fatigue, pallor, icterus, dyspnea, and circulatory symptoms (in cases of cAIHA). Laboratory tests show increased reticulocytes, spherocytes in wAIHA, agglutinated RBC in cAIHA (while there is an absence of schistocytes in cAIHA), increased lactate dehydrogenase (LDH) and bilirubin, and decreased haptoglobin. Immune hematological examinations include direct antiglobulin test (DAT, Coombs test), cold agglutinin testing, elution and adsorption techniques [23].

Diagnosis of subtypes of AIHA

In warm AIHA: IgG auto-antibody, mostly against common blood group antigens (Rh, Rhesus antigens), positive Coombs test (DAT) shows presence of IgG or IgG+C3 complement on the surface of the RBCs.

In cold AIHA: IgM auto-antibody against blood group i/I, and positive Coombs test (DAT) with the presence of complement on the surface of the RBCs.

In mixed AIHA, there is combined wAIHA and cAIHA.

In atypical AIHA, DAT (Coombs test) is IgA- or IgM-driven, although this may be negative (Table I) [14].

Differential diagnosis

The following pathologies should be included: ABO incompatibility, TA-TMA, acute or chronic GvHD, infections causing marrow suppression, drug-induced myelosuppression, drug-induced immunological distraction, graft failure and relapse of primary disease [24].

ABO incompatibility should be considered as a primary differential diagnosis of hemolysis after allo-HCT, as it occurs in about 30–50% of patients [23]. ABO incompatibility can be associated with acute hemolysis and pure red cell aplasia (PRCA) in cases of major ABO incompatibility, or with passenger lymphocyte syndrome (PLS) in cases of minor ABO incompatibility. This phenomenon is not related to donor human leukocyte antigens (HLA) match, as there is an independent heritance of ABO blood groups (chromosome 9) and HLA genes (chromosome 6). Diagnostic tests in cases of ABO incompatibility include DAT and titration

Table I. Diagnosis of autoimmune hemolytic anemia (AIHA)

	Warm AIHA (wAIHA)	Cold AIHA	Mixed AIHA	Atypical AIHA
Antibody specificity	IgG	IgM	IgG and IgM	IgA or IgM (in wAIHA)
DAT (monospecific DAT)	IgG ±C3	C3 ±IgM	IgG +C3 ±IgM	IgA or IgM, DAT negative

IgG — immunoglobulin G; IgM — immunoglobulin M [14]; DAT — direct antiglobulin test; C — complement

Table II. Differential diagnosis of post-transplant autoimmune-mediated [autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP)]

Differential diagnosis	ABO incompatibility	GvHD	Graft failure, relapse	Infections	TA-TMA
Symptoms and signs	Hemolysis; PRCA; PLS	Acute GvHD; chronic GvHD	Persistent cytopenia; symptoms of primary disease	Clinical symptoms and signs	Tissue microvascular injury (kidneys, lungs, brain, GI)
Diagnostics	DAT and elution techniques; isohemagglutinins; bone marrow	Clinical diagnosis; histology examination	CBC; chimerism; bone marrow	Microbiological testing, imaging	CBC (schistiocytes); platelets (↓); proteinuria; sC5b-5 (↑)

GvHD — graft-versus-host disease; TA-TMA — transplantation-associated thrombotic microangiopathy; PRCA — pure red cell aplasia; PLS — passenger lymphocyte syndrome; GI — gastro-intestinal; DAT — direct antiglobulin test; CBC — cell blood count

of isohemagglutinins. Bone marrow biopsy can be useful (Table II) according to Baur et al. [1].

TA-TMA is usually difficult to diagnose. Its incidence ranges between 10–35% after allo-HCT, and it is associated with chronic organ injury and high mortality. Thrombocytopenia and hypotension are typical early signs of TA-TMA. TA-TMA is a multi-system disorder of endothelial injury and organ damage (mainly kidneys, gastro-intestinal tract, and lungs) that can be triggered by chemotherapy, irradiation, immunosuppressive agents, GvHD and infections. Dysregulation or activation of the complement system, including complement gene variants, is another possible mechanism. Diagnostic confirmations include presence of schistiocytes in blood smear, thrombocytopenia, proteinuria, and increase of soluble sC5b-9 protein.

Treatment of AIHA

Treatment should be individualized depending on the disease course and underlying diagnosis, because of the higher relapse risk. In mild compensated forms of AIHA, close observation may be appropriate. However, AIHA after allo-HCT can be life-threatening and even fatal, and therefore in some patients early diagnosis and prompt intervention is mandatory. The principles of treatment derive from those of primary AIHA (Table III) [10, 14, 25–31].

Treatment of warm AIHA:

- In first line, steroids (prednisolone 1 mg/kg/day) combined with rituximab (375 mg/m^2/week) and intravenous immunoglobulins (2 g/kg) are used. The first-line treatment can be repeated in non-responding patients;
- In second line, additional options involve plasma-cell directed therapies including daratumumab (16 mg/kg/week) or bortezomib (1.3 mg/m^2/week) together with steroids and other immunosuppressive drugs, particularly in patients with malignant disease and high risk of relapse or transplanted patients in non-complete remission. Since response to rituximab, daratumumab or bortezomib may be achieved after several weeks, bridging with steroids is mandatory, although steroid tapering should be performed quickly;
- In third line, the first- and second-line options can be combined. Additionally, plasma exchange (TPE) can be considered. Due to a high risk of infectious and thrombotic complications, splenectomy is not an attractive option and should be delayed. New options include abatacept and sirolimus [9, 32].

Treatment of cold AIHA:

- First line treatment includes rituximab (375 mg/m^2/week) or rituximab combined with bendamustine (90 mg/m^2);
- For second line, eculizumab (600 mg/week) or bortezomib (1.3 mg/m^2/week) are proposed;
- In third line, TPE can be considered in severe cAIHA. It has an immediate but transient effect.

Transfusions of RBCs should be carried out if necessary. Antibodies in wAIHA are usually directed against common blood group antigens, and so excluding anti-RBC allo-antibodies is time-consuming. Even with this approach, cross-matching is usually positive. In cAIHA, transfusions should be applied warm [25, 27, 33]. Supportive care includes hydration, transfusions, supplementation with vitamin B_{12}, folate, iron, and avoidance of cold exposure. There is an increased risk of thromboembolic complications [14, 25–27, 29].

Table III. Treatment of autoimmune hemolytic anemia (AIHA) after allogeneic hematopoietic cell transplantation

Treatment	wAIHA	cAIHA
First-line	Steroids ±rituximab ±IVIG	Rituximab ±bendamustine
Second-line	Daratumumab*	Eculizumab*
	Bortezomib*	Bortezomib*
	Immunosuppressive drugs	
Third-line	Combination of first- and second-line; therapeutic plasma exchange; splenectomy	Therapeutic plasma exchange (immediate but transient effect)
Supportive treatment	Transfusions (leukocyte-reduced and irradiated red cell concentrates) [sufficient to reach 6–8 g/dL]; hydration; folate	Avoid cold exposure; hydration; transfusions (leukocyte-reduced and irradiated red cell concentrates) (recommended: warmed transfusions and infusions); folate

*Off-label use [10, 14, 25–31]; wAIHA — warm AIHA; cAIHA — cold AIHA; IVIG — intravenous immunoglobulins

Immune thrombocytopenia

Diagnosis

ITP after allo-HCT occurs in 0.5–2% of patients. It is usually a diagnosis of exclusion. Typical symptoms and signs include bleeding. Laboratory tests usually show isolated thrombocytopenia, absence of schistocytes in blood smear, and increased LDH. Tests for infectious causes should include CMV, Epstein-Bárr virus (EBV), human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Coagulation tests are necessary if microangiopathy is suspected. Testing for antibodies against (human platelet antigen (HPA) and HLA are indicated only in selected patients [34].

Differential diagnosis

Differential diagnosis is similar to AIHA: graft failure, infections, GVHD, relapse, TA-TMA, and drug-induced side-effects (Table II). Additionally, DIC should be used in differential diagnosis of bleeding or/and thrombotic complications, although DIC is always secondary to the underlying condition, such as severe infections, malignancies or trauma.

Treatment of ITP

Essentially, treatment is based on that in primary immune thrombocytopenia. Treatment is aimed at preventing bleeding. A watch-and-wait strategy is advised in patients without severe thrombocytopenia.

- In first line, steroids, either prednisolone or dexamethasone, together with immunoglobulins and rituximab, are used (Table IV) [10, 34–37];
- In second line, thrombopoietin receptor agonists (eltrombopag or romiplostim);
- In third line, the first- and second-line treatments can be combined. The use of daratumumab is a new option. Splenectomy is a last resort and should be delayed as far as possible due to increased risk of infections and thrombotic complications.

Supportive measures include platelet transfusions in life-threatening bleeding, and tranexamic acid. There is no platelet threshold value for treatment, and this can vary depending on age, comorbidities and other drugs used including anticoagulants [34].

Autoimmune neutropenia

Definition

AIN can be seen as an isolated phenomenon, or in association with autoimmune diseases, or as a secondary manifestation of infections, drugs, or malignancies. As a primary disease, it occurs most frequently in infants and young children, and is a relatively benign disorder. It can range from mild neutropenia [absolute neutrophil count (ANC); <1.0 G/L] to severe (ANC <0.5 G/L) and very severe or agranulocytosis (ANC <0.2 G/L) [5]. Monocytosis is common. Cell destruction is usually extravascular.

Diagnosis

Infections and fever feature in a typical clinical presentation. Laboratory work-up includes cell blood count and blood smear and possibly testing of antibodies with specificity of human neutrophil antigen (HNA). DAT usually has no practical value in the diagnosis of AIN [38].

Differential diagnosis

Differential diagnosis in a transplant setting is similar to AIHA and ITP: drugs, disease relapse, infections, graft failure, and GvHD.

Treatment

Granulocyte colony-stimulating factor should always be the first line of therapy, with steroids and/or IVIG as the second-line approach (Table V).

Table IV. Treatment of immune thrombocytopenia (ITP) after allogeneic hematopoietic cell transplantation

Treatment	ITP
First-line	Steroids (prednisolone or dexamethasone) ±IVIG ±rituximab*
Second-line	Thrombopoietin receptor agonist (eltrombopag, romiplostim)
Third-line	Combinations (first- and second-line); immunosuppressive drugs; daratumumab; splenectomy
Supportive treatment	Transfusions (irradiated or pathogen-reduced platelet concentrates); tranexamic acid

*Off-label use [10, 34–37]; IVIG — intravenous immunoglobulins

Table V. Treatment of autoimmune neutropenia (AIN) after allogeneic hematopoietic cell transplantation

Treatment	AIN
First-line	G-CSF
Second-line	Steroids ±IVIG; possibly rituximab
Third-line	
Supportive treatment	Antimicrobial prophylaxis; treatment of infections

G-CSF — granulocyte colony-stimulating factor; IVIG — intravenous immunoglobulins

Authors' contributions

All authors contributed to design of study, writing of manuscript, critical review, and final approval.

Conflict of interest

All authors have nothing to disclose with respect to this paper.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Implementation of CAR-T technology into clinical practice: challenge for cell bank

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Abstract

This publication is based on experience gained during the accreditation and certification process of the first Polish center (Department of Hematology and Bone Marrow Transplantation in Poznan University of Medical Sciences) to perform the procedure of treating a patient with chimeric antigen receptor T therapy.

It focuses on the functioning of the quality assurance system in the cell bank both on a general and a detailed level, concerning in particular the processing of the autologous lymphocyte product by the cell bank, i.e. its preparation for further processing steps by the manufacturer of the marketing authorization: advanced therapy medicinal product/advanced therapy investigational medicinal product (MA-ATMP/ATIMP). It also provides practical guidelines to help other cell banks to successfully meet national requirements expressed by the accreditation of the Ministry of Health for the processing and release for circulation of autologous lymphocyte product and the certification pathway of the MA-ATMP/ATIMP manufacturer (companies: Kite/Gilead, Novartis and Janssen).

Key words: chimeric antigen receptor, CAR-T, advanced cellular therapy

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Introduction

Research on chimeric antigen receptor, primarily targeting T cells (CAR-T), has led to a revolution in the treatment of patients with relapsed/refractory B-cell hematological malignancies. The first CAR-T drug used worldwide was tisagenlecleucel (Kymriah®, Novartis). Treatment of patients with diffuse large B-cell lymphoma with this therapy resulted in an overall response rate of 52% and over 80% for patients with B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma. In a study using axicarbtagene ciloleucel (Yescarta®, Kite/Gilead), an overall response was achieved in 82% of patients [1].

The Department of Hematology and Bone Marrow Transplantation at Poznan University of Medical Sciences has over 30 years of experience in the field of cellular therapies. From the early 1990s until 2019, hematopoietic stem cell transplantation procedures and related activities (e.g. donor lymphocyte infusions) were the main focus of this therapy. For all of these highly specialized procedures, each cell product was processed by a single processing unit. Since 2012, this unit has been accredited by Poland's Ministry of Health as a Stem Cell Bank (SCB). It prepares cells for various cell therapies according to a quality assurance system (QAS). Since 2012, the SCB has worked under the ISO 9001 quality management system certification. The procedures for processing hematopoietic cells and donor lymphocytes at the SCB are overwhelmingly derived from practical experience based on activities within the Hematology Diagnostic Laboratory.

Over the past 30 years, the profile of cell therapy, in terms of stem cell transplantation performed by Transplant

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Centers (TC) around the world and by the Department of Hematology and Bone Marrow Transplantation of Poznan University of Medical Sciences has changed. At the same time, the range of procedures performed in SCBs has expanded. Initially, only a few preparations were made, mainly of autologous hematopoietic cells. Currently, most of the procedures in our TC are allogeneic stem cell transplants from unrelated, haploidentical and family donors. The specimens prepared at SCB allow us to treat over 130 patients per year at our center. Additionally, approximately 30 donor lymphocyte infusions are performed annually at the TC in Poznan. This activity is extended to the collection, processing and distribution of cells for over 180 patients treated at other TCs.

In 2019, the first collection of autologous lymphocytes for CAR-T therapy was performed in our unit [2]. SCB now has experience of processing and releasing 13 autologous lymphocyte products. For all of the above cell therapy activities performed at our and other TCs for hematopoietic stem cell transplantation procedures, donor lymphocyte infusions, and CAR-T therapy, more than 800 cell products are prepared annually at SCB.

QAS in a cell bank

In Poland, the procedures for cell processing (steps such as collection, storage, quantitative and qualitative assessment, processing) and distribution/release for circulation are strictly regulated by the Transplantation Act (TA), which is based on European Union (EU) directives (European Parliament Directive 2004/23/EC, Commission Directive (EU) 2015/565 and Commission Directive (EU) 2015/566) [3]. According to the TA, all these procedures can only be performed by a cell bank. Accreditation by the Ministry of Health, preceded by a positive decision of the Transplantation Council and a positive opinion from the National Center for Tissue and Cell Banking (NCTCB) is required for each type of cells and for each process related to their processing by this entity. This requirement also applies to the individual stages of processing autologous lymphocyte preparations. A very important element of cell bank operations is the implementation and subsequent maintenance and improvement of QAS, i.e. the organizational structure of procedures and processes affecting the achievement and maintenance of high quality of cells processed in the cell bank. The QAS enables processes to be carried out in a reproducible and repeatable manner, thereby maintaining the traceability of cell product processing.

In the early 2000s, we witnessed another, perhaps breakthrough, milestone in Polish medicine. A patient's autologous lymphocytes were collected and then released for MA-ATMP production according to banking procedures. The cell product at each stage of MA-ATMP/ATIMP production carries the status 'for use by intended recipient only'. By genetically modifying the T-lymphocyte, these cells gain the ability to recognize and destroy cancer cells when administered to a patient. In other words, the cells obtained from the patient in the TC are properly prepared (including cryopreservation) and released for circulation by the cell bank to the MA-ATMP/ATIMP manufacturer. This product, as MA-ATMP or ATIMP, will then be returned to the hospital where it will be administered to the patient via the hospital pharmacy. The autologous lymphocyte product collected from the patient will undergo multiple processes at multiple sites. At each, it is important to follow QAS and maintain all processes to identify the product at each stage of preparation. The audit of Kite/Gilead, Novartis or Janssen concerns the control of compliance with both company requirements and those stated in EU regulations.

Our experience in the certification processes conducted by the above companies indicates that the QAS system in place at SCB, and its general elements such as e.g. training procedure, qualification of equipment and environments, and process validations, are common to the processing of hematopoietic cells for transplantation and autologous lymphocyte product to the company. Nevertheless, past experience with cellular therapy is not everything. All cell bank personnel involved in CAR-T procedures must complete the manufacturer's entire training program.

One of the most important points of cell bank certification is the procedure for reporting serious adverse events. According to this procedure, the audit analyzes the cell bank's experience with the adverse event and analyzes the corrective and preventive actions taken by the unit. Corrective actions allow the CB to minimize the consequences of serious adverse events. Preventive actions allow the CB to minimize the likelihood of a recurrence. The corrective and preventive actions need to be aligned with CAR-T therapy.

Implementation of new procedures in the unit's existing QAS requires its adaptation to the new requirements. The new procedures must be consistent with the unit's quality system. When creating a QAS for CAR-T procedures, there are some general and unique manufacturer requirements. One of these is the requirement for what is known as the 'four eyes' rule. According to this 'gold standard', all cell processing points must be performed by two people trained in banking procedures. It should be noted that this rule was already in place at SCB early on, even before the introduction of CAR-T therapy. Importantly, for most collaborators, each detailed step in the preparation of the product for release must be confirmed on the appropriate forms provided by the company, while also being documented in the bank's forms. An equally important criterion for the preparation of autologous lymphocyte product is to minimize the risk of cross-contamination. This involves the need to ensure working in a laminar flow chamber and cryopreservation of cells obtained from a single patient. Hence, given the bank's involvement in the processes of preparing cells for transplantation, especially

in the case of banks supplying large TCs with material for transplantation, proper equipment is extremely important. To a large extent, this requirement is ensured by the need to have dual, so-called critical, equipment which overlaps with the tools required for CAR-T procedures.

Receipt, coding, processing, quantitative and qualitative assessment, and release for circulation of autologous lymphocyte product for further processing

The draft QAS created for autologous lymphocyte preparations at the initial stage of the Ministry of Health accreditation process must include the manufacturer's requirements.

The main banking procedures for processing lymphocytes for CAR-T therapy are: product receipt, quantitative and qualitative assessment, coding, storage, cryopreservation, and release for circulation.

At SCB, an additional section of standard operating procedures dedicated to autologous lymphocyte product for CAR-T therapy has been implemented into the functioning quality system. The main procedure entitled: 'Storage, quantitative and qualitative assessment and release for circulation of autologous lymphocytes' is described in detail in the form of instructions. Due to differences in manufacturers' requirements for banking apheresis products, especially with regard to quantitative and qualitative product evaluation and release for circulation, very detailed instructions with the manufacturer's name were introduced. In addition, new forms were created for each autologous lymphocyte processing.

Receipt of autologous lymphocyte product

Procedures and requirements vary from company to company. There are several processing points for autologous lymphocyte products in the cell bank required by all of them. Upon receipt of the product, after the apheresis process, while adhering to the 'four eyes' rule, bank personnel validate the identity of the product. To meet traceability requirements, it is necessary to enter accurate data on the Receipt Form. Date, time, temperature and humidity values (actual, minimum and maximum) must be recorded at each stage of cell processing in the cell bank. All measuring devices at each stage of cell processing should allow for notification when the required limit range is exceeded. This function is crucial, especially for short- and long-term storage of autologous lymphocyte product at the correct temperature. All critical equipment and technical devices must be identified and qualified, regularly inspected and preventively maintained according to manufacturers' instructions.

The above solutions are critical to maintaining regulatory requirements. According to EU Directive 2006/86/EC: "Where equipment or materials affect critical processing or storage parameters, e.g. temperature (...), they must be identified and must be subject to appropriate monitoring, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that critical parameters are always maintained within acceptable limits. All equipment with a critical measurement function must be calibrated against an identifiable standard, if available" [4].

Coding and processing of autologous lymphocyte product

The key to ensuring that autologous cell product can be identified at every stage, from collection through processing, evaluation and storage, to release into circulation, is proper coding of the product.

A single European Code (SEC) is assigned to all material donated to the cell bank to ensure proper donor identification and traceability of all donated material and to provide information on the main characteristics and properties of the cells. A label is required to identify the autologous lymphocyte product. The label should include the following elements: patient's name and date of birth, product SEC code, date and time of collection and expiration (including time zone), and number of bags. Contact information with the name of the cell bank (the entity responsible for release stage) and the manufacturer's information is also essential. Other essential elements of the label are: the statements "for use by intended recipient only", "cells for human use", "biohazard", "do not irradiate" and (for fresh product) "do not freeze".

Autologous lymphocyte product must be placed in a cell bank with policies in place to prevent mixing and cross-contamination with other cell therapy products. To prevent cross-contamination, only one patient's material can be processed at any one time in a chamber with laminar flow of sterile air.

Quantitative and qualitative assessment of autologous lymphocyte product

The quantitative and qualitative evaluation of autologous lymphocyte product varies among MA-ATMP/ATIMP manufacturers. According to the MA-ATMP manufacturer's requirements, the evaluation of nuclear and CD3+ cell counts, cell viability, and microbiological evaluation of the product need only be performed once. Knowing the number of nuclear cells in the product is particularly important for the cryopreservation process. It affects how the cells are processed (e.g. volume reduction) and the appropriate partitioning of the product.

Our cell bank asked the manufacturer (ATIMP) to perform a quantitative and qualitative assessment of the product. According to its procedures, product evaluation in the cell bank is not required. However, analysis of the product in the cell bank rationalizes the decision path for

the number of cell apheresis needed by the CAR-T manufacturer and is useful in organizing the TC work schedule. The aforementioned product qualification criteria are also necessary to meet the QAS requirements of the cell bank, i.e. traceability requirements.

Release for circulation of autologous lymphocyte product

Another case of equal banking procedures for autologous lymphocyte product concerns the release process. Despite differences in manufacturers' procedures at the release stage, there is one single rule required by the TA. According to Article 37a(3) of the TA, any release of autologous lymphocyte product for a manufacturing facility located in another country requires NCTCB approval. Additionally, the cell bank must indicate the status of the product after cell manipulation (MA-ATMP or ATIMP) by submitting an application to the NCTCB. Much of the procedure for qualifying autologous lymphocytes for CAR-T therapy is based on our experience in processing hematopoietic stem cells for distribution to other cell banks. One of these procedures is cryopreservation and storage of a sample of the released product in the cell bank. Such a procedure can be very useful if, for example, discrepancies are found at different stages of product preparation and patient treatment.

Cryopreservation and storage of autologous lymphocyte product for further processing

Processing the cell therapy product according to the rules, and storing it under correct temperature conditions, allows the preservation of cell parameters even for many years. The experience of cell bank personnel who process autologous hematopoietic stem cells for transplantation provides the know-how of the cryopreservation process. This experience may be useful in the preparation of the CAR-T product. According to standard cell therapy guidelines, the cell therapy product should contain less than 2×10^8 nuclear cells/mL [5]. The final cell concentration affects the cell status expressed as cell viability after thawing the product. Too high a concentration of cells may cause the cells to clump together. On the other hand, too low a cell concentration may result in an inability to evaluate the product. To cryopreserve cells, it is necessary to prepare a cryoprotectant medium designed for cell preservation. This medium must contain appropriate protective proteins and DMSO at 10% final concentration [6]. It is important that the autologous lymphocyte preparation be cryopreserved in a bag and DMSO approved by the MA-ATMP manufacturer.

As a standard step in processing a cell therapy product with a cryopreservation step, it is necessary to collect several vials with a sample of autologous lymphocyte preparation, used to evaluate the product after the cryopreservation process. The vials must be frozen and stored with the cell product for CAR-T therapy. To avoid the risk of cross-contamination, only one patient's material and samples may be cryopreserved in the cryopreservation chamber at a time. It is essential that the cryopreservation of the material takes place in a programmed system in a chamber with automatic dispensing of liquid nitrogen vapor, according to the cryopreservation line. The cryopreservation line must be approved by the MA-ATMP manufacturer. The cryopreservation process must be monitored by the system. The duration of cryopreservation of the product must allow the product to reach temperatures below –80°C. Product so cryopreserved must be stored with the vials in liquid nitrogen auto-dispensing tanks, in a rack, in liquid nitrogen vapor phase. Cryopreserved cell therapy product must be stored in liquid nitrogen vapor for at least several hours before release to the MA-ATMP manufacturer. For safe storage of cryopreserved autologous lymphocyte products, liquid nitrogen tank mapping is critical. This qualification process helps to ensure the required temperature for the product throughout storage, even if the tank is opened, e.g. to remove another product.

As is evident in the QAS section of this manuscript, storage of the cryopreserved product must be constantly monitored, including an alarm system in the event of adverse temperature changes.

Summary

Cell bank experience is essential to the introduction of CAR-T therapy, but adaptation of QAS to conduct this therapy is indispensable. The tremendous success of implementing biotechnology solutions into clinical practice in the area of advanced cellular therapies, broadly defined, will likely result in the need to implement further procedures in cell bank practice.

Cell therapies are likely to go beyond the treatment of hematological diseases. Therefore, there will be a need to convert stem cell banks into cell therapy laboratories where material will be developed for a wide range of patients.

Authors' contributions

EB — sole author.

Conflicts of interest

None.

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Ethics

The work described in this article was performed in accordance with the World Medical Association Code of Ethics

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REVIEW ARTICLE

Leukemic stem cells: clone wars

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Abstract

Leukemic stem cells arise as the effect of mutations of normal hematopoietic cells and overgrow normal hematopoietic tissue. They may also infiltrate other organs. While they begin their life from mutations, they continue to mutate, creating daughter leukemic stem cells that harbor two, three, or more mutations, and these mutations can be different in different daughter stem cells of the same parental line in the same individual. These daughter stem cells then compete between themselves as to which one will overgrow the host tissues with its progeny, and finally will contribute to the host's death. This process can be shaped by therapy, which may preferentially eliminate some subclones and simultaneously favor others. To eliminate such stem cells, therapy is needed that will preferentially attack their self-renewal.

Key words: stem cells, self-renewal, oncogenic mutations

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Introduction

Understanding that leukemias are genetic diseases, and subsequently that they are clonal diseases i.e. they originate from a single mutated cell, has paved the way to the elaboration of a more detailed scenario of the development and course of this group of disorders.

The main question facing researchers was: what properties must a cell acquire to become the initiating cell of leukemia? Firstly, it has to have unlimited self-renewal potential, because otherwise its progeny will sooner or later become exhausted and self-eliminate, which does not occur. As the second necessary property, it has to have either a proliferation or a survival advantage over normal hematopoietic stem cells. In other words, it has to be able to successfully compete with normal cells for the limited space in the host body during subsequent generations. Otherwise, it might survive somewhere hidden but would be overgrown by normal cells, and the visible disease would not develop. As the third property, it has to acquire the capacity to omit, escape, resist or disregard host mechanisms that can put restrictions on its expansion.

Pathway of discoveries

The original paradigm of leukemia development was based on the example of chronic myelocytic leukemia where a single genetic change: translocation 9;22 was identified [1]. Attention was focused on the role of abnormally activated *ABL* gene transferred from chromosome 9 to 22, and fused to *BCR* gene [2]. *ABL* gene was earlier identified as associated with leukemia development in mice after infection with the Abelson Leukemia virus carrying this gene [3]. While viruses do not play a significant role in initiating human leukemia, their role in leukemia development in birds and other mammals was instrumental in allowing discoveries of the first oncogenes. Of note, the first mammalian oncoviruses were discovered in the 1950s by Ludwik Gross [4], a Polish-Jewish virologist who escaped to the United States in 1940. However, it soon became clear after elaborating on chronic myelocytic leukemia that such a single genetic change is insufficient to produce a more aggressive malignancy such as acute leukemia.

Then the 'two-hit' theory was proposed by Alfred G. Knudson [5] who originally (based on studies of retinoblastoma) suggested that inactivation of two antioncogenes

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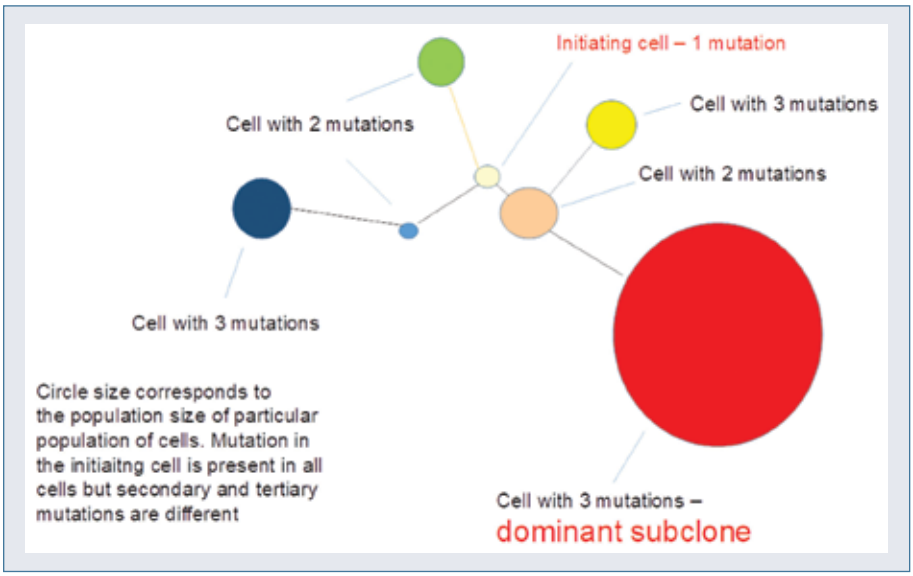

Figure 1. Hypothetical relative clonal composition of leukemic stem cell subclones. In this model, seven subclones compete but only one subclone with three mutations dominates and is clinically evident

on both chromosomes is necessary to allow neoplastic cell behavior. This theory was later modified to allow the co-occurrence of two events: activation of a protooncogene to produce an oncogene, and inactivation of an antioncogene [6].

Therefore, based on this theory, a leukemia-initiating cell should first undergo one mutation, expand, and then one of its progeny cells has to undergo a second mutation to cause further expansion of a subclone with two mutations to produce clinically visible disease. According to this theory, the original clone with one mutation that was outgrowing normal cells had to be finally overgrown by its subclone with two mutations.

This prompted vigorous research worldwide that has led to the identification of many genes mutated in various forms of leukemia. If we focus on acute myeloid leukemia, at least nine groups of genes have been identified that play a role in various forms of this group of disorders [7]. They are not all mutated in a single cell, but various compositions of mutations of these genes may produce clinically similar disorders. Furthermore, some of the genes whose mutations were initially identified in leukemias are mutated also in cells exhibiting normal behavior in subjects with completely normal blood counts.

This condition has been termed clonal hematopoiesis of indeterminate potential (CHIP) [8]. It is present in c.10% of healthy 70-year-old people, and has 1–2% yearly potential to develop into overt disease, either myelodysplastic syndrome or acute leukemia. Interestingly, genes whose mutations are responsible for CHIP which could correspond to the original 'first hit' are neither oncogenes nor antioncogenes, but usually are responsible for DNA methylation [9]. Altogether, this has expanded the 'two-hit' theory to become 'three-hit'.

The introduction of next-generation sequencing allowed the sequence of entire genomes of many subclones of the same leukemia in individual patients to be obtained. Firstly, this has led to our understanding that mutations occur much more frequently than originally anticipated, probably during each cell division. But most of them affect non-coding portions of the genome, or affect cellular functions that are irrelevant for hematopoietic cells [10]. However, they can modify the background on which leukemia-relevant mutations may occur. Consequently, the same leukemia-relevant mutations in cells with different background mutations can produce slightly different effects. It is, for instance, known that the same mutation in different strains of mice (different background) would produce different phenotypes [11].

Clone wars

Then, it was found that in fact in the same patient with leukemia not just two but more subclones of the original leukemic clone coexist, but the only visible one is the one with the best survival advantage (Figure 1). There is constant competition between various subclones. A subclone that once was dominating can be replaced by a new subclone that has acquired another mutation providing either a survival or a proliferation advantage [12–15]. This is additionally influenced by therapy. Depending on the mechanisms of action of a particular drug, different subclones may be eliminated or inhibited, and others may get a survival advantage.

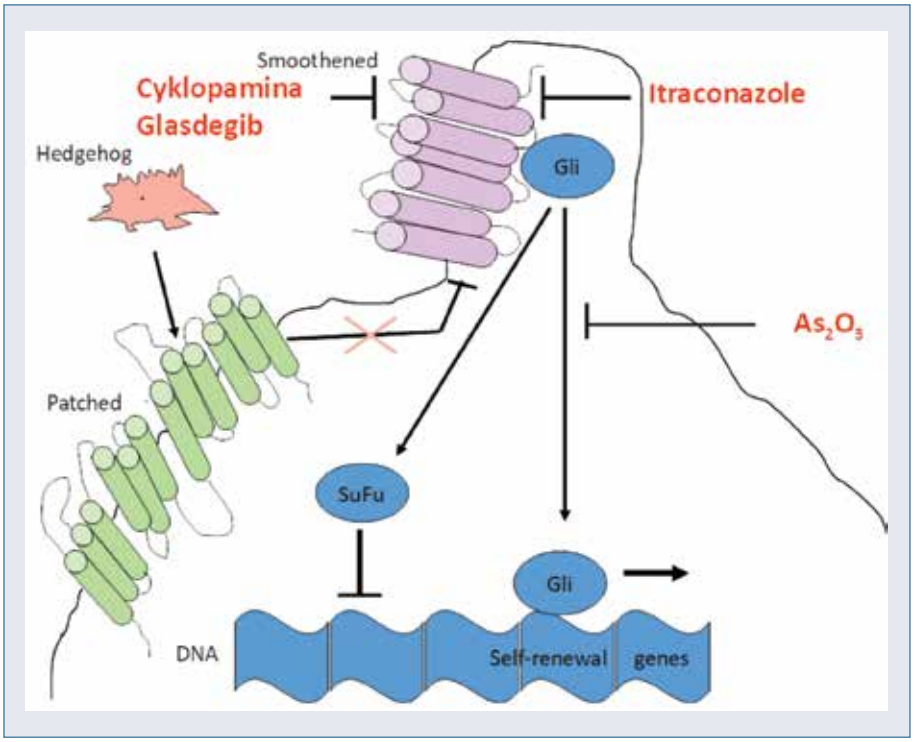

Figure 2. Some known possibilities of interference with self-renewal pathways in leukemic stem cells; Gli, and SuFu — positive and negative regulators of genes controlling self-renewal of stem cells, respectively; AS_2O_3 — arsenic trioxide

In order to operationally cure leukemia, the complete elimination of leukemic clones may not be necessary. Returning to the CHIP level could be sufficient in many cases to allow a patient to survive to his or her normal life expectancy.

Moreover, currently available therapies focus on mechanisms active relatively late in molecular machinery that allow leukemic stem cell expansion. Coming back to the first necessary property of leukemic stem cell that is self-renewal, new therapies should act on this level. Several self-renewal pathways have already been identified including Hedgehog, WNT, NOTCH, and BMP. There is evidence for the role of each of them in leukemic stem cells, but it is usually activation through indirect mechanisms and not by direct mutation. Nevertheless, some of the inhibitors of these pathways are in advanced stages of clinical trials in acute myeloid leukemia [16–19] and some are compounds already used in the clinic for other indications (Figure 2) [20].

Conclusion

Leukemia is a clonal disease in which various subclones of the original clone first outgrow normal hematopoietic cells and their progeny, and later compete between themselves until one of them wins the war and becomes resistant to therapy that will kill the host, thus committing suicide.

Author's contributions

WWJ — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Myeloid/lymphoid neoplasms with eosinophilia: clinical picture and therapeutic approaches

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Abstract

Myeloid/lymphoid neoplasms with eosinophilia (M/L_{eo}) and tyrosine kinase (TK) fusion genes constitute a separate category within the 2016 World Health Organization (WHO) classification. All these are characterized by blood or tissue eosinophilia and the presence of a unique genetic abnormality. M/L_{eo} may have diverse clinical manifestations with variable response to TK inhibitors (TKI). *PDGFRA*-rearranged neoplasms (usually with detectable *FIP1L1-PDGFRA*) are found to be extremely sensitive to low dose of imatinib (IM at 100 mg daily) with nearly 100% hematological complete response rate. Moreover, >90% of IM treated patients may achieve long-term molecular response. IM discontinuation may result in sustained remission in c.50–60% of patients. An excellent response to IM (but at 400 mg/day) was also demonstrated for patients with *PDGFRB* rearrangements, but trials on IM cessation were not attempted. The *FGFR1*-rearranged neoplasms are associated with an aggressive disease course and allogeneic stem cell transplantation (allo-SCT) is the only potentially curative approach. Participation in clinical trials should be recommended. Recently, pemigatinib was found to be effective in a proportion of *FGFR1*-rearranged individuals. An aggressive outcome with rapid blast transformation is also characteristic for the *JAK2*-rearranged neoplasms. These patients should be included in clinical trials or attempted with ruxolitinib or fedratinib as a 'bridge' to allo-SCT. A new category of neoplasms with eosinophilia and *FLT3* and *ABL1* rearrangements has not yet been incorporated into the WHO 2016 classification. The prognosis is poor with a tendency to evolve into resistant acute leukemia. The treatment includes TKI with known activity against *FLT3/ABL1* followed by allo-SCT.

Key words: myeloid, lymphoid, neoplasms, eosinophilia, *PDGFRA, PDGFRB, FGFR1, JAK2, FLT3, ABL1*, imatinib, treatment

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Introduction

Hypereosinophilic syndromes (HES) constitute a group of rare disorders presenting with blood or tissue hypereosinophilia (HE) associated with eosinophilia-attributable organ damage/dysfunction [1]. Rapid development in molecular findings within HES has led to the discovery of several dysregulated tyrosine kinase (TK) fusion genes which were then incorporated into the 2016 World Health Organization (WHO) classification of tumors. These neoplasms created a new category of 'myeloid/lymphoid neoplasms with eosinophilia and gene rearrangements of platelet derived growth factor receptor alpha/beta (*PDGFRA/B*), fibroblast growth factor receptor 1 (*FGFR1*) and with *PCM-JAK2*' [2] (Table I). Moreover, two novel rearrangements of *FLT3* and *ABL1* (both commonly partnered by *ETV6*) are still under investigation and have not yet been added to the WHO classification [3]. All these abovementioned entities may

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Table I. World Health Organization classification of myeloid/lymphoid neoplasms with eosinophilia (modified from [2])

Category	Presentation and characteristic findings
PDGFRA-rearranged neoplasm	1) CEL, MPN or AML, rarely T-LBL or myeloid sarcoma; eosinophilia common, but can be absent 2) Presence of *FIP1L1/PDGFRA* fusion gene (by FISH or RT-PCR) or with other fusions of *PDGFRA* gene
PDGFRB-rearranged neoplasm	1) CMML, MPN/MDS, MPN; monocytosis; eosinophilia is not invariably present 2) Presence of t(5;12) or a variant translocation or demonstration of an *ETV6-PDGFRB* fusion gene or other fusions of *PDGFRB* gene
FGFR1-rearranged neoplasm	1) MPN, MDS/MPN, AML or T-/B-cell LBL with eosinophilia (not invariably present) and/or neutrophilia and/or monocytosis and 2) Presence of t(8;13) or a variant translocation leading to *FGFR1* rearrangement
JAK2-rearranged neoplasm	1) MPN with eosinophilia (not invariably present), AML, T-cell LBL/ALL or MPAL 2) Presence of t(8;9) or a variant translocation leading to *JAK2* rearrangement

ALL — acute lymphoblastic leukemia; AML — acute myeloid leukemia; CEL — chronic eosinophilic leukemia; CMML — chronic myelomonocytic leukemia; FISH — fluorescence *in situ* hybridization; LBL — lymphoblastic lymphoma; MDS — myelodysplastic syndromes; MPAL — mixed phenotype acute leukemia; MPN — myeloproliferative neoplasms; RT-PCR — reverse transcriptase polymerase chain reaction

clinically present in the chronic phase, most commonly as chronic eosinophilic leukemia (CEL) or other myeloproliferative neoplasms (MPN) or at *de novo* blast phase with aggressive disease course and poor response to therapy (clinically as acute leukemia/lymphoma). Of interest, not all these neoplasms have prominent blood eosinophilia. The sensitivity to TK inhibitors (TKI) is variable; from remarkable responses in cases with *PDGFRA/B* rearrangements, to poor in the remaining neoplasms [4].

The characteristics of myeloid/lymphoid neoplasms with eosinophilia are set out in Table I.

PDGFRA-rearranged neoplasms

Epidemiology: male predominance, age 20–50 years, single cases reported in females and children. Incidence in developed countries: 10–20% in patients with unexplained HE. The most common partner gene of *PDGFRA* is *FIP1L1* (F/P); other fusions rarely detected.

Manifestation: skin involvement in 57%, spleen (52%), lungs (45%) and heart (35%).

Clinical presentation: CEL, acute myeloid leukemia (AML) with eosinophilia, T-cell lymphoblastic leukemia/lymphoma (T-cell ALL/LBL), extramedullary disease (EMD).

Typical findings in blood: HE, but normal eosinophil count can rarely be present. Blast cells rarely observed, but may occur. Marked elevation of serum vitamin B_{12} and tryptase is common.

Typical findings in bone marrow: in chronic phase (CP) — hypercellular with increased number of eosinophil precursors, loosely distributed spindle-shaped mast cells, reticulin fibrosis. In blast phase (BP) — depending on type of leukemia/lymphoma

Diagnosis: F/P fusion is not visible on conventional cytogenetics. It results from deletion in chromosome 4q12. Diagnosis can be set up by fluorescence *in situ* hybridization (FISH) and/or reverse transcriptase polymerase chain reaction (RT-PCR). Both peripheral blood and bone marrow can be used for assessment. RT-PCR detects most breakpoints within *PDGFRA* and *FIP1L1* but misses rare cases of *PDGFRA*-associated neoplasms with alternate fusion partners.

Treatment: in CP — imatinib (IM) 100 mg daily with concurrent use of corticosteroids in patients with cardiac involvement. Complete hematological response (CHR) expected within days, complete molecular response (CMR) within weeks or months. CHR and CMR rates ~100% and >90% respectively. IM 100 mg daily to 100 mg weekly as response maintenance. In BP — IM 100–400 mg daily plus chemotherapy.

Response assessment: RT-PCR or FISH every 3 months for the first 3 years, then every 3–6 months. Imaging studies for extramedullary presentation. In IM resistance, screen for *PDGFRA* T674I or D842V acquired mutations.

Imatinib discontinuation: IM cessation may lead to durable remissions. Molecular relapse-free survival (MRS) survival was 91% at 12 months and 65% at 24 months after stopping IM. Dose and duration of IM treatment as well as CMR duration did not impact on MRS. Twenty out of 46 patients (57%) relapsed after median 45 months in a recent report. Time to IM initiation and duration of IM administration were independent factors of relapse.

Prognosis: Excellent in CP, variable, but usually favorable in BP [4–16].

PDGFRB-rearranged neoplasms

Epidemiology: male predominance, median age 49 years (range 20–80). Single cases reported in children. Incidence is low (<2% of all MPN). More than 30 gene partners of *PDGFRB* have been identified, but *ETV6-PDGFRB* resulting from t(5;12)(q32;p13.2) is the commonest.

Manifestation: most patients have splenomegaly, but hepatomegaly can also be observed. Dermatological manifestation is rare.

Clinical presentation: chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia (aCML), CEL, MPN or AML. EMD can also be present.

Typical findings in blood: eosinophilia is common (58%), most patients show moderate anemia or thrombocytopenia. Cases without eosinophilia have been reported.

Typical findings in bone marrow: in CP — hypercellular due to neutrophilic and eosinophilic proliferation. Spindle-shaped mast cells can be present. In BP — depending on type of leukemia/lymphoma.

Diagnosis: standard cytogenetics on bone marrow/peripheral blood cells remains preferred method to confirm diagnosis. Cytogenetic analysis usually shows t(5;12)(q32;p13.2). Breakpoints of *PDGFRB* are located in chromosomal region 5q31~q33, but rare cases harboring *PDGFRB* rearrangements may reveal normal karyotype. FISH can be used to demonstrate all *PDGFRB* rearrangements, but cannot identify partner fusion genes. RT-PCR can detect small clones, complex and/or cryptic cases not evident on cytogenetics. RT-PCR useless outside of *ETV6-PDGFRB*.

Treatment: IM 400 mg daily in CP and combined with chemotherapy in BP. CHR and complete cytogenetic remission (CCR) rates for CP are 100% and 86%, respectively. After IM duration of 7 years, six-year progression-free survival rate of 88%. CHR is usually achieved by 1 month and CCR by 3 months of IM treatment. Reduction of IM to 100 mg daily can be considered after CHR/CCR.

Response assessment: standard cytogenetics and/or FISH every three months during the first 3 years, then every 3–6 months. RT-PCR can be used to document molecular response (in patients with known fusion genes). Imaging studies for extramedullary presentation. In resistant cases, screen for C843G mutation.

Imatinib discontinuation: single reports with variable outcome.

Prognosis: excellent in CP, variable but usually favorable in BP [4, 17–22].

FGFR1-rearranged neoplasms

Epidemiology: moderate male predominance. Median age 32 years, but this neoplasm can occur in children and older people (7–84 years). Incidence is low (<1% of all MPN). To date, 15 partner genes of *FGFR1* have been detected. Common rearrangements include: 1) t(8;13)(p11;q12) which results in the fusion of *ZMYM2* with *FGFR1*; 2) t(8;9)(p11;q33) leading to *CNTRL/FGFR1*; and 3) t(6;8)(q27;p11) with *FGFR1OP/FGFR1* fusion.

Manifestation: in CP — splenomegaly and hepatomegaly, mediastinal lymphadenopathy is usually absent. In BP — depends on clinical presentation (*see below*).

Clinical presentation: CEL, AML, T-cell LBL [mainly in association with t(8;13) fusion gene], CML [t(8;22)], CMML [t(6;8) and t(8;9)], mixed phenotype acute leukemia (MPAL).

Typical findings in blood: eosinophilia, neutrophilia, occasionally monocytosis.

Typical findings in bone marrow: MPN-like with eosinophilia and considerable variability in CP, blast infiltrations in acute leukemias/lymphomas.

Diagnosis: standard cytogenetics identifies *FGFR1*-related translocations which can be confirmed by FISH and/or RT-PCR.

Treatment: in CP — clinical trial or TKI with activity against *FGFR*: pemigatinib or midostaurin or ponatinib. In BP — treatment depends on clinical presentation: chemotherapy (AML/ALL-like) plus TKI. Imatinib, nilotinib and dasatinib are ineffective. Allogeneic stem cell transplantation (allo-SCT) is only curative therapeutic approach and should be considered early in eligible patients.

Response assessment: PB/BM including conventional cytogenetics/FISH and RT-PCR (if available). Imaging studies for extramedullary presentation.

Imatinib discontinuation: not applicable.

Prognosis: aggressive clinical course with poor prognosis, rapid transformation of CP to BL (within 1–2 years of diagnosis) [3, 4, 19, 23, 24].

JAK2-rearranged neoplasms

Epidemiology: marked male predominance, median age 47 years (range 12–75). Incidence is low (<1% of all MPN). Commonly includes cases with t(8;9)(p22;p24.1) resulting in fusion of *PCM1-JAK2*. Alternative partners of *JAK2* may contain t(9;12)(p24.1;p13.2) and t(9;22)(p24.1;q11.2) with fusions of *ETV6-JAK2* and *BCR-JAK2*, respectively.

Manifestation: hepatosplenomegaly.

Clinical presentation: atypical CML, CEL, primary myelofibrosis (PMF), MPN/MDS, AML, B/T-cell LBL.

Typical findings in blood: eosinophilia (not commonly observed), neutrophil precursors.

Typical findings in bone marrow: in CP — eosinophilia, dyserythropoiesis and dysgranulopoiesis (MPN/MDS), increased fibrosis (PMF) is frequent. BP — depending on clinical presentation.

Diagnosis: standard cytogenetics identifies *JAK2*-related translocations which can be confirmed by FISH and/or RT-PCR.

Treatment: In CP — clinical trial or TKI with activity against *JAK2*: ruxolitinib or fedratinib. In BP — treatment depends on clinical presentation: chemotherapy (AML/ALL-like) plus TKI. Imatinib, nilotinib and dasatinib are ineffective. Allo-SCT is only curative therapeutic approach, and should be considered early in eligible patients.

Response assessment: PB/BM including conventional cytogenetics/FISH and RT-PCR (if available). Imaging studies for extramedullary presentation.

Imatinib discontinuation: not applicable.

Prognosis: highly variable from weeks (BP) to years (CP) [3, 4, 19, 25–27].

ETV6-FLT3 and *ETV6-ABL1* gene fusions

These fusion genes constitute 'provisional' categories which have not yet been added to the WHO 2016 classification. The cases with *FLT3* fusions occur extremely rarely, and usually involve t(12;13)(p13;q12) leading to fusion with *ETV6*. To date, 17 cases and seven genes have been identified as partners of *FLT3*. Clinically present as CEL, T-cell LBL or peripheral T-cell lymphoma. Rearrangement can be detected by standard cytogenetics but FISH and RT-PCR are useful. Clinical course is aggressive. Patients should be recommended to participate in a clinical trial or receive TKI with activity against *FLT3*: midostaurin, sorafenib, sunitinib or gilteritinib. In BP — chemotherapy with TKI. Allo-SCT should be considered as soon as possible [3, 4, 28, 29].

ALL remains the most common presentation in *ABL1*-rearranged neoplasms, however various clinical phenotypes have been reported. Eosinophilia is not invariably present (in all MPN/AML, but in a minority of ALL). The common abnormality includes t(9;12)(q34;p13) resulting in *ETV6-ABL1* fusion. Rearrangement can be detected by standard cytogenetics, but FISH and RT-PCR are highly recommended. This neoplasm is characterized by an aggressive disease course with poor response to therapy. Patient should be treated within clinical trials or receive TKI with activity against *ABL1*. In BP — chemotherapy plus TKI. Early recommendation to allo-SCT [3, 4, 30].

Step-by-step algorithms with treatment options for myeloid/lymphoid neoplasms with eosinophilia are summarized in Figure 1 [31].

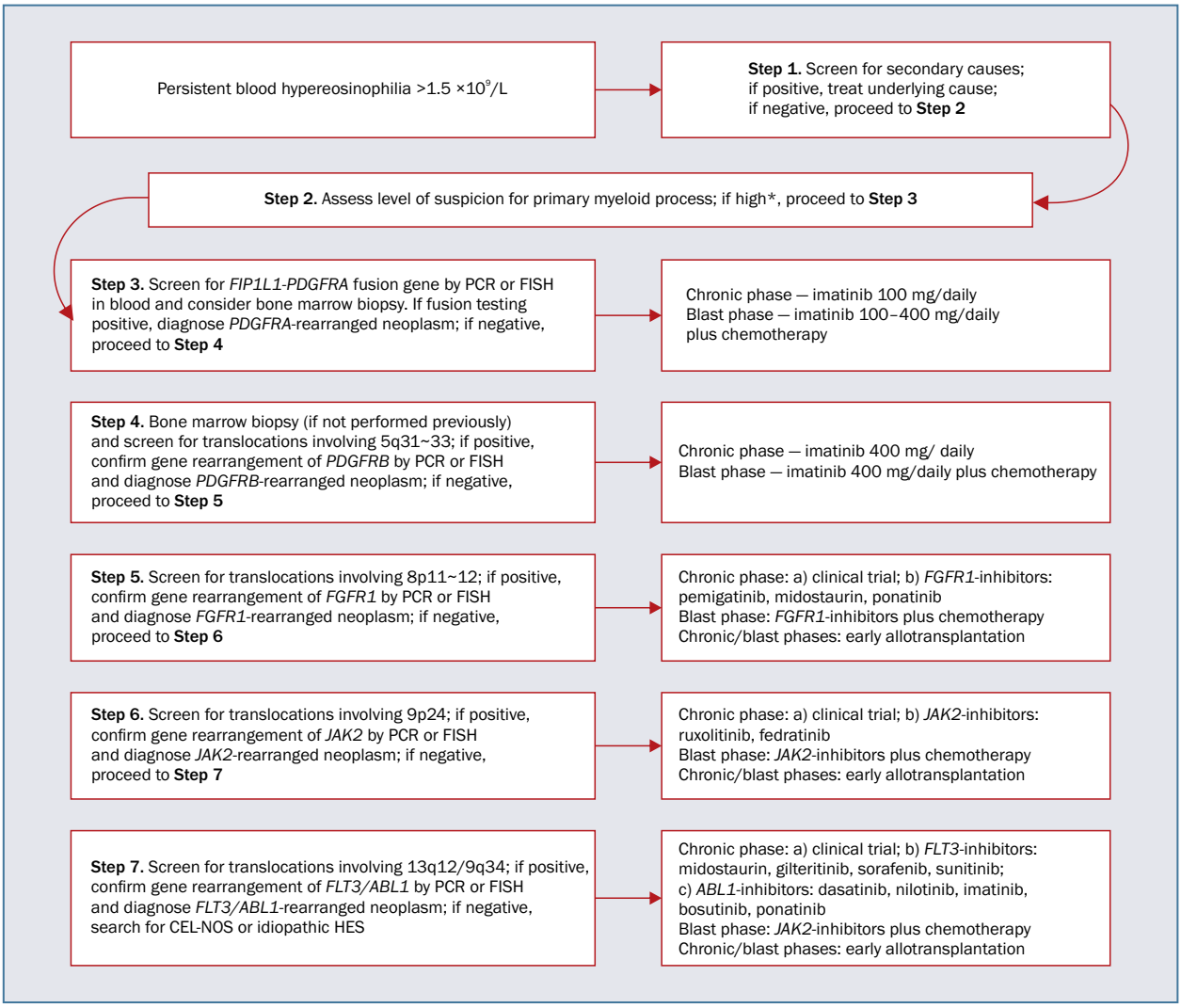

Figure 1. Step-by-step diagnostic ladder with therapeutic options (modified from [31]); *including elevated serum B_{12} or tryptase, splenomegaly, dysplastic eosinophils or blasts in peripheral blood, unexplained anemia/thrombocytopenia, or known steroid-refractory eosinophilia); PCR — polymerase chain reaction; FISH — fluorescence *in situ* hybridization; CEL-NOS — chronic eosinophilic leukemia-not otherwise specified; HES — hypereosinophilic syndromes

Conclusions

Molecular findings have led to the discovery of several novel mutations involving dysregulated tyrosine kinase genes. These findings have resulted in better characteristics of several myeloid and lymphoid neoplasms with eosinophilia which has created a separate category within the WHO classification. The results of molecular profiling will enable more targeted therapy and precise monitoring.

Author's contributions

GH — sole author.

Conflicts of interest

Advisory board: Novartis, Abbvie. Speaker's fee: Novartis.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Therapy of Philadelphia-negative myeloproliferative neoplasms in the blast phase

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Abstract

All four Philadelphia negative myeloproliferative neoplasms: essential thrombocythemia, polycythemia vera, pre-fibrotic myelofibrosis, and myelofibrosis, are at risk of transforming to blast phase disease. The risk is highest in the case of myelofibrosis and amounts to c.20%. In the case of essential thrombocythemia, the transformation rate is 1%, and in polycythemia vera it is 5–10%. The prognosis of patients during the blast crisis is poor, with a median survival time of a few months. For patients who qualify for intensive therapy, the basis of treatment are cycles analogous to those in acute myeloid leukemia and allotransplantation of hematopoietic stem cells. In the remaining patients, hypomethylating drugs such as azacitidine and decitabine can be used. Some hope has been raised by new drugs approved for the treatment of patients with acute myeloid leukemia such as venetoclax, IDH1 and IDH2 inhibitors ivosidenib and enasidenib. It is very important that patients with myeloproliferative neoplasms, especially those with myelofibrosis, properly assess the risk of blast transformation and qualify them early enough for allotransplantation of hematopoietic stem cells. New prognostic scales taking into account molecular factors can be very helpful in the assessment. This article discusses the risk factors of blast transformation, and prognostic scales as well as therapies that can be used during the blast crisis, including new drugs.

Key words: MPN blast phase, blast phase risk factors, treatment

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Introduction

The World Health Organization (WHO) classification system distinguishes four classic Philadelphia negative myeloproliferative neoplasms (MPN): primary myelofibrosis (PMF), pre-fibrotic PMF (pre-PMF), essential thrombocythemia (ET), and polycythemia vera (PV) [1]. In addition, 5–30% patients with ET or PV experience fibrotic progression of their disease over time, referred to as post-ET and post-PV myelofibrosis (MF), respectively [2]. All of these entities may evolve into blast phase disease (MPN-BP), defined by the presence of ≥20% blasts in the blood or bone marrow [2]. A second but closely related entity is accelerated phase (MPN-AP), defined as an elevation of peripheral or bone marrow blasts of between 10% and 20% [3].

The transformation frequency is the lowest for ET at roughly 1%, and highest for PMF and post ET/PV MF at about 20%. In the case of PV and pre-PMF about 5–10% of patients transform to the blast phase (BP) [4–6]. MPN-BP is associated with an aggressive course and very poor prognosis, with salvage chemotherapy and allogeneic stem cell transplant (allo-SCT) being the only curative treatment options [7, 8].

This paper discusses new prognostic scales that can facilitate the proper prognosis and selection of risk adapted therapy for patients with MPN, which may prevent at least some of them from progressing to the blast phase. The current possibilities of treating patients with MPN-BP are also discussed.

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Assessment of risk factors for blast transformation in patients with ET and PV

In PV and ET, leukemic transformation is a rare, usually late, complication. The interval between diagnosis and evolution to acute myeloid leukemia (AML) is highly variable, from a few years to 20 years [5, 6].

It is very important to distinguish ET from pre-PMF, which is the entity defined for the first time by the 2016 WHO criteria [1]. This is possible only with the close correlation of clinical, molecular and histopathological data. Compared to ET, patients with pre-PMF have a higher risk of transformation to AML and shorter overall survival (OS) [9]. Passamonti et al. [10] analyzed the course of disease among 605 patients with ET (follow-up 4,596 person-years). Leukemia occurred in 14 patients (2.3%) at a median 11 years after diagnosis of ET; the risk was 2.6% at 10 years. Age >60 years (p =0.02) was significantly correlated with the development of leukemia. Cytotoxic treatment did not imply a higher risk of leukemia. Among 605 patients with ET analyzed by Gangat et al. [5] followed for a median of 84 months, leukemic transformation was observed in 20 patients (3.3%). In multivariate analysis, hemoglobin level below normal and platelet count ≥1,000 $\times 10^9$/L were identified as independent risk factors for leukemic transformation.

The European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) prospective project included 1,638 patients with PV [6]. AML/myelodysplastic syndrome (MDS) was diagnosed in 22 patients after a median of 2.5 years from recruitment in the study and a median of 8.4 years from the diagnosis of PV. Older age was confirmed as the main independent risk factor. Exposure to radioactive phosphorus (P32), busulphan, and pipobroman were also identified as risk factors of progression to AML compared to treatment with phlebotomy or interferon. Tefferi et al. [11] analyzed the course of PV in a group of 545 patients. A total of 50 (3%) cases of post-PV AML were documented and occurred at a median of 10.8 years (range 0.5–22.3) from diagnosis. Cumulative hazard of leukemic transformation was 2.3% at 10 years and 5.5% at 15 years. Risk factors included older age, abnormal karyotype, and leukocytes >15 $\times 10^9$/L. Leukemic transformation was associated with treatment exposure to pipobroman or P32/chlorambucil. Similarly to previous large retrospective and population-based studies, they did not observe an association between leukemic transformation and hydroxyurea use [6, 12].

Bonicelli et al. [12] observed transformation to AML in 30 (9.2%) of 327 PV patients (median follow up 11 years). The median time from PV diagnosis was 55.4 months (range: 27–262 months) and the cumulative risk of leukemia was 8%, 14% and 17% after 10, 15 and 20 years, respectively. Using Cox multivariate analysis, only female sex was identified as a risk factor of AML, whereas age >70 years, leukocytosis >13 $\times 10^9$/L and thrombosis at diagnosis remained significant predictors of survival.

In recent years, the introduction of the NGS (next-generation sequencing) technique has allowed the identification of many additional (except for driver mutations) somatic mutations in patients with MPN. These include mutations of genes involved in the post-translational modification of histones (*ASXL1*; frequency 10–35%, *EZH2*; frequency 7–10%), DNA methylation (*TET2, DNMT3A, IDH1 /2*), mRNA splicing (*SRFS2, SRF3B1, U2AF, ZRSR2*) and DNA repair processes (*TP53*). Recent publications have highlighted the prognostic contribution of so-called high molecular risk (HMR) mutations (*ASXL1, EZH2, SRSF2, IDH1/2, U2AF1*) [13–17]. Tefferi et al. [14] showed that spliceosome mutations *SF3B1, SRSF2* in ET and *SRSF2* in PV adversely affect OS. They also revealed that *TP53* mutations predicted leukemic transformation in ET. Luque Paz et al. identified three molecular groups associated with a distinct time to leukemic transformation in PV and ET [13]. Short-term transformations were mostly characterized by a complex molecular landscape and mutations in *IDH1/2, RUNX1*, and *U2AF1* genes, whereas long-term transformations were associated with mutations in *TP53*, *NRAS*, and *BCORL1* genes. Considering the important role of molecular landscape on prognosis in PV and ET, Tefferi et al. [14] constructed the three-tiered mutation-enhanced international prognostic systems (MIPSS) which takes into account male sex, leukocyte count ≥11 $\times 10^9$/L, HRM in ET, and age >60 years, thrombosis history, leukocyte count ≥15 $\times 10^9$/L in PV.

Assessment of risk factors for blast transformation in patients with MF

Among classic myeloproliferative neoplasms, the highest risk of blast transformation concerns patients with MF and amounts to approximately 20% [4, 7]. Myelofibrosis is a disease with a very heterogeneous course; therefore, it is important to properly assess the risk of blast transformation and implement an appropriate, risk-adjusted therapy.

It seems that the type of driver mutation influences the course of PMF. Patients with type 1 *CARL* mutation are younger, have a higher platelet count, lower leukocytosis, require less frequent red blood cell transfusions, have fewer unfavorable epigenetic mutations, are in lower risk groups, and have significantly longer OS compared to patients with *JAK* (+) and *MPL* (+) [15–17]. On the other hand, 'triple negative' patients have a particularly poor prognosis as they have a significantly shortened OS and an increased risk of leukemic transformation [18, 19]. Also, patients with the previously described HMR mutations have a shorter OS and a higher risk of blast transformation [15–17].

In a significant percentage of patients with PMF (30––50%), karyotype abnormalities occur at the time of diagnosis. The most common aberrations include del (13q), del (20q), trisomy 8, trisomy 9, del (12p), and 1q abnormalities. Complex karyotypes occur in about 15% of cases [20]. The presence of certain cytogenetic abnormalities, such as complex karyotype, chromosome 5 and 7 abnormalities, are associated with a significantly higher risk of transformation to AML [20].

So far, prognostic indices such as IPSS (International Prognostic Scoring System), and DIPSS (Dynamic International Prognostic Scoring System), and DIPSS plus, have been used to assess the prognosis of patients with PMF [21–23]. Due to the growing understanding of the prognostic significance of mutations, several prognostic indices have been published that also take into account molecular changes. In 2018, Guglielmelli et al. [24] proposed the MIPSS70 and MIPSS70 plus indices, taking into account both clinical data and molecular and cytogenetic tests. The MIPSS70 index takes into account the following risk factors: Hb <100 g/L, leukocytes >25 $\times 10^9$/L, platelets <100 $\times 10^9$/L, peripheral blood blasts >2%, bone marrow fibrosis >grade 2, presence of constitutional symptoms, absence of type 1 *CALR* mutation, presence of HMR epigenetic mutation, and presence of at least two HMR mutations. Depending on the number of risk factors, patients are classified into three risk groups: low, intermediate, or high, with median OS of 27.7; 7.1, and 2.3 years, respectively. The MIPSS70-plus index additionally takes into account changes in karyotype. Currently, it is recommended to use the new version of MIPSS70+ (MIPSS70+ vs. 2.0 index) [25]. This additionally takes into account the division into very unfavorable and unfavorable karyotype as well as moderate and severe anemia. Tefferi et al. [26] proposed a prognostic model that takes into account only molecular and cytogenetic changes. This is known as GIPSS (Genetically Inspired Prognostic Scoring System for primary myelofibrosis). As risk factors, it considers changes in karyotype, absence of type-1 *CALR* mutations, and presence of epigenetic mutations *ASXL1, SRSF2* and *U2AF1Q157.*

In the case of myelofibrosis secondary to PV or ET, a separate prognostic scale, MYSEC-PM (Myelofibrosis Secondary to PV and ET-Prognostic Model), is recommended [27].

It should be emphasized that, whenever possible, molecular risk factors should be taken into account, especially when deciding whether to qualify patients for allo-SCT. New prognostic indices allow for a more accurate assessment of the expected survival time. It has been shown that, using the MIPSS70 index, nearly 30% of patients with low and intermediate risk-1 according to IPSS are in a high-risk group, with an expected OS of only 2.3 years [24]. All patients with high-risk myelofibrosis eligible for transplantation should be offered this treatment option before the disease progresses to an accelerated or blast phase.

Treatment of MPN blast phase

Patients in the blast phase of MPN have a poor prognosis, with an expected OS of several months. Post MPN AML is more often characterized by unfavorable changes in karyotype than in *de novo* disease [4, 7, 8]. Tefferi et al. [8] retrospectively reviewed the results of treatment of 410 MPN-BP patients: 248 from the Mayo Clinic and 162 from Italy. Among 248 patients with MPN BP from the Mayo Clinic, cytogenetic information was available in 172 cases, of which 140 (81%) were reported as abnormal and 32 (19%) as normal; among the 140 abnormal cases, 56 (40%) were labelled 'high risk' based on the presence of monosomal karyotype or monosomy 7 (n =46), or single or multiple abnormalities including inv(3)(q21.3q26.2)/t(3;3)(q21.3;q26.2) (n =5), or i(17)(q10) (n =5). Median OS in the entire group of patients was only 3.6 months, with no improvement over the last 15 years. Multivariate analysis performed on the Mayo cohort identified high risk karyotype, platelet count <100 $\times 10^9$/L, age >65 years and transfusion need as independent risk factors for survival. Intensive chemotherapy (AML-like induction chemotherapy) resulted in complete remission (CR) or CR with incomplete count recovery (CRi) rates of 35% and 24%, respectively; treatment-specified 3-year/5-year survival rates were 32%/10% for patients receiving allo-SCT (n =24), 19%/13% for patients achieving CR/CRi but who were not transplanted (n =24), and 1%/1% in the absence of both allo-SCT and CR/CRi (n =200) (p <0.01). Similar results were presented by Kennedy et al. [28]: among 75 patients with MPN-BP, 39 received AML-like induction chemotherapy followed by allo-SCT in eligible patients (17 of 39). The 36 other patients were treated with hypomethylating agents (HMA), novel agents, or supportive care. Two-year survival was 25.6% in the intensive treatment group compared to 3% for the rest. Moreover, survival was significantly better in the transplant group (2-year survival of 47% vs. 15%; p =0.03). The MPN Subcommittee of the Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation studied 46 patients with MPN-BP who received allo-SCT [30]. Before SCT, 42 patients (91%) received induction chemotherapy. Of the 38 patients evaluable for response, nine (24%) achieved CR, 10 (26%) achieved partial response (PR), and 19 (50%) were refractory or had progressive disease at the time of SCT. The 3-year progression-free (PFS) and OS rates were 26% and 33%, respectively. The only significant factor for survival was CR vs. no CR before transplantation (69% vs. 22%, p =0.008); however, CR was achieved only in eight patients.

A new liposome formulation of cytarabine and daunorubicin used in AML induction therapy is CPX-351. This drug has proven efficacy in the treatment of the elderly, especially in the case of therapy-related AML and with antecedent MDS or chronic myelomonocytic leukemia (CMML) [30]. Therefore, it would be advisable to use it in the case of MPN-BP.

In patients who are not eligible for intensive chemotherapy, HMA such as azacytidine or decitabine can be used [31, 32]. Thepot et al. [31] reported the azacytidine treatment outcomes of 52 patients with MPN-BP who transformed to AML (n =26) or MDS (n =28). Overall response rate (ORR) was 52% (24% CR, 11% PR, 8% marrow CR or CRi, 9% hematologic improvement) and median OS was 11 months. Prognostic factors for CR achievement were the underlying MPN (14% CR for PV vs. 43% for ET; p =0.040) and type of transformation (36% vs. 12% CR in MDS and AML, respectively; p =0.038). Badar et al. [32] conducted a retrospective study of 21 patients with MPN--AML and 13 with MPN-AP treated with decitabine. Six patients (29%) with MPN-AML responded to decitabine (three CR, two CRi, and one PR); median response duration was 7 months. Median OS was significantly higher in those who responded (10.5 vs. 4 months). Among patients with MPN--AP, eight (62%) benefited; median response duration was 6.5 months. Median OS was 11.8 months in responders vs. 4.7 months in non-responders.

Although ruxolitinib monotherapy has very limited efficacy in the advanced stages of MPN [33], its addition to HMA or low doses of cytosine arabinoside may be a therapeutic option [34].

New targeted therapies have recently been approved for treating AML patients, such as venetoclax, IDH1 and IDH2 inhibitors ivosidenib and enasidenib [35–37]. Considering that MF is a disease characterized by the overexpression of the antiapoptotic BCL-2 family of proteins, and IDH mutation occurs in approximately 30% of patients with MF blast phase, it seems that they may turn out to be valuable drugs also for patients with MPN-BP [38–40]. So far, experience with the new drugs is limited, but promising.

Morsia et al. retrospectively analyzed 14 consecutive MPN-BP patients who received venetoclax plus HMA and observed a high rate of ORR [41]. Venetoclax was administered in combination with azacitidine (n =5) or decitabine (n =9). Median age of patients was 67 years with poor-risk cytogenetics in 69% of patients. In 1/2 patients with myeloid sarcoma, partial resolution of the extramedullary tumor was observed. Among the remaining 12 patients, ORR was 42% (n =5) and included CR in three patients (25%) and PR in another two (17%). Cahill et al. retrospectively assessed 15 patients with *IDH1/2*-mutated AML arising from antecedent MPN (seven MPN-BP, one MPN-AP, five MDS--AML, and two CMML-AML) [42]. Thirteen *IDH2* mutated patients received enasidenib as monotherapy (n =12) or combined with azacytidine (n =1). Two *IDH1*-mutated patients received ivosidenib as monotherapy (n =1) or combined with azacytidine (n =1). ORR rate to IDH inhibitor therapy was 40% for the entire group, and 75% for eight patients with MPN-AP/BP (when using the 2012 MPN-BP response criteria). Median OS for all patients was 235 days, and for patients with MPN-AP/BP was not reached.

Conclusions

Allo-SCT, preceded by AML-like induction chemotherapy, is proven to be the only treatment modality that improves at least short-term survival of patients with MPN-BP. However, in the majority of patients SCT is not a feasible option due to advanced age, co-morbidities and poor performance status. For them, the best treatment option remains azacytidine or decitabine. New drugs such as venetoclax and *IDH1/2* inhibitors are raising hopes.

Due to the very poor prognosis of patients in the blast phase of MPN, and the lack of effective treatment options in this phase, care should be taken to prevent transformation.

It is very important that drugs that have leukemogenic potential, such as pipobroman, chlorambucil, and radioactive phosphorus, should be avoided during the chronic phase of the disease. It is also very important to properly assess the risk of transformation (correct diagnosis, new prognostic scales) and select the appropriate therapy early in the course of the disease.

Author's contributions

AG-T — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Calreticulin, a multi-faceted protein: thrombotic and bleeding risks in CALR mutation positive essential thrombocythemia

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Abstract

Essential thrombocythemia (ET) is a clonal disorder of a multipotent hematopoietic progenitor cell. In most patients, a driving mutation of Janus kinase 2 gene, calreticulin gene or myeloproliferative leukemia virus oncogene is detected. The occurrence of thrombotic and/or bleeding complications is very typical in manifestations of ET, with many cases of both occurring in the same patient. The thrombotic or bleeding phenotype can be a consequence of the coexistence of driving and non-driving molecular mutations and polymorphisms, affecting the platelet number and function. This paper discusses the nature of this disease, paying special attention to calreticulin gene function.

Key words: CALR, JAK2, MPL, acetylsalicylic acid, thrombosis, bleeding, ERp57, calnexin pathway, store-operated calcium entry, platelet function

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Introduction

Essential thrombocythemia (ET) is a clonal disorder of a multipotent hematopoietic progenitor cell. In 75–89% of ET cases, the driving mutations of Janus kinase 2 gene (*JAK2*), calreticulin gene (*CALR*) or myeloproliferative leukemia virus oncogene (*MPL*) are detected with frequencies of 61–65%, 13–22%, and 1–2%, respectively [1–6]. All of the mutations (*JAK2*, *CALR*, *MPL*) identified to date share the common characteristics of constitutive activation of tyrosine kinase-dependent signaling pathways and cytokine independent cellular proliferation [7, 8].

The clinical disease manifestation differs depending on the driving mutations and co-operating mutations in the myeloid genes status. ET patients are at risk of polycythemic transformation (*JAK2*V617F positive cases) and myelofibrotic transformation (*CALR* mutation positive cases, and patients with co-operating mutations in the myeloid genes). Leukemic transformation is rare, but possible due to the 'transforming' mutations acquisition (*TP53*, *RUNX1)* or overexpression of MDM2/MDM4 by hematopoietic progenitor cell(s) [9–11]. The leukemic transformation risk is also higher in ET patients with extreme thrombocytosis [12].

The main factors influencing the overall survival of ET patients are a previous thrombosis episode, leukocytosis, and the presence of co-operating mutations in the myeloid genes [13].

The risk of thrombosis is especially high in ET patients with *JAK2* mutation, a history of previous thrombosis, and advanced age (≥60 years). It has been also postulated that *JAK2* variant allele frequency (VAF) can influence venous thromboembolism [14]. A detailed analysis of thrombotic risk, depending on the type of driver mutation status, showed 5-year thrombosis-free survival rates of 93%, 91% and 88% for patients carrying the *JAK2*V617F, *MPL*

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and *CALR* mutation, respectively [15]. Recently published data documents a lower risk of venous thrombosis in ET patients carrying the *CALR*-type 2 mutation [16]. Interestingly, also the lower incidence of arterial thrombosis is consistent mostly with *CALR* mutations and/or extreme thrombocytosis [17].

In 3–18% of ET patients, bleeding occurs as an initial presenting symptom [18, 19]. The annual frequency of bleeding and major bleeding complications amounts to 4.6 and 0.79 per patient per year, respectively [20]. A report by Stuckey et al. [21] showed that the major bleeding complications frequency after an average follow-up period of 87.7 months was 6.6% (91 hemorrhagic events in 1,380 patients observed). Of 249 patients from the very-low risk group, 12 with an unknown driver mutation status experienced severe bleeding (4.8%). Interestingly, nine of them (75%) were treated with an anti-aggregatory drug.

The results of large clinical trials have revealed that major bleeding during follow-up occurs in 6% of WHO-ET defined patients, at the rate of 0.79% of patients per year. A detailed analysis of the WHO-ET patients confirmed previous hemorrhage and acetylsalicylic acid (ASA) as independent risk factors for bleeding complications. It should be mentioned that the study did not include the mutational status of the ET patients studied (*JAK2* vs. *CALR* vs. *MPL* positive) [19]. In a post hoc survey of 311 patients diagnosed with ET (mostly included in the prospective UK-PT1 trial) and receiving ASA with either hydroxyurea or anagrelide, an increased grade of bone marrow reticulin fibrosis predicted higher rates of major hemorrhage during the follow-up [22, 23].

Thrombosis and bleeding scoring systems for risk stratification in ET patients

Thrombosis risk in ET patients can be calculated with the help of the IPSET, IPSET-thrombosis and revised IPSET thrombosis scores based on age, previous history of thrombosis, *JAK2* mutation positivity, and the presence of cardiovascular risk factors [24, 25]. Recently, a mutation-enhanced international prognostic system has been proposed (Table I).

There is as yet no final agreement regarding the bleeding risk factors in ET patients. The bleeding risk is higher in ET patients with a history of previous major bleeding and high platelet count (≥1,500 $\times 10^9$/L) [13]. The bleeding risk in individual cases however may be also influenced by acquired coagulation abnormalities. This is observed in ET patients with extreme thrombocytosis and the symptoms of acquired von Willebrand syndrome (AvWS) due to consumption coagulopathy. For this reason, the administration of ASA is not recommended, if the ristocetin cofactor activity is <30% [26, 27]. The bleeding risk assessment in ET cases should be made with caution, because AvWS symptoms can also present in patients with near-normal platelet counts [28, 29] (Table II).

In a prospective study of the myeloproliferative neoplasms (MPN) registry of the Study Alliance Leukemia, bleeding events were rarely diagnosed before the MPN diagnosis, and their frequency was constant over a period of 160 months. However, the study was limited by the fact that the analysis was performed in a group of both ET and polycythemia vera (PV) patients, independently from the driver mutation status [30]. Another unresolved problem is the issue of hemorrhagic complications severity assessment in patients with ET due to the use of different bleeding intensity scales, e.g. International Society on Thrombosis and Haemostasis (ISTH), ISTH-like, World Health Organization and Common Terminology Criteria for Adverse Events. This may be the reason for the underestimation of the low and moderate bleeding frequency in ET patients. The data concerning the severe complication frequency is more accurate, and confirms that 13.7% of deaths in ET and PV patients was caused by bleeding, especially by fatal cerebral hemorrhage [20]. The pathogenesis of bleeding complications in ET patients is likely multifactorial, including alterations of primary hemostasis (mainly related to vascular endothelial cells dysfunction), AvWS, as well as quantitative and qualitative platelets abnormalities. It has been also postulated that anti-platelets drug and/or anticoagulants administration may influence the bleeding risk in individual patients.

It has been shown that bleeding episodes are more frequently observed in MPN patients who have been treated with anti-platelet or anticoagulant drugs (61.3% at time of diagnosis vs. 72.4% at time of bleeding) [20]. The risk of bleeding with prominent thrombocytosis is even more evident than an increased risk for thrombosis [31], and major bleeding risk is higher in patients with platelet count >1,000.0 $\times 10^9$/L receiving anti-platelet therapy [32]. Recent data shows that prophylactic administration of ASA exacerbates the risk of bleeding, particularly in *CALR*-mutated ET patients, independently from the platelet count [20]. Interestingly, in *JAK2*V617F-mutated ET patients, low-dose ASA administration is associated with no effect on the risk of bleeding [32].

Clinical significance of extreme thrombocytosis in ET patients

At the time of diagnosis, extreme thrombocytosis (ExT, defined as a platelet count ≥1,000.0 $\times 10^9$/L) is present in 22% of ET patients [33, 34]. In the Mayo Clinic MPN database, 18% of adult patients (192/1,070) with ET were aged below 40 and 50% of them presented ExT at the time of diagnosis. Driver mutational status analysis revealed that young patients with ExT harbored the *CALR* gene mutation

Table I. Thrombotic risk factors and thrombosis risk categories in essential thrombocytosis patients

Scale/risk	IPSET-thrombosis	Revised IPSET-thrombosis	Mutation-Enhanced International Prognostic System (MIPSS-ET)
Factors	Age >60 years =1 point Cardiovascular risk factors (tobacco use, diabetes, hypercholesterolemia, hypertension) =1 point Previous thrombosis =2 points *JAK2*V617F =2 points	Thrombosis Age *JAK/MPL* mutation	Adverse mutations *SRSF2, SF3B1, U2AF1, TP53* =2 points Age >60 years =4 points Male sex =1 point Leukocyte count ≥11.0 ×10^9/L =1 point
Category	**Low:** 0–1 point **Intermediate:** 2 points **High:** ≥3 points	**Very low** No thrombosis history Age ≤60 years No *JAK2* or *MPL* gene mutation **Low** No thrombosis history Age ≤60 years *JAK2* or *MPL* mutation **Intermediate** No thrombosis history Age >60 years No *JAK2* or *MPL* mutation **High** Thrombosis history Age >60 years *JAK2* or *MPL* mutation	**Low:** 0–1 point **Intermediate:** 2–3 points **High:** ≥4 points

Table II. Postulated bleeding risk factors in essential thrombocythemia patients

Author	Bleeding risk factor
Rumi et al. [13]	1. History of previous major bleeding 2. Platelet count ≥1,500.0 ×10^9/L
Tefferi et al. [26, 27]	3. Ristocetin cofactor activity <30%
	4. *CALR* mutation*#
	5. Clonal hematopoiesis indeterminate potential (CHIP) associated mutations — i.e. *IDH2**
	6. Germline polymorphisms predisposing for bleeding

*Postulated, #documented in the case of antiplatelet drug administration

more frequently than the *JAK2* mutation (46% vs. 35%). The frequency of arterial thrombosis and major hemorrhage rates at, or prior to, diagnosis also differs between young ET patients with and without ExT (2% vs. 8% and 15% vs. 7%, respectively). Previous data implied that ExT was an independent risk factor for leukemic transformation of ET [12]. This was confirmed by recently published results showing that ExT is an independent predictor of leukemia-free survival and overall survival in ET patients aged below 40 [35].

Role of driver mutations in pathogenesis of bleeding in patients with essential thrombocythemia

It cannot be excluded that driver mutation-specific abnormalities of platelet function play an important role in the pathogenesis of bleeding complications in ET patients. The data published so far in this field is limited. It has been documented that abnormal function of Janus kinase 2, the signal transducer and activator of transcription pathway

(*JAK2*-STAT pathway), may be responsible for abnormal platelets function in platelet aggregation studies. The phosphorylation of JAK2 in thrombin stimulated human platelets was previously reported by Rodriguez-Linares et al. [36]. Also, the regulatory role of STAT3 in collagen-induced platelet aggregation was confirmed by Zhou et al. in 2013 [37]. The involvement of *JAK2*-STAT3 pathway in the process of collagen-induced platelet activation through the activation of *JAK2*-JNK/PKC-STAT3 signaling was documented by Lu et al. [38]. The critical role of JAK2 in this process was supported by the observation that *JAK2* inhibitor AG490 (tyrphostin) attenuated collagen-induced platelet aggregation and calcium mobilization in a concentration-dependent manner [38].

Potential role of calreticulin in bleeding predisposition

CALR is made up of three protein domains: 1) an amino N-terminal lectin binding domain containing an endoplasmatic reticulum (ER) targeting signal sequence; 2) a proline-rich P-domain containing high-affinity binding sites for Ca^{2+}; and 3) a C-domain containing multiple low-affinity Ca^{2+}-binding sites and an ER retention signal (KDEL). Within the endoplasmatic reticulum, CALR participates in the control process of newly synthesized proteins (conformational dependent molecular sorting). Misfolded or unfolded proteins are retained in the ER, thereafter transported to the cytosol, and finally ubiquinated and degradated by the proteasome [39]. Due to the physiological role of CALR and the key role of calcium ions homeostasis and calcium ions flow in platelets, it is possible that abnormal cellular localization of CALR and abnormal CALR-associated cellular storage of calcium ions (including megakaryocytes and platelets) may be responsible for abnormal platelet function and an increased risk of bleeding. In 2009, Reilly et al. [40] demonstrated that calreticulin in platelets was localized to the granulomere. Co-immunoprecipitation techniques, however, did not show an interaction between calreticulin and platelet glykoprotein $\alpha_{IIb}\beta_3$ under various platelet activation states.

In 2013, Klampfl et al. [41] and Nangalia et al. [42] described new genetic variants of *CALR* in patients with ET and primary myelofibrosis. More than 50 different types of *CALR* exon 9 mutants have been found in ET patients. All of these mutants lead to a 1-bp frameshift and loss of the KDEL sequence (endoplasmatic reticulum retention peptide) and the original *CALR* stop codon [43]. The most frequent variants, type 1 (c.1092_1143del) and type 2 (c.1154_1155insTTGTC), account for c. 80% of all *CALR* mutations. Type 1 mutations are more frequent, accounting for c.50% of *CALR*-mutated cases of ET. Recently, it was shown that *CALR* mutations promoted the formation of abnormal protein chaperone complexes, which resulted in its mislocalization to the nucleus to enhanced *MPL* transcription due to increased recruitment of Friend leukemia integration 1 transcription factor (FLI1), ERp57, and *CALR* to the *MPL* promoter [44–47].

The abovementioned abnormalities may have resulted in an increase in platelets production. However, the role of mutant *CALR* protein on platelet function is still an open question. Recently published data has shed light on this field, stressing the role of abnormal interaction between proteins in the calnexin pathway. The calnexin pathway includes, among others, thiol isomerase ERp57 (ER protein 57, ERp57), calnexin and its soluble homolog, calreticulin, and is dedicated for N-glycosylated proteins folding in ER [48]. Under physiological conditions, ERp57 is mobilized to the surface of activated platelets, regulating their function (platelet aggregation, dense granule secretion, fibrinogen binding, calcium mobilization and thrombus formation) [49, 50]. Moreover, ERp57 modulates store-operated calcium (Ca^{2+}) entry (SOCE) activity, a key regulator of megakaryopoiesis. The abovementioned process is mediated by the C-terminal domain of *CALR* protein which is deleted in the case of *CALR* mutants [51, 52]. The regulatory role of the C-domain of *CALR* on SOCE was confirmed by experimental results documenting significantly increased SOCE in megakaryocytes positive for the *CALR* mutation [47], and interactome data confirming that *CALR*wt binds directly to ERp57, but *CALR*mut does not [44, 53].

The hypothesis that *CALR* mutants can affect not only the platelet number, but also their function, was confirmed by Hauschner et al. [54], who showed that after ADP stimulation aggregation of *CALR* mutated platelets was less pronounced that in the case of normal or *JAK2* mutated platelets. Moreover, *CALR* mutated platelets attachment to immobilized fibrinogen and the number of *CALR* mutated platelets achieving the fully spread state is lower than in the case of normal and *JAK2* mutated platelets. This is accomplished by an increased and more dispersed localization of intracellular free Ca^{2+} in the case of *CARL* mutation positive platelets. The abovementioned data may, at least in part, explain the increased bleeding frequency observed in *CALR* mutation positive MPN patients who have been treated with anti-aggregatory drugs.

Other potential molecular aberrations affecting thrombotic and bleeding risks

The occurrence of thrombotic and/or bleeding complications is very typical in manifestations of ET, with cases of both occurring in the same patient. The thrombotic or bleeding phenotype may be a consequence of the coexistence of driving and non-driving molecular mutations and polymorphisms, affecting the platelet number and function. Lindstrom et al. [55], with the help of a genome-wide association study (GWAS) and a transcriptome-wide association study (TWAS),

identified 16 novel susceptibility *loci* for venous thromboembolism. Some of them (*GP6*, *ZFPM2*) have been associated with megakaryopoiesis and platelet biology [55].

In 2020, Veninga et al. [56] documented a predisposition for thrombosis and bleeding in patients with clonal hematopoiesis of indeterminate potential (CHIP). According to this concept, in ET patients carrying the CHIP-associated gene mutations, the risk of thrombosis may be affected by elevated platelet counts (i.e. *ABCB6*, *ASXL1*, *DNMT3A*, *GATA1*, *SF3B1*, *SH2B3*) or elevated platelet counts and hyper-reactive platelet phenotype (*ABCB6* and *SH2B3*). On the contrary, the coexistence of CHIP-associated *IDH2* mutations may result in an increase in the platelet count and bleeding phenotype.

Conclusion

Thrombotic and bleeding risk assessment is an essential part of the treatment strategy in ET patients. However, laboratory and clinical data should be interpreted with caution, especially in *CALR* mutation positive individuals who can experience bleeding episodes during anti-platelet therapy. Also, molecular study results should be carefully analyzed, since data from the COSMIC database has revealed 155 different *CALR* variants, including the newly created class E (about 10% of *CALR* variants) which seems not be to associated with ET [57].

Author's contributions

KL — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Old and new targets for immunotherapy of B cell acute lymphoblastic leukemia

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Abstract

B cell-specific antigens such as CD20 and CD19 are the leading examples of clinically utilized targets for cancer immunotherapy. Rituximab, the anti-CD20 monoclonal antibody (mAb) approved for the treatment of B cell lymphoma in 1997, was the earliest mAb drug ever registered in cancer immunotherapy. The clinical success of chimeric antigen receptor (CAR)-modified T cells has been demonstrated in patients with B cell acute lymphoblastic leukemia (B-ALL), and CD19-directed CAR-T cells were the first CAR therapy ever approved to treat cancer patients. While surface antigen-targeting immunotherapies play a significant role in the therapy of B-ALL, in particular in the treatment of relapsed and refractory patients, they have some limitations and face continuous challenges. Herein, I review the types of antigen-specific immunotherapies that are used in the treatment of B-ALL, including naked mAbs, antibody-drug conjugates, B cell-specific T cell engagers, and CAR-modified T cells. I discuss the requirements for good immunotherapy targets and summarize the main methods used to identify novel putative targets. I present an overview of B cell-specific and non-B cell-specific target antigens, both already used in clinics and tested in preclinical models. I also discuss limitations of current B-ALL immunotherapy, attempts to overcome these limitations, and future directions of immunotherapy research.

Key words: acute lymphoblastic leukemia, B cell, immunotherapy, antigen, target, CD19, CD22, CD20, CD72

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Introduction

Surface antigen-targeted immunotherapies were first introduced in the treatment of B cell malignancies. The availability of B cell-specific target antigens such as CD19, CD20 and CD22 that are not expressed in other tissues has greatly contributed to success. CD19 is the main target for chimeric antigen receptor (CAR) T cell immunotherapy and for blinatumomab, a bispecific T cell engager (BiTE). Both therapies are already approved for the treatment of relapsed/refractory (R/R) B cell acute lymphoblastic leukemia (B-ALL). Although CD19 CAR-T cells have shown unprecedented response rates, exceeding 80% in R/R B-ALL patients, the durability of response is limited, and many patients relapse with CD19-negative disease [1, 2]. Other B cell-specific antigens such as CD22 and CD20 are already available as second-line therapies of CD19-negative relapses and their efficacy has been tested in clinical trials [3, 4]. However, emerging results of these trials are revealing at best transient responses, hence novel target antigens need to be identified and verified.

B-ALL is a heterogeneous disease with dozens of genetic abnormalities identified to date, and it develops in both children and adults. Although the survival prognosis is good for pediatric B-ALL (~90%), the treatment outcome in adults is much worse (40–50% overall survival) [5]. Patients harboring specific genetic translocations respond poorly to conventional therapy but also to modern

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immunotherapies. The subtype with the poorest outcome is **mixed lineage leukemia**-rearranged B-ALL (MLLr B-ALL), which frequently escapes CD19-targeted immunotherapy due to lineage switch from MLLr B-ALL to MLLr acute myeloid leukemia (AML), with a loss of B cell phenotype-associated markers [6–9]. This precludes the use of B cell-specific antigens as immunotherapy targets and encourages the selection of novel candidates as alternative targets for immunotherapy.

In this review, I describe the types of antigen-specific immunotherapy currently used in the treatment of B-ALL. I define the features of appropriate immunotherapy targets, and list ways to identify novel targets for immunotherapy. In addition, I summarize the target antigens already utilized in R/R B-ALL therapy as well as novel, alternative targets tested in preclinical models. Finally, I discuss the limitations and challenges of B-ALL immunotherapy.

Types of antigen-specific immunotherapy employed in B-ALL

Conventional B-ALL therapy is composed of intensive, multiagent chemotherapy delivered in several cycles of treatment over 2–3 years, and is associated with numerous side effects and long-term consequences [5]. Naked monoclonal antibodies (mAbs), such as rituximab, can be added to the chemotherapy of a subset of adult B-ALL patients at the induction phase, due to their low price, broad availability and mild side effects. Other, more advanced, immunotherapies such as BiTe and CAR-T cells play an important role in the treatment of R/R B-ALL patients, as a bridge therapy to allogeneic hematopoietic stem cell transplantation (allo-HSCT). Some attempts are also being made to apply CAR-T cells to treat relapse after allo-HSCT [10].

Currently available immunotherapy options in the treatment of B-ALL are extensively described in [11]. Briefly, the vast majority of registered immunotherapies target surface antigens specific for B cells, namely CD19, CD20, CD22, with mAbs recognizing these antigens. Apart from naked mAbs that work mainly through the induction of host's effector cell-dependent mechanisms (immunophagocytosis, antibody-dependent cytotoxicity), mAb derivatives such as antibody-drug-conjugates (ADC) or BiTE are also used in clinical practice [12]. Additionally, cellular therapy using autologous, patient-derived T cells genetically modified with chimeric antigen receptors (CARs) has been available since 2017 [13]. CARs are synthetic constructs composed of several domains: 1) an extracellular domain responsible for the recognition of tumor-specific targets, which is derived from mAb; 2) a transmembrane part; and 3) intracellular domains responsible for the transmission of activating signals and co-stimulation, CD3ζ, CD28, 4-1BB.

During CAR-T cell therapy, autologous T cells are collected from a B-ALL patient by leukapheresis, genetically modified *ex vivo* with CARs, and infused back into the patient's circulation, where they specifically recognize cells expressing target antigens, mainly leukemic cells. Importantly, the recognition of malignant cells by CAR-T cells and induction of the cytotoxic responses are major histocompatibility complex (MHC)-independent. The construction of CAR molecules, e.g. the choice of costimulatory domains as well as types of hinge and transmembrane domains, significantly affect CAR-T cell functionality [14].

Although antigen-targeted immunotherapy is more specific than conventional chemotherapy, adverse side-effects associated with CAR-T cell and BiTE therapy have been frequently reported. B cell aplasia, a direct consequence of on-target off-tumor toxicity, impairs antibody production and increases susceptibility to infection, but is manageable with immunoglobulin infusion. A treatment-induced life-threatening complication is cytokine release syndrome (CRS), which leads to multiple organ dysfunction and neurotoxicity [15]. This can be mitigated with the use of corticosteroids and tocilizumab, an antibody blocking interleukin 6 (IL-6) receptor. Further information about the efficacy, challenges and ways to address obstacles to CAR-T cell therapy can be found in [16, 17].

Immunotherapy targets

What makes a good target for cancer immunotherapy?

An ideal target for cancer immunotherapy should be expressed exclusively on malignant cells and should be essential for cancer cell proliferation and survival. However, none of the targets currently in use meets these stringent criteria. The vast majority of antigens utilized in the immunotherapy of B-ALL are B cell-specific proteins that occur both in malignant and normal B cells, but are rarely present in other tissues. This usually ensures sufficient efficacy and safety of the targeted immunotherapy. The resulting on-target off-tumor toxicity to normal B cells is an unavoidable but manageable side effect. However, B-ALL subtypes derived from early stages of B cell development, with more stem cell-like features such as MLLr- or TCF3-ZNF384 fusion-B-ALL, in response to CD19-directed immunotherapies were shown to undergo lymphoid-to-myeloid lineage switch [7, 8, 18]. This resulted in the loss of B cell phenotype and precluded further immunotherapy targeting, not only CD19 but also other B cell-specific antigens.

Stability, sustainability, and abundance of a target antigen in all leukemic clones are other important features. Indeed, the outcome of immunotherapy usually correlates with high antigen density on malignant cells [1, 19]. Another important issue is the lack of expression of the target antigen on activated T cells. This is particularly important for CAR-T cell immunotherapy targets. Fratricide

elimination of CAR-T cells expressing the corresponding antigens has been observed in the case of CD38 [20], and is a major obstacle to CAR-T cell manufacturing and their subsequent efficacy.

The usefulness of a surface protein as an immunotherapy target also depends on the type of immunotherapy in which it is employed. Comparisons of the intracellular transport and efficacy of CD22- and CD19-targeted immunotoxins revealed that CD22 is much more efficiently internalized and hence may serve as a better target for ADC [21, 22]. In contrast, antigens that internalize slowly, such as CD20, are better targets for naked antibodies due to prolonged exposure of the Ab crystallizable fragment (Fc) and therefore efficient activation of Fc-dependent effector mechanisms [23].

Methods of target identification

To date, combined transcriptomic and proteomic approaches have been successfully used to select candidates for novel immunotherapy targets in various cancer models including B-ALL [24–26]. Identifying protein-coding mRNAs that are specifically expressed in cancer cells is feasible by comparing malignant primary cells and cancer cell lines to normal counterparts. However, as the correlation between mRNA and protein expression on the cell surface is moderate, transcriptomic data must be integrated with cell surface proteomics. Quantitative mass spectrometry has been successfully employed to generate human cell surface proteome [24, 26]. The integrated proteomic and transcriptomic approach has been recently used to identify CD72 as an optimal target in MLLr B-ALL by comparing cell surface proteins in cell lines representing MLLr to other subtypes of B-ALL [25].

B cell-specific targets used in B-ALL immunotherapy

B-ALL arises from B cell lineage-committed progenitors at various stages of their differentiation, such as pro-B or pre-B cells. The use of B cell-specific antigens including CD19, CD22, and CD20 as targets in B-ALL therapy has already proved very successful in clinical studies. On the other hand, CD72 was recently proposed as an alternative B cell-specific target, and proved its efficacy in preclinical models. The main features of these antigens and the corresponding immunotherapies are summarized in Table I and briefly described below.

CD19

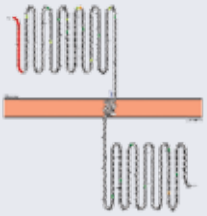
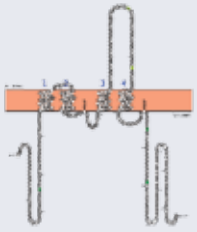
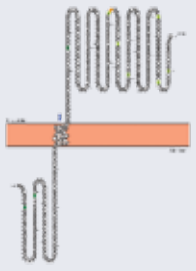
CD19 is a B cell-specific molecule considered as a marker of B cells. Its expression starts during the B lineage commitment from hematopoietic progenitors, and continues throughout all stages of B cell development up to plasma cells. CD19 is a type-I transmembrane protein, with a single transmembrane domain belonging to the immunoglobulin superfamily. It is a co-receptor of the B-cell receptor (BCR) and is involved in modulating BCR signaling [28]. Although its role in the promotion of the proliferation and survival of mature B cells is well-documented, its role in immature B cells is unclear. Importantly, CD19 expression is neither crucial for B-ALL cells viability and proliferation rate *in vitro* nor for B-ALL lymphoblasts' engraftment and progression *in vivo* [29].

Nevertheless, as CD19 is ubiquitously expressed in B-ALL cells independent of the genetic subtype, it is utilized as the main target for B-ALL immunotherapy. Upon binding to an antibody, CD19 internalizes, which makes it a suitable target for immune-conjugate therapy rather than for naked mAb [12]. Denintuzumab mafodotin and coltuximab ravtansine are ADC that have been already tested in clinical trials, but initial results have revealed low clinical responses in patients with R/R B-ALL [30]. Much better clinical responses have been achieved with the use of blinatumomab, a BiTE. Blinatumomab is a single-chain, dual-specificity construct with the ability to recognize CD3 molecules on T cells and CD19 molecules on B cells, thus activating T cells to kill proximal B cells. The efficient renal clearance of blinatumomab results in its short half-life and enforces continuous infusions over several days [31]. Blinatumomab provides clear benefits over conventional consolidation chemotherapy [32] and is effective even in patients with therapy-related and congenital T cell impairments [33].

CAR-T cells recognizing CD19 antigen are pioneering, breakthrough therapy [2] and since 2017, CD19-CAR-T cells (tisagenlecleucel, kymirah) have been approved for the treatment of R/R B-ALL. However, despite remissions reaching up to 90% in some studies, the durability of this treatment is limited, with overall survival reaching only 12.9 months [2]. There are several reasons behind CAR-T cell therapy failure and both CD19-positive and CD19-negative relapses have been detected. CD19-positive relapses occur due to insufficient CAR T-cell expansion, lack of memory T cell formation resulting in poor CAR-T cell persistence, and immunosuppressive microenvironment. Extensive studies are underway aimed at optimizing CAR construction and their *ex vivo* manufacturing in order to increase their persistence and overcome the inhibitory environment [17].

CD19-negative relapses are the most frequent causes of CD19 CAR-T cell treatment failure in B-ALL. As mentioned, CD19 is dispensable for B-ALL cell survival, therefore various processes leading to CD19 loss, such as selection of CD19-negative clones, downregulation of CD19 mRNA, antigen masking, and trogocytosis have been detected in patients undergoing CD19-targeted therapy [34]. The challenges surrounding CAR-T cell therapy have been summarized in a recent review [35].

Table I. B cell-specific surface antigens used in clinics and tested in preclinical models as targets in B cell acute lymphoblastic leukemia (B-ALL) immunotherapy. Visualization of transmembrane topology performed with Protter [27]

Target antigen	Membrane topology	Function	Available immunotherapy	Stage of development/ /references
B cell-specific targets				
CD19	Single-pass type I membrane protein 	Co-receptor for the BCR B cell differentiation and proliferation	BiTE — blinatumomab ADC Denintuzumab mafodotin Coltuximab ravtansine CAR-T cells — tisagenlecleucel	Registered drug Clinical trials Registered cellular therapy
CD22	Single-pass type I membrane protein 	Involved in regulation of BCR signaling, both positive and negative	ADC Inotuzumab ozogamycin CAR-T cells mAbs Epratuzumab	Registered drug Clinical trials Cinical trials
CD20	Tetraspanin 	Development, differentiation, activation of B cells Ca^{2+} signaling	mAbs Rituximab Ofatumumab CAR-T cells	Registered drug Registered drug Clinical trials
CD72		Negative regulation of BCR signaling Interaction with T cells	CAR-T cells	Preclinical studies *in vitro* and *in vivo*

ADC — antibody-drug-conjugates; BiTE — bispecific T cell engagers; mAbs — monoclonal antibodies

CD22

CD22, like CD19, is a B cell-restricted protein. CD22 expression starts in the early stages of B cell development. In pro-B cells, it occurs as a cytoplasmic protein. At the late pre-B cell stage, CD22 appears on the cell surface, where it persists during all subsequent stages of B cell differentiation. CD22 is a type-I, single-pass membrane protein with the ability to bind sialic acid, and therefore it is also known as sialic acid-binding immunoglobulin-like lectin (SIGLEC-2). Interactions with sialylated ligands regulate the ability of CD22 to modulate B cell function. CD22 contains intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIM) and plays a role in the negative regulation of BCR signaling, by recruiting a cytoplasmic SRC homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) [36]. Upon mAb binding, CD22 is promptly internalized [37], which makes it an ideal target for ADC. Indeed, it has been demonstrated that CD22 may shuttle between endosomal compartment and a cell surface, enabling continuous transportation and intracellular accumulation of pH-sensitive cargo, which contributes to the efficacy of CD22-targeting immunotoxins [38–40].

CD22 is expressed in the majority of B-ALL subtypes, hence it is employed as a target in B-ALL immunotherapy. Inotuzumab ozogamycine, a conjugate of cytotoxic drug ozogamycin with anti-CD22 mAb, is approved for the treatment of adult and pediatric B-ALL patients who do not respond to conventional chemotherapy [41]. Although it has a role in the treatment of R/R B-ALL, it can cause severe, life-threatening hepatotoxicity, which limits its use [42]. CARs targeting CD22 have also been developed [43–46]. Importantly, CD22-specific CAR-T cells exert cytotoxicity also in CD19-negative R/R B-ALL. The results of clinical trials testing the efficacy of CD22-CAR-T cells conducted mainly on B-ALL patients who relapsed from previous CD19 CAR-T cell therapy revealed more than70% complete remission, but only 13.4 months overall survival [47]. In contrast, the very recent results of another two clinical trials (NCT02588456, NCT02650414) have reported very low response rates [48]. Both the previous and the more recent clinical studies employed CARs with single chain variable fragment derived from the same antibody, and the length of the linker between the heavy- and the light-chain variable domains was the only difference. Detailed preclinical investigations have confirmed the impact of the linker length on tonic CAR-T cell signaling and consequent clinical efficacy, indicating that only fine differences in CAR construction can significantly affect the clinical outcome [48].

CD20

CD20 is also a B lineage-restricted antigen, but it is ubiquitously expressed only in mature B cells, hence it is extensively used as an immunotherapy target in malignancies derived from mature B cells. The expression of CD20 starts already during B cell development, at the pre-B cell stage, and persists until B cells terminally differentiate into plasma cells. CD20 also occurs in B-ALL cells, but its expression is heterogeneous, often present only in a small proportion of the leukemic population, therefore only 25% of patients qualify for treatment with anti-CD20 immunotherapy [12]. CD20 is a type II transmembrane protein with four transmembrane helices. It is localized in lipid rafts, in close proximity to BCR, CD40, MHC-II, CD53, CD81, and other receptors. Although the precise physiological role of CD20 remains unclear, both human and animal studies suggest its involvement in B cell activation, Ca^{2+} signaling, and interaction of B cells with T cells and other cells of the microenvironment. A summary of CD20 structure, function, and gene regulation has been recently published [49].

In adult, but not in pediatric, B-ALL, the expression of CD20 is associated with a poor prognosis [50, 51]. A phase III trial conducted in Philadelphia (Ph)-negative B-ALL patients with CD20 expression revealed that the outcome of young adults can be improved by a combination of chemotherapy with rituximab, the anti-CD20 mAb approved for medical use in 1997 [52]. Rituximab is one of the best-studied immunotherapy drugs with low toxicity and manageable side effects, but it is not effective in monotherapy. Hence, rituximab is being added to chemotherapy of adult B-ALL patients when at least 20% of leukemic cells are CD20-positive. In pediatric B-ALL, the benefit of the addition of anti-CD20 mAbs to chemotherapy has not been evaluated in a comprehensive way in clinics. Anti-CD20 mAbs other than rituximab have not been extensively investigated in B-ALL patients. Some preclinical studies suggest that obinutuzumab, a class II anti-CD20 mAb, is superior to rituximab *in vitro* and *in vivo* [53]. CD20 may also be targeted by CARs. CAR-T cells simultaneously targeting CD19 and CD20 antigens were designed to overcome CD19-negative relapses. Indeed, in preclinical models, CD19-CD20-bispecific CAR-T cells were more effective than any single antigen-specific CAR-T cells [54]. These dual CD19/CD20 CARs are already tested in clinical trials in patients with advanced R/R B cell malignancies (NCT04700319, NCT04007029).

CD72

Recently, Nix et al. [25] identified unique surfaceome of MLLr B-ALL subtype, with significant upregulation of adhesion-related proteins and downregulation of MHC-I and MHC-II molecules. This study also revealed significant overexpression and cell surface upregulation of CD72 in MLLr B-ALL.

CD72 is a B cell-specific protein which contains intracellular ITIM domains and is involved in negative regulation of BCR signaling. It binds CD5 molecule on T cells, suggesting its role in the crosstalk between B and T cells. CD72 is abundantly expressed in normal B cells and B cell-derived neoplasms, including B-ALL and B cell lymphomas [25]. Using an *in vitro* yeast display library, the authors developed CD72-binding nanobodies and inserted the sequences recognizing CD72 to lentiviral backbone derived from tisagenlecleucel to generate CAR-T cells targeting CD72. The CD72-directed CAR-T cells effectively killed CD72-expressing B-ALL and B cell lymphoma cell lines including CD19-negative cells, and were not toxic against normal cells including PBMC, IVECs, MSC, iPSC. Importantly, the CD72 CAR-T cells were also effective *in vivo* against MLLr B-ALL cell lines and patient-derived xenografts, without toxicity against normal tissue other that B cell ablation [25].

Overall, considering the aforementioned preclinical results, CD72 CAR-T cells are very promising candidates to be tested in clinical trials as a second-line treatment in patients relapsing after CD19-targeted immunotherapy.

Other targets

Other B cell-specific antigens such as CD23 [55], CD79b [56], CD37 [57], BAFFR [58], and BCMA [59] are currently being tested as CAR targets in malignancies derived from mature B cells, such as chronic lymphocytic leukemia,

non-Hodgkin lymphoma, and multiple myeloma. Most of these molecules are not abundantly present on normal immature B cells or in B-ALL cells [60–62]. BAFFR was shown to be expressed in some B-ALL subtypes, mainly E2A-PBX rearranged cells, but the levels were usually low to moderate [63].

Alternative targets in MLLr B-ALL

Particular efforts to identify targets alternative to CD19 have been made in MLLr B-ALL, the extremely poor prognosis subtype. The susceptibility of this subtype to undergo lineage switch and to lose B cell-specific antigens has prompted efforts to identify alternative, B cell-unrelated, targets. One such protein, chondroitin sulfate proteoglycan (CSPG4), also known as neuron-glial antigen 2 (NG2), has been already tested as a putative CAR target in MLLr B-ALL cell line KOPN8 in a proof-of-concept study [64]. NG2 is a diagnostic marker of MLLr B-ALL which is associated with leukemia invasiveness, central nervous system infiltration, and poor patient survival [65]. It was also found that NG2 is important for MLLr B-ALL engraftment to NSG mice and that blockage of NG2 with mAbs leads to relocation of leukemic blasts from the bone marrow to peripheral blood, increasing sensitivity to chemotherapy [66]. However, NG2 is present in only about 50% of leukemic blasts [66] and is also expressed in normal tissues [67], which are significant drawbacks limiting the utility of the antigen as a clinically relevant immunotherapy target.

Another approach proposed in MLLr B-ALL is the simultaneous targeting of two antigens, CD19 and CD133 [68]. CD133, also known as prominin 1 (PROM1), is a stem cell marker and a target gene of the MLL-AF4 oncoprotein [69]. CD133 is abundantly expressed in MLLr B-ALL and is maintained in CD19-negative leukemic cells. Tandem CARs targeting CD19 and CD133 killed leukemic cells expressing only one of the antigens, but the CARs' cytotoxic activity was superior when both antigens were present simultaneously, both *in vitro* and *in vivo*. However, as CD133 is also present on normal hematopoietic stem and progenitor cells (HSPC) at similar levels, the occurrence of on-target off-tumor cytotoxicity has already been reported [70]. Further studies are needed to address the safety issue of these tandem CAR-T cells.

Summary, perspectives, concluding remarks

The success of CD19- and other B cell antigen-targeted therapies in B-ALL has already proved that cell type-specific antigens are appropriate targets for immunotherapy. However, as antigens currently applied in clinics are dispensable for B-ALL cell survival, antigen loss is the major drawback and limitation of B-ALL immunotherapies. This can result from clonal heterogeneity of leukemic population and the selection of antigen-negative subclones or from treatment-related downregulation of target antigens [71]. In CAR-T cell therapy, the emerging approach to overcome this limitation is the use of bi- and tri-specific CARs [72]. Combinatorial targeting has already presented superior efficacy in preclinical models [68, 73] and dual-specificity CAR-T cells directed to CD19 and CD20/CD22 are already being assessed in clinical trials for the treatment of B cell malignancies. In this context, better characterization of clonal heterogeneity, predominantly with respect to target antigens expression, may translate into rational design of combinatorial immunotherapies and may diminish the risk of relapse.

Finally, novel surface antigens crucial for malignant cell survival should be identified and tested as potential alternative immunotherapy targets. This is particularly needed in subtypes derived from B cell precursors at early stages of B cell development and displaying phenotypic plasticity, such as MLLr B-ALL or TCF3-ZNF384 fusion. The broader range of possible immunotherapy targets may pave the way towards more effective and durable immunotherapies.

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Author's contributions

MF — sole author.

Conflicts of interest

The author declares no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Genomic studies of Hodgkin lymphoma using circulating cell-free DNA

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Abstract

The revolutionary finding of cell free DNA (cfDNA) circulating in the bloodstream had a huge impact on the development of non-invasive prenatal testing (NIPT) (obstetrics) and liquid biopsies (oncology). The latter, combined with the sequencing of tumor DNA-containing cfDNA, have been widely applied in cancer research, demonstrating the potential of these techniques to improve prognostication and guide individualized treatment strategies. During routine NIPT analysis of more than 88,000 pregnant women performed in our institution, 14 abnormal genomic profiles suggestive of maternal tumor have been identified. Interestingly, one patient was further diagnosed with classic Hodgkin lymphoma (cHL), a tumor characterized by a low content (<2%) of neoplastic cells in tumor mass. To examine whether circulating cfDNA can be informative about genomic imbalances in neoplastic Hodgkin/Reed-Sternberg (HRS) cells, we performed a pilot study of nine prospective cHL cases. This study showed that genomic profiles of cfDNA correspond to the profiles of HRS cells. To get further insights into the genome of cHL, a large study on cfDNA from 177 prospective cHL patients was subsequently established. Based on ultra-low pass sequencing of cfDNA from this cohort, we built a comprehensive catalog of genomic abnormalities, as well as their frequencies and patterns. Besides the known recurrent imbalances, such as gain/amplification of 2p16/*REL-BCL11A* and 9p24/*JAK2-CD274-PDCDLG2*, novel recurrent abnormalities were identified in cHL. Altogether, we have provided evidence that cHL is characterized by consistent and recurrent genomic imbalances and we have shown the potential of genomic profiling of cfDNA as a novel and non-invasive tool in the diagnosis and follow up of cHL patients.

Key words: circulating cell-free DNA, liquid biopsy, genomic profiling, DNA sequencing, Hodgkin lymphoma

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Introduction

Cancer is a genetic disorder driven by accumulating genetic abnormalities, including oncogenic DNA mutations and chromosomal defects in somatic cells [1]. Acquired aberrations result in deregulated expression of involved genes (oncogenes and tumor suppressor genes) and consequently lead to malignant transformation of affected cells. To date, more than 500 driver genes, activated or inactivated by genetic and epigenetic aberrations, are known to be involved in carcinogenesis.

The major source of neoplastic material used for cancer research have been traditional biopsies taken for diagnostic purposes. Sampling tumor tissue, however, is invasive, associated with procedural risks, sampling errors and the potential inability to capture special heterogeneity in the setting of multifocal disease. Over the last two decades, another fraction of tumor DNA known as circulating cell-free tumor DNA, has attracted attention of the scientific community. Circulating cell-free DNA (ccfDNA), first reported by Mandel and Metais in 1948 [2], comprises genome fragments that float freely through the bloodstream. This

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fraction of DNA became particularly interesting when it was discovered that fetal DNA, mainly originating from placental trophoblast cells, can cross the placenta and be identified in the plasma of pregnant women [3]. This revolutionary finding launched the era of non-invasive prenatal testing (NIPT), a screening of fetal genome in maternal ccfDNA using next generation sequencing [4, 5]. Interestingly, it appears that tumors also shed DNA to the circulation and that ccfDNA of patients with cancer contains circulating tumor DNA (ctDNA) which can serve as a 'liquid biopsy' [6].

The liquid biopsy' terms a minimally invasive technique of extraction of tumor-derived material (circulating tumor cells, as well as DNA, RNA, microRNA and proteins) from non-solid biological tissue like blood, saliva and urine, for further genomics and proteomics analysis in patients with cancer. Circulating cfDNA fragments are stable in circulation, but their half-life is relatively short. DNA is shed primarily through apoptosis and necrosis, but also through secretion. ccfDNA in the blood of healthy individuals is present at low levels, but is increased in oncological patients, being shed from both normal and malignant cells.

Given that ctDNA harbours cancer-specific modifications, including point mutations and chromosomal copy number abnormalities (CNA) (gains and losses), and sampling can be easily done at each stage of disease, interest of non-invasive diagnostic of tumors has grown enormously [7–12].

Detection of incipient tumors in pregnant women by screening of plasma cell-free DNA

NIPT has been recently widely applied in routine practice. Since then, occasional reports of discordant NIPT results indicating for detection of tumor-derived CNAs in plasma cfDNA of pregnant women have been published [13, 14]. In University Hospital Leuven, Belgium, NIPT was introduced in 2013. Over 6.5 years, more than 88,000 pregnant women underwent NIPTing. Application of the Genome-wide Imbalance Profile sequencing pipeline (GIPSeq) developed in our institution allows unbiased detection of CNA in cfDNA [5].

Upon analysis of the 88,294 NIPT results, 14 women without a previous medical history of cancer were identified with a GIPSeq result suggesting an occult maternal malignancy [15, 16]. The patients underwent further clinical investigations, including whole-body diffusion-weighted magnetic resonance imaging (WB-DWI MRI), which led to identification of maternal tumors in 12 pregnant women. In one case, the cancer diagnosis (primary mediastinal large B-cell lymphoma) was made three years after the detection of an aberrant result by NIPT/genome-wide imbalance profile sequencing (GIPSeq). More than 66% of tumors were of hematological origin, mainly Hodgkin (75%) and non-Hodgkin lymphomas (25%). One-third (33.3%) of cancers were solid tumors (ovarian and breast carcinoma, high grade osteosarcoma). In several cases with confirmed cancer diagnoses, further genetic analyses, using array comparative genomic hybridization (aCGH), fluorescence *in situ* hybridization (FISH), or low-pass whole genome sequencing (0.1×) of biopsy DNA, evidenced that the ccfDNA-detected CNAs represent genomic modifications of tumor DNA. In two patients with an aberrant GIPSeq profile and lack of suggestive malignancy, clinical follow-up was advised. Although the detected maternal malignancies were occult, it is possible that the symptoms of cancer (such as fatigue, nausea, abdominal discomfort, and vaginal blood loss) easily could have been misinterpreted as physiological gestational symptoms.

Genome-wide studies of classic Hodgkin lymphoma (cHL) using plasma cfDNA

Initial discovery and pilot study of cHL

The identification of genomic abnormalities in plasma cfDNA of a pregnant woman further diagnosed with early-stage cHL (Ann Arbor stage IIA) [15] was unexpected, because cHL is hallmarked by a minority of neoplastic cells (0.1–2%) amidst an overwhelming majority of non-malignant immune cells [17]. For this reason, detection of HRS cell-derived DNA in plasma was intriguing. The rarity of neoplastic cells in cHL tumor mass complicates the analysis of somatic genetic alterations in this malignancy and hampers the elucidation of its pathogenesis, biology and diversity [18]. Genetic features of HRS cells has been initially studied in HL-derived cell lines or sporadically in original tumor samples by FISH [19–21]. More recently, challenging attempts were undertaken to investigate genomics of cHL using HRS cells isolated by laser microdissection or cell sorting after whole genome DNA amplification [22–28]. The discovery that HRS-derived DNA is present in a patient's plasma opened a new avenue to remotely sample the HRS genome in a minimally invasive way and to impact HL research.

To validate our initial observation of HRS cells-related chromosomal imbalances in patient's ccfDNA [15], we undertook a pilot study of nine prospective cases of biopsy-proven nodular sclerosis Hodgkin lymphoma, which is the most frequent subtype of cHL [29]. Eight cases were recruited at time of diagnosis and one at first relapse. The patients presented at stage IIA (7/9) and IVB (2/9) disease. The collected ccfDNA was subjected to low-coverage massive parallel sequencing. The downstream analysis detected genomic gains and losses in eight cases. The most frequent gains, found in ≥5 cases, affected 2p (n =7), 3q, 5q and 9p (n =5), while recurrent losses detected in 4–5 patients involved 1p, 6q, 7q, 9q, 10q, 11q, 13q and 22q. Some of these imbalances were extensively validated by FISH with probes representing affected chromosomal regions on HRS cells from either cytogenetic harvests or

biopsy samples. All patients, including the pregnant woman, underwent chemotherapy with or without the involvement of node radiotherapy. All responded, as shown by early clinical evaluation. Importantly, GIPSEq analysis performed in subsequent samples taken between days 15 and 43 after treatment initiation, showed normal genomic profiles in all cases, confirming clinical observation of complete metabolic remission. To gain insights into mechanisms underlying an abundant release of ccfDNA by scarce HRS cells, we analyzed the expression of cell cycle indicator Ki67 and cleaved caspase 3 by immunohistochemistry (IHC) in all nine cases. Coexpression of both molecules in HRS cells and the presence of necrosis in biopsy samples suggest a high turnover of neoplastic HRS cells in cHL. The finding of most complex genomic profiles in both patients with advanced disease (stage IVB) suggests an association between the HRS cell burden and the level of HRS cell derived DNA in ccfDNA. These pilot data indicates for potential of ccfDNA profiling as a novel non-invasive tool for diagnosis and monitoring of the disease.

Landscape of copy number variations in cHL

After the technical proof-of-principle study proving that circulating cfDNA from HL patients contains circulating tumor DNA (ctDNA) from HRS cells, and that the DNA can be profiled by massive parallel sequencing [29], we established a large collaborative project of KU Leuven and the Lymphoma Study group (LYSA) of cHL on cell-free DNA [30]. The project aimed at screening of genomic imbalances in HRS cells using ultra-low pass sequencing of cfDNA in a large series of cHL patients. Between 2014 and 2018, we prospectively collected plasma cfDNA from 177 new cHL patients (mostly early stage). To profile genomic imbalances, cfDNA samples were sequenced at low coverage (0.26×) and subjected to downstream bioinformatic analysis. In addition, we attempted to estimate the clonal fraction of cfDNA (presumably derived from HRS cells) and analyze a possible correlation of ctDNA with the known prognostic risk factors and tumor burden, and monitor the CNA evolution after treatment initiation.

The subsequent genome-wide analysis of cfDNA from this cohort allows the construction of a comprehensive catalog of the types of CNA, their frequencies and patterns in cHL. More than 90% of patients (164/177) exhibited CNA in cfDNA. The remaining cases displayed balanced CN profiles at diagnosis, likely due to a low content of tumor fractions, below the detection threshold of the applied assay. CNA was detectable in 94% (140/149) and 92% (22/24) of cases with stage I–II disease and III–IV disease, respectively. Most cases (152/164) revealed complex profiles with five or more CNAs. The most prevalent gains affected 2p16 (69%), 5p14 (50%), 12q13 (50%), 9p24 (50%), 5q (44%), 17q (43%) and2q (41%). Genomic gain or amplification usually impacts expression of harbored genes, as show in the case of 2p16 and 9p24 amplification targeting the known lymphoma-related driver genes, *REL/BCL11A* and *JAK2/ /CD274/PDCDLG2,* respectively. Other candidate genes (unmutated or mutated) deregulated by genomic gains in cHL include *IL-10* (1q32), *XPO1* (2p15), *NFKB1* (4q24), *CASP6* (4q25), *CSF1R* (5q34) and *STAT6* (12q13). Genomic losses most frequently targeted 13q (57%), 6q25q27 (55%), 4q35 (50%), 11q23 (44%) and 8p21 (43%). The known tumor suppressor genes affected by losses of 6q and 13q include *TNFAIP3* and *FOXO1*, respectively. In addition, we identified loss of 3p13p26 and 12q21q24, and gain of 15q2-q26 as novel recurrent CNA in cHL.

FISH was used to validate genomic gains or losses detected in cfDNA. Using DNA probes representing the gained/lost regions of interest which were applied on stored cytogenetic harvests from 10 biopsies, we confirmed the imbalances in HRS cells of 9/10 cases. Notably, most CNAs detected in our series have been already reported in smaller studies on purified HRS cells or on tumor sections, but their occurrence and frequencies varied in different studies [19, 20, 22–25].

Our study, the largest study on CNA in cHL to date, provides a comprehensive landscape of CNAs with a reliable rating of their relative frequency. We found that occurrence of gain of 2p16, 5p14 and 12q13 and loss of 6q25 and 13q is similar to gain/amplification of 9p24 affecting *CD274/PD-L1* and *PDCDLG2/PD-L2*. The latter aberration is an important predictor for favorable outcome after anti-PD-1 driven immune checkpoint blockade, e.g. nivolumab [15, 31]. Significantly, we found loss of 3p13-p26 and 12q21-q24 and gain of 15q21-q26 as three novel non-random CNAs in cHL. Postulated candidate oncogenes harbored by chromosome 15q include several genes promoting cell proliferation and acting downstream of NF-κB, JAK-STAT and cytokine receptor signaling (*AKAP13, FES, STRA6* and *PIAS1*) and genes showing anti-apoptotic activity (*MAP2K1* and *MAP2K5*). Candidate tumor suppressor genes mapped on 3p and 12q include negative regulators of cell proliferation (*RHOA, VHL* and *TLR9*/3p, *SOCS2*/12q) and apoptosis (*TRAIP*/3p, *APAF1*/12q). Further investigations studying whether and how these novel CNAs are implicated in the pathogenesis of cHL are required.

In addition, we found that ctDNA concentration at diagnosis was associated with HRS cell burden and tumor mass volume. Notably, ctDNA and related CNA rapidly diminished upon treatment initiation, while persistence of CNA correlated with an increased probability of relapse.

Our study showed that cfDNA could be a gateway to the genome of HRS cells and serve as substrate for the monitoring of early disease response.

Altogether, we have provided evidence that cHL is characterized by consistent and recurrent genomic imbalances. The aberrations were detected by massive parallel sequencing of ccfDNA from 93% of patients with early and

advanced stages of newly diagnosed tumors. Besides the known recurrent gains and losses, loss of 3p13p26 and 12q21q24 and gain of 15q21q26 were identified as novel recurrent CNA in cHL. Genomic aberrations were correlated with disease burden and evolution. The rapid normalization of ccfDNA profiles on therapy initiation suggests a potential role for ccfDNA profiling in early response monitoring. These findings confirmed our pilot studies, and showed the potential of genomic profiling of cfDNA as novel and non-invasive tool at diagnosis and follow up of cHL patients. Moreover, our discoveries and those by other groups show several new possibilities for exploring the molecular pathogenesis of HL, as illustrated by recent publications [32–35] and have potential implications for the prospective clinical development of biomarkers and precision therapy for this tumor.

Authors' contributions

All authors contributed in the discussed research and writing of the article

Conflict of interest

The authors have no competing interests to declare.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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Hodgkin lymphoma: differences and differential diagnosis

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Abstract

Hodgkin lymphoma accounts for approximately 15% of all lymphomas. The World Health Organization 2017 classification lists two main types as separate entities: classic Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).

The morphology of neoplastic cells differs slightly in both types, while these cells in typical cases show a different immunophenotype. Overlapping histological images and immunophenotypes cause diagnostic difficulties and the need for detailed differential analysis. The classic form accounts for about 90% of cases and occurs in four histoclinical subtypes, requires differentiation from other lymphomas and immunoproliferation, and neoplasms with other differentiation. NLPHL occurs in six immunohistochemical patterns. An increase in the number of T lymphocytes and histiocytes, and a disappearance of the nodular structure, are associated with a more aggressive course and the possibility of transformation into diffuse large B-cell lymphoma, most often T-cell/histiocyte rich large B-cell lymphoma. Recent reports demonstrate the importance of stroma in maintaining tumor viability. Accurate histopathological diagnosis must be based on representative material that allows the assessment of the architecture of the tissue.

Key words: classic Hodgkin lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, gray zone lymphoma

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Introduction

Thomas Hodgkin presented a lecture entitled 'On some morbid appearances of the absorbent glands and spleen' at a meeting of the Medical and Chirurgical Society of London in January 1832, and then published a paper in Medical-Chirurgical Society Transactions. It is noteworthy that in the era of numerous infectious lesions of lymph nodes, Hodgkin distinguished a clinical course and an autopsy picture of the disease, which was named after him, and he did it without the benefit of microscopic examination. The name of Hodgkin's disease was finally introduced by Samuel Wilks in 1865, when he published material extended to include his own cases with clinical and pathological details [1].

Understanding of the disease continued to grow after the use of microscopic examination of tumors and the description of the histology of Hodgkin's disease by Theodor Langhans (1872) and characteristic diagnostic cells by Carl Sternberg (1898), and Dorothy Reed (1902) [2]. It took another 100 years to elucidate the neoplastic nature of the disease, detailed microscopic and immunophenotypic description, histological subtypes, and combining them with etiological factors and the clinical course. For the last

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20 years, researchers have been interested in the relationship between cancer cells and a niche microenvironment that they can create themselves [3–5].

General information

The current World Health Organization (WHO) 2017 classification, based on the histological picture and immunohistochemical staining, distinguishes two types of Hodgkin lymphoma (HL), i.e. the classic form, classic Hodgkin lymphoma (cHL), which accounts for about 90%, and the non-classical form, nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) [6–8]. Distinguishing these types allows for proper correlation with clinical data as well as different treatments. In the histological picture of both types, neoplastic cells constitute a small percentage of tissue and occur in their niche microenvironment.

Diagnostic for cHL are large multi-lobated Reed-Sternberg cells (RS) and mononuclear Hodgkin (H) cells, hence sometimes the joint term Hodgkin/Reed-Sternberg (H/RS) is used. Diagnostic for NLPHL are smaller cells with polylobular nuclei (LP) with nucleoli, commonly called popcorn cells [6, 7].

Neoplastic cells

Neoplastic cells of both types of HL originate from the B-cell germinal center line [2, 3, 9], but they present other differentiation stop points, and thus differ in antigens used in histopathological diagnostics, and recently also in the treatment of patients. In cHL, cells typically show the transcription factor PAX5 (low expression), CD30 and CD15, but negative CD45 and B-cell antigens, in contrast to NLPHL CD45 positive with B-cell antigens (PAX5-high expression, CD19, CD20, CD79a, OCT2 , BOB.1, J-chain) but CD15 and CD30 negative [6]. In some cases, the immunophenotypes differ from the typical ones, which makes diagnosis difficult.

Neoplastic cells, as cells of origin from the immune system, retain the ability to produce many active cytokines and interleukins, influencing the cellular composition of the stroma they surround them with [3, 4, 6, 7]. This influence translates into histological images, and these also into the clinical course.

Cell survival is possible thanks to stimulation of multiplication, blocking apoptosis and creating a useful microenvironment to protect against the immune system. Genetic studies have shown activations of signaling pathways, especially JAK/STAT, NF-kappaB, P13K/AKT, which affect the cell cycle and block the apoptotic pathways. Changes in PDL1/PDL2 genes affect the regulation of the immune system through check point inhibition, in which T helper lymphocytes, NK cells and macrophages are involved [3, 4, 10–13]. H/RS cells can transfer important receptor proteins, PDL1 ligands, to other cells in the process of trogocytosis, blocking the aggressive action of the immune system [14]. Epstein-Bárr virus (EBV) is involved in the etiopathogenesis of cHL through the epigenetic action of the LMP1 and LMP2 proteins [15–17]. Contrary to the classical form in NLPHL, EBV is not detected in neoplastic tissue, but recent reports indicate the similarity of *Neisseria catarrhalis* and its possible involvement in the pathogenesis of NLPHL similar to *Helicobacter pylori* in gastric marginal lymphoma [18].

Classic Hodgkin lymphoma

The WHO classification distinguishes cHL in its classical form into four histo-clinical subtypes:

1) nodular sclerosis (NSCHL);
2) mixed cellularity (MCCHL);
3) lymphocyte-rich (LRCHL);
4) lymphocyte depleted (LDCHL).

Nodular sclerosis classic Hodgkin lymphoma (NSCHL) is characterized by intensive fibrosis of the stroma causing thickening of the lymph node capsule and division of the tissue into nodules. H/RS cells take the form of lacunar cells and are often found in diffuse areas with necrotic foci. There are numerous eosinophils and histiocytes in the background. In a longer-lasting disease, the stromal lesions may be so strong that the neoplastic tissue disappears and, with small sections, diagnostic material may not be obtained. The diagnosis becomes a challenge for the surgeon and pathologist. This is the most common type of cHL (70%) in countries with higher socioeconomic status. Young people (especially in II–III decade) suffer most often, men and women with similar frequency. The disease most often affects the mediastinum and is often diagnosed during X-ray screening. The prognosis is better than for the other subtypes [6, 7, 10].

Mixed cellularity (MCCHL) subtype accounts for about 25% of the cHL. In the histological picture, it is characterized by diffuse mixed-cell stroma with dispersed H/RS cells, most often with typical morphology. In this type, a bimodal peak in incidence is observed among children and adults aged over 60. Of all the subtypes, it is most often associated with EBV infection (about 75%); in developing countries it affects children and is EBV positive. It occurs more often in patients with HIV infection and immunodeficiency. The group of patients is dominated by men (c.70%). Peripheral lymph nodes are most often affected. The prognosis is intermediate between NSCHL and LDCHL [6, 7, 10].

Lymphocyte-rich (LRCHL) subtype accounts for approximately 5% of cHL cases, and is characterized by the presence of dispersed H/RS cells in nodular, less often diffuse, small lymphocyte-abundant stroma. The histological picture requires differentiation from NLPHL, especially that in immunohistochemical staining, antigens characteristic for NLPHL, such as transcription factors BOB.1, OCT2, bcl6, may be present atypically. EBV antigens are less common

than in MCCHL, but more often than in NSCHL. The disease affects peripheral lymph nodes in patients most commonly in their 30 s, with a 2:1 male predominance. The prognosis is better than for other cHL subtypes [6, 7, 10].

Lymphocyte-depleted (**LDCHL**) subtype is the rarest among cHL and accounts for less than 1%. It is associated with HIV infection and similarly to MCCHL with EBV latent infection. In the histological structure, numerous atypical forms of H/RS cells are present, the stroma is dominated by histiocytes and fibrosis, and lymphocytes are sparse. From the point of view of a pathologist, other neoplasms, including sarcomas, should be considered in everyday work. The age of patients varies widely, the disease occupies the retroperitoneal space, and bone marrow involvement is frequent. The prognosis is worse than in the other cHL subtypes [6, 7, 10].

Establishing a diagnosis of cHL subtype requires appropriate diagnostic material, preferably surgical biopsy. Core-needle biopsy and fine-needle aspiration biopsy can be used only at the initial stage of tumor diagnosis in order to direct further diagnosis [non-Hodgkin lymphoma (NHL), HL, melanoma, carcinoma, sarcoma]. Flow cytometric diagnosis is not efficient in the diagnosis of HL [10], but it is useful if there is a need to differentiate from selected NHL. WHO classification allows the diagnosis of cHL without specifying the subtype, but requires differentiation from NLPHL [6].

Differential diagnosis of cHL

In typical images and immunophenotypes, making an accurate diagnosis is quite simple. Overlapping histological images and the appearance of atypical antigenic constellations is a challenge for a pathologist. WHO 2017 has accepted a new entity: B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma and cHL also called gray-zone lymphoma (GZL), especially for mediastinal tumors that share common features for cHL and primary mediastinal large B-cell lymphoma, co-express CD20 and CD30, which can be used in the treatment of patients. Molecular studies indicate a closer association of the gray-zone lymphoma with cHL [6, 7, 10]. However, GZL does not release the pathologist from trying to diagnose both entities if possible.

Other diseases requiring differentiation from cHL are EBV-dependent lymphomas and lymphoproliferation, such as EBV-positive diffuse large B-cell lymphoma (DLBCL), muco-cutaneous ulcer, and polymorphic forms of lymphoproliferation associated with age-related or iatrogenic immunosuppression. Anaplastic large cell lymphoma, especially ALK negative, as well as peripheral T-cell lymphoma may be a diagnostic problem [9, 10].

Apart from neoplasms of the immune system, the initial diagnosis should take into account neoplasms with other differentiations, in particular melanoma metastases into lymph nodes. Thanks to immunohistochemical staining, diagnostics is possible. LDCHL may be more difficult in a few cases to differentiate sarcomas in which the CD30 antigen may be present.

Non classic Hodgkin lymphoma — nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)

NLPHL accounts for c. 5–10% of all HLs. It has a separate histological picture in relation to cHL, its own immunophenotype, it is EBV-independent, and it requires differentiation from other tumors. It typically presents as a long-lasting peripheral lymphadenopathy with no systemic symptoms. The mediastinal and mesenteric nodes may be involved in a few cases. Patients usually present first and second stage disease. Long-lasting disease is associated with a more aggressive course [6, 10].

In the histological picture, LP cells constitute a small percentage of cells with the immunophenotype mentioned above. Six immunohistochemical patterns have been distinguished, of which C-D-E are associated with the appearance of T lymphocytes in the background, in the E pattern resembling T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) [8, 19]. Differentiation of diffuse NLPHL with abundance of T lymphocytes and macrophages from THRLBCL is difficult and sometimes impossible in the absence of even one typical nodule. Transformation of NLPHL into DLBCL has been reported in recent actuarial studies in up to 30% of cases, especially into THRLBCL [8]. Transformation is now believed to be a continuum of changes depending on the environment in which lymphoma cells exist.

The WHO classification indicates that the presence of one common nodule is sufficient to diagnose the disease as NLPHL, and the presence of diffuse T-cell and histiocyte-rich tissue should be noted in the report as an E-pattern [6, 10]. A diagnosis of THRLBCL is possible when the histological picture shows the presence of dispersed single large B cells, CD20 positive, sometimes resembling H/RS cells in a background rich in T lymphocytes and/or histiocytes and without small B lymphocytes [6, 7]. Genetic and molecular studies do not differentiate tumors. Due to diagnostic difficulties, some clinicians believe that the stage of disease is a more important prognostic and predictive factor than the histological picture of NLPHL [10]. In advanced cases, patients require intensive treatment.

Conclusions

The development of diagnostic methods over the years has allowed for the expansion of information about HL and the separation of cHL from NLPHL. These diseases have different etiological factors, a different histological picture and immunophenotypic profile, and they require differentiation

from other neoplasms and lymphoproliferations. Diagnostics requires obtaining representative material, preferably the entire lymph node or a surgical biopsy sample. Current knowledge allows for the application of different therapeutic procedures. Contemporary treatment is based not only on the cytotoxicity of drugs, but also on interference in the microenvironment in which cancer cells live.

Author's contributions

AG — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Treatment of Hodgkin lymphoma relapse after autologous hematopoietic cell transplantation

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Abstract

Despite the high response rate to first-line treatment, approximately 10% of patients with Hodgkin lymphoma (HL) develop primary resistance to chemotherapy, and 10–30% of patients experience relapse. Today, salvage chemotherapy with subsequent autologous stem cell transplantation (ASCT) remains the standard of care for those patients with relapsed/refractory HL (RRHL). Treating patients with HL who relapse following ASCT continues to be a difficult clinical challenge. For many years, allogeneic hematopoietic stem cell transplantation was the only therapeutic option in this patient population. The last decade has brought new treatment options for RRHL patients with immunotherapy, including: brentuximab vedotin anti-CD30 monoclonal antibody, or the checkpoint inhibitors nivolumab and pembrolizumab, or advanced immune therapies such as bispecific antibodies, or chimeric antigen receptor T-cell therapy.

Key words: Hodgkin lymphoma, brentuximab vedotin, nivolumab, autologous stem cell transplantation, allogeneic stem cell transplantation

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Introduction

Hodgkin lymphoma (HL) accounts for approximately 10% of all diagnosed lymphoproliferative disorders [1]. Most patients with HL achieve disease remission after first-line chemotherapy, but c.5–10% of patients remain refractory to treatment, while 10–30% of patients are found to have rapid relapse of the disease (RRHL) [2].

The standard of care for patients with RRHL continues to be salvage chemotherapy with subsequent autologous stem cell transplantation (ASCT) [3, 4]. However, more than 50% of patients undergoing ASCT are found to have disease progression or relapse. Over the years, multivariate prognostic models have been developed to assess survival probabilities in RRHL patients undergoing ASCT. Poor prognostic factors include: relapse <12 months after the completion of first-line treatment, primary refractory disease, the number of prior lines of therapy, time from diagnosis to ASCT, extra-nodal disease, cancer stage, anemia, and the presence of B cell symptoms at relapse [5–7].

Chemosensitivity prior to ASCT is crucial in the patient population with primary refractory HL. A study by Moskowitz et al. [8] found a difference in 10-year overall survival (OS) of 66% vs. 17% in patients who were chemosensitive compared to chemoresistant prior to ASCT. Similarly, Sirohi et al. [9] demonstrated the significance of the depth of response to therapy before ASCT, highlighting the 10-year OS of 72% for patients who achieved complete remission (CR), 54% for patients who achieved partial remission (PR), and only 11% for patients who were chemoresistant [9]. Treatment of relapse after ASCT in patients with HL remains a major clinical challenge, with allogeneic stem cell transplantation being the only therapeutic option in this patient population [10].

Encouragingly, the 2010s brought new treatment options for RRHL patients through immunotherapy including

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BV anti-CD30 monoclonal antibody, or the immune checkpoint inhibitors nivolumab and pembrolizumab, or advanced immune therapies such as chimeric antigen receptor T-cell (CAR-T), or bispecific antibody therapy.

Today, immunotherapy is a promising and increasingly popular treatment option for cancer patients. The genome of cancer cells contains numerous mutations and epigenetic modifications that lead to the generation of immune tolerance and inhibition of the anti-tumor immune response. Therefore, the goal of immunotherapy is to break down immune tolerance of cancer cells and develop optimal immune responses to the tumorigenic process. This review summarizes the current therapeutic options in the HL patient population with relapse following ASCT.

Brentuximab vedotin

Brentuximab vedotin (BV) is an anti-CD30 monoclonal antibody that was approved in 2011 by both the Food and Drug Administration and the European Medical Agency for the treatment of patients with HL who relapse after ASCT, or those who have not responded after two lines of therapy and are not eligible for ASCT. Results of phase II clinical trials demonstrated the efficacy and safety of BV therapy in 102 patients with RRHL following ASCT. Encouragingly, the overall response rate (ORR) was 75%. 34% of patients achieved metabolic complete remission (mCR) [11], and the 5-year OS and progression-free survival (PFS) were 41% and 22%, respectively [12]. BV therapy has also been shown to play an important role in first-line treatment in combination with standard chemotherapy options [13–15].

Consolidation treatment is used in lymphoma patients to maintain and strengthen the response to therapy. Optimal consolidation treatment should have a low toxicity that will not affect hematopoietic recovery after ASCT. The efficacy of BV for patients with high-risk HL in the early consolidation phase following ASCT was demonstrated in the phase III AETHERA trial. Patients who were included in this study had at least one of the following risk factors: primary refractory HL (no CR after first-line treatment), relapse of HL <12 months after completion of therapy, or an extra-nodal disease location [16]. The 5-year PFS in the group receiving BV was 59% compared to 41% in untreated patients [17].

The results of the AETHERA trial contributed to the revision of both the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network guidelines for the management of post-ASCT HL patients. According to the ESMO, consolidation with BV after ASCT is recommended in patients with at least one of the following: primary refractory HL, disease relapse <12 months after treatment completion, or extra-nodal disease. Consolidation treatment with BV after ASCT is not recommended in cases of prior BV resistance [18]. Retreatment with BV in patients with HL and in patients with anaplastic large cell lymphoma resulted in an ORR of 60% with an mCR rate of c.30%, with a median response time of 9.5 months [19].

Checkpoint inhibitors

Immune disorders that stimulate Reed-Sternberg cells to overproliferate play a significant role in the pathogenesis of HL. The amplification of chromosome 9 (9p24.1) has been shown to lead to dysregulation of PD-1 ligand and JAK2 kinase [20]. The PD-1 receptor is expressed on activated T and B lymphocytes, NK/T cells, and monocytes as a result of antigen binding by the T or B cell receptor (TCR or BCR). The primary function of PD-1 is to inhibit the activity of T cells and reduce their effector functions, by inhibiting the production of IFN-gamma, IL-2 and TNF-alpha. The presence of PD-1 ligands on Reed–Sternberg cells and tumor-associated macrophages, as well as the expression of PD-1 receptors on T cells, suggest a significant suppression of T cells due to PD-1/PD-L1/PD-L2 interactions, and justifies the use of a PD-1 blockade in the treatment of cancer. As a therapeutic measure, PD-1 inhibitors will block the attachment of PD-L1 and PD-L2 ligands to the PD-1 receptor, thereby enhancing the T-cell anti-tumor response [21].

Nivolumab and pembrolizumab are two anti-PD-1 antibodies that have been approved for RRHL therapy. In a phase II clinical trial, nivolumab was used in HL patients after failed ASCT and BV treatment. The study population included 80 patients, with a median four different treatments (range 3–15), and an average of 16 nivolumab cycles that resulted in a PFS of 76.9% and OS of 98.7% at 6-month follow-up. The reasons for discontinuation of nivolumab therapy were: disease progression (16% of patients), stem cell allotransplantation (6%), and autotransplantation in 1% [22]. In the phase II CHECKMATE 205 trial, which included 243 patients with RRHL, nivolumab was used in three patient groups: patients not treated with BV, patients treated with BV after ASCT, and patients in whom BV was used before and after ASCT. The ORR in the entire study group was 69%, with an mCR of approximately 16%, and a one-year OS of 92% [23]. Following this successful trial, a subsequent analysis of the CHECKMATE 205 trial results confirmed the efficacy and safety of nivolumab therapy in patients with RRHL. After 18 months of follow-up, the 12-month OS was 98%, while the 31-month follow-up showed a median PFS of 15 months and OS rates of 86–90%, depending on the study group [24].

The phase III KEYNOTE-204 trial evaluated pembrolizumab versus BV in RRHL patients. PFS was 13.2 months for pembrolizumab and 8.3 months for BV. This study achieved an objective response rate of 65.6% in the pembrolizumab group and 54.2% in the BV group [25].

Currently, there are numerous trials investigating concomitant immunotherapy in patients with RRHL. The treatment of BV with nivolumab resulted in an ORR of 95% and an mCR of 65% [26]. Adding ipilimumab, an anti-CTLA-4 antibody, to BV and nivolumab resulted in ORR in 95% and mCR in 84% of cases. However, triple immunotherapy is not currently recommended due to potential grade 3 immunological complications [27].

Allogeneic hematopoietic stem cell transplantation in era of new drugs

Allotransplantation of hematopoietic stem cells continues to play a role in the therapy of patients with multidrug-resistant HL. Historical analyses have shown that patients with HL who received myeloablative treatments had a 50% non-relapse mortality (NRM) rate, although the use of reduced intensity conditioning regimens significantly reduced NRM and prolonged both PFS and OS [28, 29]. However, even with the introduction of novel targeted molecules such as BV and PD-1 inhibitors which facilitate long-term remissions, there is still a group of patients for whom allo-HSCT is the only therapeutic option. Interestingly, novel drugs such as nivolumab and pembrolizumab used before and after allotransplantation may actually increase complications, with PD-1 inhibitors shown to increase the risk of veno-occlusive liver disease and graft-versus-host disease (GvHD) when administered shortly before allotransplantation. Moreover, the risk of acute GvHD is higher in patients who receive PD-1 inhibitors after allotransplantation [30, 31].

As yet, the optimal time between the discontinuation of PD-1 inhibitors and the execution of allo-HSCT has not been determined, and there is still discussion around whether a patient who achieves CR, or at least PR, after PD-1 inhibitor therapy should receive consolidation treatment with allo-HSCT. Six weeks appears to be a safe period after the discontinuation of PD-1 inhibitor therapy. The authors of the recommendations also address the situation of using PD-1 inhibitors after allo-HSCT in the case of relapsed disease. In this patient population, a reduced-dose PD-1 inhibitors therapy is recommended [32]. Post-transplant cyclophosphamide as GvHD prophylaxis is recommended in HL patients undergoing haploidentical stem cell transplantation [33].

New therapies in RRHL

CAR-T therapy

CAR-T therapy is a novel, advanced genetic engineering technology that represents a new therapeutic option for patients with RRHL. In a phase I/II clinical trial with 22 RRHL patients after multiple lines of treatment, 53% of patients achieved CR within six weeks following CAR-T administration. However, cytokine release syndrome (CRS) developed in four patients during the trial (three with grade 1 disease and one with grade 2 disease) [34]. In another phase I/II trial, when CD30. CAR-T therapy was preceded by lymphocyte depletion with fludarabine, ORR was achieved in 72% of RRHL patients, with a CR rate of 59%. Most patients in the study population had been previously treated with ASCT, BV, and PD-1 inhibitors, with a one-year PFS of 36% [35]. Again, CRS was observed in 24% of patients from this trial, most commonly at grade 1. In patients who show progression after CAR-T treatment, retreatment with PD-1 inhibitors has been implemented with fairly good results [36]. Current research is evaluating CAR-T therapy based on the co-expression of CD30 and CCR4 (CD30.CCR4.CAR-T) [37]. Finally, CAR-T therapy that targets latent membrane proteins 1 and 2 (LMP1 and LMP2) has also shown encouraging results, with an ORR of 62% and a CR of 52% in patients with refractory lymphoma [38].

JAK2 inhibitors

A common disorder in HL is amplification of chromosome 9p24 that leads to dysregulation of the JAK2 signaling cascade, and contributes to abnormal cell proliferation [39]. Therefore, JAK2 inhibition has emerged as another potential target for therapy in the RRHL patient population. The JAK1/2 inhibitor ruxolitinib was used in a phase II trial in patients with RRHL, although the resulting ORR was 9.4%, response duration was only 7.7 months, and the PFS was 3.5 months [40]. It has therefore been concluded that ruxolitinib monotherapy has no benefit for patients with RRHL. It is possible that the use of JAK1/2 inhibitors in combination with other treatment options will allow for better therapeutic outcomes.

Novel antibodies

Camidanlumab tiserine is an anti-CD25 antibody conjugated with cytotoxic agent pyrrolobenzodiazepine. In a phase II study, this antibody was administered to 51 heavily pretreated HL patients with a median seven previous lines of therapy including BV and PD-1 inhibitors. ORR and CR rates were 83% and 38.3% respectively.

A bispecific, tetravalent AFM13 antibody directed against CD30 and CD16 antigens is undergoing phase I trials. Its mechanism of action is based on activating NK cells through the CD16 antigen and on Reed–Sternberg cells through the CD30 antigen. In a phase Ib trial, AFM13 was used in combination with pembrolizumab in patients with RRHL not previously treated with PD-1 inhibitors. The ORR in the study group was 87% and the CR was 39%, making this a promising therapeutic option in need of further development [41].

BTK inhibitors

The use of Bruton's tyrosine kinase (BTK) inhibitors in patients with RRHL is also the subject of clinical trials. The literature reports a therapeutic effect of ibrutinib in patients

with RRHL after allo-HSCT [42]. Ibrutinib in monotherapy, as well as in combination with BV or nivolumab, is the subject of phase II trials.

Conclusion

In recent years, the therapeutic approach to patients with RRHL has changed with the introduction of new molecules using immunological mechanisms of action. The ASCT procedure is still the primary treatment for RRHL, but with new therapeutic tools such as BV, many patients are achieving optimal response prior to hematopoietic stem cell transplantation.

Moreover, consolidation therapy after ASCT significantly improves outcomes in patients with high-risk HL. Advanced genetic engineering technologies such as CAR-T, or novel bispecific antibodies with a complex mechanism of immune action, provide a potential therapeutic option for multidrug-resistant patients after ASCT and allo-HSCT, but there is a need for further development.

Author's contributions

Equal contribution in design of the article and preparing manuscript.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Management of nodular lymphocyte-predominant Hodgkin lymphoma: recommendations and unresolved dilemmas

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Abstract

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare malignancy of young adults characterized by an indolent and recurrent course. Although relapses or transformation to aggressive B cell lymphoma can occur decades after the primary diagnosis, the prognosis of NLPHL is relatively good, with as much as a 90% 10-year overall survival rate. The rarity of NLPHL makes it difficult to conduct multicenter prospective studies to establish separate guidelines for the diagnosis and treatment of this disease.

Therefore, the recommendations for the treatment of NLPHL have for many years been the same as for classic Hodgkin lymphoma, except for early stages without risk factors. The registration of anti-CD20 monoclonal antibody for the treatment of CD20-positive B-cell lymphomas has opened up new perspectives for NLPHL patients. Modern and accurate histopathological examinations as well as imaging diagnostics, especially positron emission tomography/computed tomography has allowed a more precise distinction to be made between the indolent NLPHL and the transforming-to-aggressive lymphoma forms. This review is intended to provide readers with the clinical features, course, outcome and methods of standard treatment in patients with NLPHL. The author in particular wishes to draw attention to unresolved issues regarding standard management and also the use of active surveillance, anti-CD20 immunotherapy, less aggressive regimens of chemotherapy, and indications for new treatment options.

Key words: NLPHL, radiotherapy, immunochemotherapy, new agents

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Introduction

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) accounts for 5% of Hodgkin lymphoma (HL). In contrast to classic Hodgkin lymphoma (cHL), it is characterized by an indolent and recurrent course. Although late relapses and transformation to diffuse large B cell lymphoma (DLBCL) can occur, NLPHL prognosis is relatively good. Based on long term data from multicenter registries, 10-year overall survival is 57–99% depending on the clinical stage of the disease and the time of treatment initiation (i.e. whether within 12 months of diagnosis) [1]. Unlike cHL, NLPHL relapses can occur many years after initial or subsequent lines of therapy. NLPHL is a rare neoplasm, with a crude incidence in Europe of 2.3 per 100,000 per year [2]. In 2018, 659 cases of HL were registered to the Polish National Cancer Registry. This corresponds to up to 30 new cases per year of NLPHL in Poland [3]. Adequate surgical biopsy of lymph node for formalin-fixed sample is required for a diagnosis of NLPHL. In the histological examination malignant cells termed lymphocyte-predominant cells (LP cells, popcorn cells)

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are located in the background of small B lymphocytes, rosetting PD1+ T-cells, epithelioid histiocytes, and follicular dendritic cells. LP cells are germinal centers of origin with B cell markers expression (positive for CD20, CD45, CD79a, PAX5, OCT2, BOB1, BCL6; negative for CD15 and CD30).

According to the 2016 revision of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues, the variant of growth pattern should be noted in the diagnostic report: 75% of NLPHL cases show typical nodular growth pattern (classical B-cell rich nodular: A or serpiginous: B), but 25% present different histology patterns (C, D, E, or F) with diffuse infiltrates or nodules dominated by surrounding T-cells. Cases of typical histological growth patterns demonstrate localized disease and a better prognosis in terms of increased response rate and progression-free survival (PFS) compared to variant histology patterns. NLPHL of variant histology has to be carefully distinguished from aggressive B-cell T cell-rich lymphoma [4, 5].

NLPHL is typically recognized in children and young males aged 30–40. Approximately 75% of cases are limited according to Ann Arbor stage I/II with lymph nodes involvement. Mediastinal, extranodal, bulky disease or B symptoms are uncommon [1, 6, 7].

Transformation to aggressive B cell lymphoma is a constant feature of NLPHL. It may be present at initial diagnosis simultaneously with the NLPHL pattern. Therefore, representative surgical biopsy is essential for a reliable diagnosis.

The risk of transformation to aggressive B cell lymphoma has been reported to be 31% over 20 years of NLPHL follow up [8]. Because the transformation can occur at any relapse and in follow up, even decades after the initial NLPHL diagnosis, re-biopsy is necessary whenever a relapse is suspected.

NLPHL still poses a challenge for hemato-oncologists. Treatment recommendations by the European Society of Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) are partly in line with cHL guidelines. But indolent presentation of NLPHL could favor non-aggressive approaches such as surgical resection with a watch-and-wait strategy (IA), local radiotherapy, or rituximab monotherapy [2, 9]. On the other hand, the tendency for recurrence and the occurrence of transformations may require more aggressive treatment: immunochemotherapy with rituximab (R) combined with radiotherapy (RT) [10]. The rare occurrence of NLPHL, and the unavoidably long follow-up, somewhat preclude organizing prospective trials which could result in establishing evidence-based recommendations for first or salvage lines of treatments. This review discusses the current treatment options for patients with NLPHL.

Staging and risk factors

Clinical staging of NLPHL is based on the Lugano classification based on Ann Arbor staging with Cotswolds modification [11]. The risk groups are often extrapolated from cHL according to EORTC/LYSA and German Hodgkin Study Group (GHSG) scales [2]. Additionally, the German Study Group has proposed a prognostic scale including three adverse risk factors: male gender (2 points); low serum albumin level (1 point); and variant growth pattern C–F (1 point). Patients are divided into three risk groups: low (0–1 point); intermediate (2 points); and high (3–4 points) with estimated 5-year PFSs of 95%, 88% and 69% respectively. The histopathology growth pattern is an independent prognostic factor of progression or relapse, but it has not influenced the choice of NLPHL treatment thus far. The same applies to other points of the scale [5]. Also, age above 45 years at presentation, advanced stage, low hemoglobin level, and systemic symptoms are considered adverse factors in terms of overall survival (OS) [6, 8]. The risk of transformation to aggressive B cell lymphoma is approximately 7% in 10 years of observation, and 31% in 20 years of NLPHL follow up. The risk is higher in bulky disease and splenic involvement [8].

In everyday clinical practice, the type of therapy for newly diagnosed NLPHL is stratified according to disease stage and risk factors. Three groups are distinguished: non-bulky (<10 cm) early stage NLPHL; early stage; and advanced disease. In non-bulky early stage, NLPHL is in clinical stage (CS) I or II with contiguous disease without related symptoms and threat of organs compromise. CSs III and IV according to the Ann Arbor scale are defined as advanced NLPHL. Early stage with risk factors (intermediate risk group) are situations in between.

Recommendations: first line treatment

Early stages without risk factors: CS IA/ /contiguous IIA

Approximately half of patients with NLPHL are early stage without risk factors. According to the ESMO recommendation for patients with CS IA, standard treatment is ISRT (involved site radiotherapy) 30 Gy alone. Based on retrospective data of the GHSG, 8-year PFS and OS for patients with stage IA is 91.9% and 99% for involved field radiotherapy (IF-RT) [12]. Patients with CS IIA contiguous disease treated with radiotherapy alone also have good prognosis.

Moreover, observation after complete excision of lymph node can be considerable for selected groups of patients in early stages without risk factors. Retrospective studies show a 10-year OS rate of 91% in these circumstances [13].

Early stages with risk factors and advanced stages

There are several options for induction treatment of NLPHL patients in the early stages with risk factors and in advanced stages. ABVD chemotherapy can be considered. The GHSG recommends interim-PET/CT guided eBEACOPP for advanced stage [14]. Others suggest the use of

chemotherapy with anti-CD20 antibody RCHOP [10] or in some selected cases RCVP [15] or R bendamustine [16]; these issues are discussed later. Immunochemotherapy lasting 3–4 months with or without radiotherapy can be considered in early stages; longer treatment should be applied in advanced stages. According to the NCCN guidelines, observation is advised for advanced NLPHL asymptomatic patients after making individual decisions.

Transformed NLPHL

In a case of upfront transformation, RCHOP is recommended with efficiency comparable to induction strategy for DLBCL [13].

Evaluation of response after first-line treatment

According to the Cheson criteria, PET/CT should be incorporated in the staging and assessment of efficacy of induction therapy. If radiotherapy was planned, contrast-enhanced CT is mandatory in the staging period. The re-biopsy of suspected NLPHL sites should be obtained in cases of stable or progressed disease after initial treatment. Also, the verification of recurrence by histopathological examination is essential to exclude transformation to aggressive cHL in further course of the disease.

Recommendations: relapsed NLPHL

The initiation of salvage therapy must be preceded by the histopathological verification of NLPHL recurrence. To precisely describe the clinical stage of the relapsed disease, contrast-enhanced CT of the neck, chest and abdomen with pelvis, or PET/CT, is recommended.

In localized relapses, radiotherapy can be used. Also, monotherapy with rituximab can be considered [17]. Salvage systemic therapy has to be chosen individually, according to several factors: patient performance status, extent of disease relapse, disease symptoms, and type of previous treatment [18]. The optimal chemotherapy regimen used for the second line in NLPHL is not defined. The implementation of anti-CD20 monoclonal antibody is essential for cases with no previous anti-CD20 exposure and if relapse is more than six months after prior anti-CD20 therapy. The role of autologous transplant (AHCT) in not clearly defined in recommendations, but it remains a PET/CT guided therapeutic option, the same as in cHL [9]. Patients with transformation to DLBCL should be managed according to recommendations for relapsed/refractory DLBCL. In that case, the role of AHCT is clear.

Unresolved dilemmas in treatment of NLPHL

Early stages

Various treatment options have been evaluated in the early stages. According to ESMO and NCCN, radiotherapy alone is the standard treatment for early favorable NLPHL. Several reports show that the addition of chemotherapy or other variants of induction strategy in early favorable stages do not improve patient outcomes [12, 19–22]. For instance: in a multicenter retrospective database of stage I/II NLPHL diagnoses over a 20-year period, the outcome of 559 patients was analyzed. Patients underwent radiotherapy, chemotherapy, combined modality treatment (radiotherapy with chemotherapy), observation after surgical excision, rituximab and radiotherapy or as a single agent. 5-year PFS was 87.1% in the entire group, and 5-year OS was 98.3%. 5-year PFS for different kinds of induction strategies were: 91.1% in the radiotherapy group; 90.5% after chemotherapy with radiotherapy; 77.8% after chemotherapy; 73.5% in the observation group; 80.8% after rituximab with radiotherapy; and 38.5% after rituximab alone [23].

For selected groups of patients, total surgical resection followed by a careful watch-and-wait strategy (active surveillance) is reasonable. This kind of management is purposely chosen in pediatric and young adult groups to avoid potential acute and long-term toxicity e.g. maturation disorders or secondary malignances. In 163 consecutive patients, 37 (23%) were observed. 23 of them were in early stage, and 14 advanced. 5-year PFS was 77% after active surveillance and 87% in the group receiving active treatment, with no difference in OS. With a median follow up of 69 months, only 10 patients in the watch-and-wait group required active treatment [24]. Also, in a French multicenter study of 164 adult patients, OS did not differ between the group actively treated or observed. With a median relapse rate of 3 years, 50% of observed patients remained without treatment, and with inferior PFS. OS was equal in both groups [13]. In a prospective pediatric trial (NCT00107198), patients younger than 22 with stage IA completely resected were observed carefully. In a case of relapse, 3 cycles of doxorubicin, vincristine, cyclophosphamide and prednisone were administered. Of 52 patients after complete surgical excision, 13 relapsed at a median of 11.5 months. 5-year OS was 100% [19]. Upfront monotherapy with rituximab alone is associated with high response and relapse rates [12, 23]. In the prospective GHSG study, 28 patients with stage IA received 4 weekly doses of rituximab with an objective response rate (ORR) of 100% (85% CR) but 3-year PFS was 81.4% [25]. In another prospective phase II trial, 39 patients with recurrent or newly diagnosed NLPHL were treated with 4 weekly doses of rituximab followed by observation or 2 years of maintenance therapy. After 4 weeks of induction, ORR was 100% with CR of 67%. 5-year PFS for the rituximab-only arm was 39% whereas for rituximab with maintenance it was 58.9% [26]. However, the potential role of rituximab alone in induction treatment may be supported by the high rate of responses and no severe grade 3/4 toxicity. Anti-CD20 antibodies can be

considered individually as induction treatment for early stage NLPHL for patients in poor performance status with concomitant diseases.

Advanced disease

There have been no randomized trials directly comparing induction chemotherapy regimens, and the data is based on retrospective observations or phase II trials. The upfront use of anti-CD20 antibody is strongly recommended due to the consistent expression of that antigen on the LP cells surface. Therefore, the recommendation for ABVD alone in advanced disease may not be as relevant as it used to be. Still, the choice of chemotherapy which should be combined with antibody remains debatable. Regimens of CHOP, ABVD, CVP or bendamustine are taken into account. The BEACOPP protocol is not recommended widely outside the GHSG. RABVD consists of rituximab at a standard dose on day 1 and classical ABVD every 14 days of a 28-day cycle. In a short report, ORR was 100% with one PR out of six patients [27]. Fanale et al. [10] reported 27 patients with newly diagnosed NLPHL (16 patients with CS III/IV) who achieved a CR rate of 89%, ORR of 100 % after induction RCHOP. Median follow-up of the group was 6.7 years, and estimated 5-year PFS was 89% with no transformation event during the observation.

There is no strong evidence to support the use of immunochemotherapy regimens recommended for follicular lymphoma such as RCVP and R-Bendamustine. Published data shows very small groups with individual cases of advanced disease, often without anti-CD20 antibody [15, 16]. NLPHL with constant CD20 expression on the malignant cells, indolent nature, watch-and-wait periods, late relapses, and a risk of transformation follows the clinical course of follicular lymphoma. Histological growth patterns A and B are favorable factors in terms of outcome. Therefore, it would be very interesting to conduct a trial exploring the role of these regimens in the induction treatment of classical variant NLPHL.

Relapsed NLPHL

The treatment of relapsed or refractory NLPHL remains undefined. The management of refractory disease depends on several factors associated with the characteristics of the disease (localized or disseminated relapse, time of relapse, transformed), with the patient's status (age, general condition, concomitant diseases) and with the type of previous treatment. There are no prospective studies comparing different salvage strategies in refractory and relapsed NLPHL. Re-biopsy is necessary to distinguish NLPHL from non-malignant lymphadenopathy or transformed disease. Patients with negative biopsy results should undergo active surveillance. Biopsy-proven NLPHL relapses can be asymptomatic as recurrent indolent lymphomas. Therefore, active surveillance can be appropriate in particular cases.

Another option is monotherapy with rituximab followed (or not) by two years of maintenance treatment. Prolonged administration of rituximab may result in a longer PFS period compared to four doses of rituximab alone, but results from the single small study were not statistically significant [26]. Other preferences for refractory NLPHL are: radiotherapy alone for limited relapse, and systemic salvage chemotherapy with or without rituximab in advanced disease according to the guidelines for diffuse large B cell lymphomas.

For young patients with a disseminated and refractory (<1 year) relapse, AHCT should be considered first of all. In a retrospective analysis of 26 patients transplanted five years previously, event-free survival (EFS) was 69 and OS 76% [28]. Even better results were reported by the GHSG. Among 31 transplanted relapsed and refractory patients, 5-year PFS was 84.6 and OS 89.8% [29]. The use of rituximab plays an important role in salvage strategy containing AHCT. Akhtar et al. reported 5-year EFS of 76% in transplanted NLPHL; after rituximab salvage 100%, without rituximab 56% [30].

Transformed NLPHL

Upfront transformation should be treated identically to DLBCL. Consolidation with AHCT in the first line is debatable. In a relapse setting, there is no standard management established, and treatment strategies are determined individually.

Modern treatment approaches

The role of modern targeted approaches in NLPHL is undefined. Only small groups of patients or case reports can be presented to outline the new treatment directions for relapsed or refractory NLPHL. Radioimmunotherapy selectively delivers radiation from radionuclides to tumor cells. In one prospective study, murine anti-CD20 antibody radiolabeled with Yttrium-90 (ibritumomab tiuxetan) was used in the treatment of CD20 positive relapsed lymphomas (including three patients with NLPHL). The ORR was 88% including 65% complete metabolic response [31]. A case report of 2 NLPHL patients in first relapse after rituximab showed well tolerated treatment with ibritumomab tiuxetan with no relapse during seven years of observation [32]. The IRENO phase II trial is conducted by the German Study Group to evaluate the efficacy and safety of ibrutinib in patients with relapsed NLPHL (NCT02626884) [33].

Lenalidomide is the agent which can block directly tumor growth and modulate tumor microenvironment by stimulating cytotoxic T cells and NK cells.

Individual cases of NLPHL or T cell histiocyte-rich large B cell lymphomas successfully treated with lenalidomide with or without rituximab can be found [34, 35]. There is no data regarding the use of check point inhibition in NLPHL. PD-L1 expression on LP cells is heterogenous. On the other hand, PD-L1 is located on the bystander

histiocytes especially in variant histology pattern E [5]. Very likely, clinical trials with immune check inhibitors will be conducted.

Conclusions

NLPHL is a unique entity in between HL and indolent follicular lymphoma. Due to its rarity, no randomized multicenter trials have been performed so far to establish separate straightforward treatment guidelines. Data derived from single-arm studies, national registries, retrospective series and subgroup analysis of HL trials, confirm its excellent prognosis. Therefore, the treatment of NLPHL has become less aggressive over time. This is to reduce acute and late toxicity including cardio-pulmonary complications or secondary cancers, especially because NLPHL often occurs in children and young adults. New treatment strategies have focused on limiting radiation, adding anti-CD20 immunotherapy to chemotherapy, and careful use of active surveillance as an alternative to immediate or delayed treatment. The cooperation of large multidisciplinary diagnostic and therapeutic groups would be advisable to establish modern clear standards of NLPHL management and to develop new treatment strategies.

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EP-K — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Improving first-line treatment in diffuse large B-cell lymphoma

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Abstract

R-CHOP remains the standard of care in first-line treatment of diffuse large B-cell lymphoma (DLBCL), the most common lymphoma subtype. Patients who fail this therapy have a poor outcome, with relapse or refractory disease resulting in fatality in the majority.

In this short paper, we summarize recent clinical studies exploring alternative regimens and efficacy of autologous stem cell transplantation (ASCT) consolidations.

In ABC DLBCL, adequately identifying patients with poor prognosis but failed to recognize the patient for molecular target of therapy. Immunotherapy, which may potentially be used in less well genetically characterized patients, is most potent if used relative to chemotherapy protocols, therefore its optional combination remains to be determined. The hope is ultimately to move away from a universal chemotherapeutic mentality towards an individualized approach, be it through the use of a targeted small molecule or a biological drug.

We discuss the role of new monoclonal antibodies such as obinutuzumab, brentuximab vedotin, polatuzumab vedotin and bispecific antibodies (BIABs) in first-line treatment regimens. BIABs which can bind to two different antigens at the same time are under investigation. After neurotoxic blinatumomab, anti-CD20/anti-CD3 BIABs take the lead, and due to their favorable toxicity profile they can be used in elderly patients with comorbidities, causing durable responses in patients with B-cell non-Hodgkin lymphoma who otherwise have limited options, even in those relapsing or refractory to chimeric antigen receptor (CAR) T-cell therapy.

Key words: DLBCL, targeted molecular therapies, immunotherapy

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Diffuse large B-cell lymphoma (DLBCL) belongs to a group of aggressive lymphomas which, if left untreated, progress rapidly and shorten the lives of patients. First-line immunochemotherapy, usually curative in aim, should be implemented as soon as possible.

The first effective regimen was suggested in 1973 by the 'NCN gang of five' (Canellos, Chabner, Schein, DeVita and Young) when doxorubicin was added to the CVP (cyclophosphamide, vincristine, prednisone) regimen. CHOP is the single most effective protocol, and is still used nearly 50 years later with only a few modifications. With its significantly prolonged progression-free survival (PFS) and overall survival (OS), the results hold up well against so-called second generation chemotherapy regimens [1]. Introducing rituximab, a chimeric anti-CD20 monoclonal antibody, was a breakthrough, and has been the only widely accepted CHOP modification so far. This significantly increased the complete response (CR) rate, and improved 10-year PFS and OS [2].

R-CHOP-14, an attempt to intensify the R-CHOP regimen in elderly patients by shortening the interval between

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cycles to 14 days, despite initial German results, proved discouraging in properly planned randomized phase III studies [3, 4]. Efficacy was not superior and toxicity was more pronounced. Therefore, R-CHOP-21 remains the standard of care. An even greater dose escalation was explored in high-risk DLBCL, in R-MegaCHOEP, a four-arm randomized trial with or without subsequent autologous stem-cell transplantation (ASCT) consolidations [5, 6]. Despite the improvement of failure-free survival (FFS) after ASCT, there was no difference in overall survival [6, 7].

An alternative approach is the R-DAEPOCH (dose adjusted rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) protocol [8], aimed at improving the effectiveness of treatment and minimizing side effects. This aims to modulate the dose of individual cytostatics in subsequent chemotherapy cycles depending on their pharmacodynamics and individual toxicity of treatment. However, the only randomized study failed to prove its superior efficacy over an R-CHOP regimen [9]. In a non-randomized setting, the R-DAEPOCH regimen, or early consolidation of first line therapy with ASCT, is supposedly better in DLBCL with 'double hit' and possibly also with double expression of c-myc and bcl-2 or bcl-6 oncoproteins [10]. The Polish Lymphoma Study Group also recommends more intensive (i.e. R-CHOP 14) therapy in PMBCL (primary mediastinal B cell lymphoma) patients.

Our understanding of the molecular complexity of DLBCL has evolved over the years. It was previously considered as a single disease, but gene expression profiling (GEP) analysis has identified three groups based on the cell of origin: an activated B-cell (ABC), a germinal center B-cell (GCB), and a third category termed indeterminate or unclassifiable (type 3), which accommodates cases that do not fit neatly into the other categories [11–13]. Each of these subtypes is subject to a distinct molecular mechanism and oncogenic signaling pathway, and may therefore differ in response to conventional treatment. Chronic, active B-cell antigen receptor signaling, constitutive myeloid differentiation primary response gene 88 (MYD88) signaling, and phosphatidylinositol-3-kinase/serine-threonine kinase (PI3K/Akt/mTOR) pathway, subsequent antiapoptotic nuclear factor-kappa B (NF-κB) pathway and interferon pathway activation, are characteristics of ABC DLBCL [12, 14, 15]. For the GCB subtype, BCL6 and EZH2 are most common [16].

The ABC subtype shows a much worse prognosis with R-CHOP [17, 18]: 10–20% of DLBCL patients will be resistant to first-line chemotherapy, and a further 30–40% will relapse after gaining complete remission [19]. Therefore, ABC DLBCL patients may be regarded as presenting an unmet medical need for a new, more efficient first line regimen. Dysregulation of the important oncogenic drivers of ABC DLBCL, such as IRAK4, BTK, MYD88, PI3K, and NF-κB, makes them a suitable potential target. Recent phase III studies exploring the role of adding new agents to an R-CHOP regimen have been negative and failed to meet their primary target.

One of the first examples of this strategy investigated the role of bortezomib, a pleotropic proteasome inhibitor inhibiting IκB degradation, which appeared to be a suitable candidate for blocking NF-κB. The PYRAMID study [10] and the ReMoDL-B trial [20] both interrogated the merits of bortezomib plus R-CHOP in ABC-DLBCL. Neither reached their primary endpoints.

The PHOENIX study was a phase III clinical trial investigating the role of ibrutinib, a BTKi (Bruton tyrosine kinase inhibitor) added to R-CHOP in patients with non-germinal center diffuse large B-cell lymphoma. In the final analysis, with a median follow-up of 34.8 months, there were no differences either in EFS or in OS [21, 22], and therefore the study was declared negative, having not reached its primary targets.

However, in a subgroup analysis of patients under the age of 60, significant (nearly 10%) improvements in EFS, PFS and OS in the experimental arm were demonstrated. This discrepancy was explained by increased toxicity of the combination in patients over 60, which resulted in a higher rate of treatment discontinuation and a lower dose intensity in the experimental group. The ESCALADE study is an ongoing protocol with acalabrutinib, a second generation BTKi, featuring non-GCB DLBCL patients under 65. This study will probably lead to approval of acalabrutinib in this indication, although it does not address the most important question. GEP-based subtypes are not unique clinical entities. BTKi should have been investigated in the MCD genetic subtype only which accounts for c.25% of ABC DLBCL cases and as much as 75% of patients with PCNSL (primary central nervous system lymphoma) [23]. The MCD subtype has a particularly bad prognosis, and involves MYD88 and CD79B mutation both prone to BTKi [11].

Molecularly targeted drugs may address only very well and adequately characterized disease subtypes. Immunotherapy, and in particular immunomodulating agents and monoclonal antibodies, may be useful in a broader context. ROBUST [24, 25] was a multicenter, international, randomized, double-blind, placebo-controlled, phase III protocol run in 257 global sites assessing R-CHOP with lenalidomide (R2-CHOP) versus R-CHOP in ABC subtype DLBCL [26]. Although ROBUST did not meet the primary or secondary PFS endpoints for R2-CHOP, it had certain promising conclusions: positive trends for PFS favoring R2-CHOP were observed in patients with higher risk IPI ≥3 and the safety profile of R2-CHOP was consistent with that previously observed. It was disappointing that the study did not confirm the previous phase II results from the Mayo Clinic. Comparing the two studies, one should note that results of the R2-CHOP arm were comparable, while R-CHOP results were significantly better in a phase III study. It appears that in

a multicenter setting, fewer high risk cases were included, and that the average time from diagnosis to therapy initiation was prolonged.

The currently recruiting FIRST-MIND study is evaluating tafasitamab, a humanized anti-CD19 monoclonal antibody with a modified constant region (Fc) that increases Fc-γ receptor binding affinity or the addition of tafasitamab to lenalidomide in addition to R-CHOP in intermediate and high-risk DLBCL [27, 28]. Under this protocol, it is mandatory to initiate treatment within four weeks of diagnosis, to increase the number of high-risk patients and better reflect real life settings. Tafasitamab in combination with lenalidomide in relapsed or refractory DLBCL patients showed an overall response rate of 54% and complete remission rate of 32%, with median progression-free survival of 16.2 months [29].

In other investigated first-line treatment regimens, the role of new monoclonal antibodies such as obinutuzumab, brentuximab vedotin, polatuzumab vedotin and bispecific antibodies have been assessed.

The GOYA study comparing G-CHOP to R-CHOP used obinutuzumab, a second generation anti-CD20 monoclonal antibody, instead of rituximab. The results showed a comparable safety profile, but did not significantly improve investigator-assessed PFS compared to R-CHOP in these patients [30].

Early phase II results of brentuximab vedotin, an anti-CD30 monoclonal antibody linked to monomethyl auristatin E (MMAE), a microtubule disrupting agent, have been encouraging. An acceptable safety profile and high efficacy (CR with an estimated annual PFS of 82%) was demonstrated in a subgroup of DLBCL patients with CD30 antigen on the surface of neoplastic cells [31, 32].

Even more exciting have been consistent polatuzumab vedotin (pola) results. This is an anti-CD79b antibody-drug conjugate with MMAE. This compound is approved in relapsing/refractory DLBCL, after a phase II randomized study comparing bendamustine +rituximab +pola (BR-Pola) with a BR regimen. The response rate was 70% (BR-Pola) versus 33% (BR) [33], and median PFS and median OS were significantly prolonged. In a phase I study, setting an optimal dose of polatuzumab vedotin in combination with R-CHOP, an acceptable safety profile in previously untreated DLBCL patients was demonstrated [34]. Of the 10 DLBCL patients enrolled, seven had an end-of-treatment response: five CRs, one partial response (PR), and one data unavailable [35]. This regimen was properly explored in the Polarix study, a randomized, placebo-controlled, double-blind phase III trial in newly diagnosed DLBCL patients with IPI ≥2, comparing R-CHOP to Pola R-CHP (polatuzumab vedotin, rituximab, cyclophosphamide, vincristine, prednisone) [36]. A similar study (POLAR BEAR) is being conducted in elderly patients subjected to an R-mini CHOP regimen with reduced doses of cytostatics. Preliminary results will be published in 2022 [37].

So far, little is known about bispecific antibodies (BIABs), which may represent the future. They are antibodies that can bind to two different antigens at the same time. They 'combine' the target (tumor cell) and the effector cell of the immune system (lymphocyte or macrophage), promoting the destruction of the target cell. Blinatumomab is the first CD19/CD3 bispecific T-cell engager antibody construct approved for the treatment of refractory Philadelphia chromosome-negative acute B-lymphoblastic leukemia. However, the development of all other BIABs directed against CD19 has been halted, due to neurotoxicity adverse events [38].

In their place, we have anti-CD20/anti-CD3 BIABs: mosunetuzumab, odrenextamab, epcoritamab and glofitamab, to name only the compounds most advanced in their development. They all lead to durable responses in patients with B-cell NHL, who otherwise have limited options, even those relapsing or refractory to chimeric antigen receptor (CAR) T-cell therapy [39]. Their favorable toxicity profile allows them to be used in elderly patients with comorbidities, as demonstrated in the recent report summarizing the results of a first line chemotherapy-free regimen [40]. Their potential role in PR consolidation, or as an additional compound added to Pola-R-CHOP, is currently being investigated in multicenter randomized studies.

Numerous attempts have been made to improve the first-line treatment of DLBCL patients. Using new compounds, adding to or replacing an R-CHOP regimen, is probably more effective than escalating the dose or intensity of classical chemotherapy. Molecularly targeted drugs such as BTKi have proved to be effective in very well characterized genetic subsets of patients which cannot be identified by the routine histopathological methods used in 2021. In this respect, the ABC DLBCL subtype describes patients with an adverse prognosis, but cannot be used to select patients for targeted therapies. Immunotherapy may be effective in less accurately defined genetic subtypes, but its mechanism of action may be compromised by intensive chemotherapy regimens. Obinutuzumab is evidently a 'better' monoclonal antibody than rituximab, but CHOP abrogated its efficacy, as demonstrated in the GOYA study [30]. We are still exploring the role of lenalidomide added to a (modified) R-CHOP regimen, but the most fascinating results in DLBCL so far were achieved in the L-MIND protocol, where it was combined only with the monoclonal antibody tafasitamab.

Our patients with DLBCL still await solutions to improve their outcome. The failure of several phase III studies has proved that this is the only way to verify the new protocols. Although ASCT consolidation is widely used in high-risk DLBCL patients, none of the randomized studies has confirmed its efficacy. Furthermore, this idea is no longer being explored in any ongoing clinical trial.

Author'scontributions

Equal contribution in design of the article and preparing manuscript.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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XOSPATA – WYBIERZ DŁUŻSZE PRZEŻYCIE*

*U PACJENTÓW Z NAWROTOWĄ LUB OPORNĄ NA LECZENIE OSTRĄ BIAŁACZKĄ SZPIKOWĄ Z MUTACJĄ FLT3 W PORÓWNANIU DO CHEMIOTERAPII RATUNKOWEJ

Mediana całkowitego czasu przeżycia

to 9,3 miesiąca przy stosowaniu leku XOSPATA (n = 247)

w porównaniu do 5,6 miesiąca dla chemioterapii ratunkowej (n = 124)
[HR = 0,637 (95% CI: 0,490-0,830); P = 0,0004][1]

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- Jest wskazana u pacjentów z FLT3m+ R/R AML, w tym FLT3-ITD, FLT3-TKD oraz mutacjami FLT3-ITD i FLT3-TKD[1]

Doustna monoterapia w dawce raz na dobę oferująca możliwość leczenia ambulatoryjnego w R/R FLT3m+ AML[1]

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astellas

AML – ostra białaczka szpikowa
CI – przedział ufności
CR – całkowita remisja
CRh – całkowita remisja z częściową regeneracją hematologiczną
FLT3m+ – mutacja dodatnia FLT3
FLT3-ITD – wewnętrzna duplikacja tandemowa FLT3
FLT3-TKD – domena kinazy tyrozynowej FLT3
HR – współczynnik ryzyka
OS – przeżycie całkowite
R/R – nawrót/oporność

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Informacja o leku

▼Niniejszy produkt leczniczy będzie dodatkowo monitorowany. Umożliwi to szybkie zidentyfikowanie nowych informacji o bezpieczeństwie. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane. Aby dowiedzieć się, jak zgłaszać działania niepożądane – patrz punkt 4.8 ChPL.

Nazwa produktu leczniczego: Xospata 40 mg, tabletki powlekane. **Skład jakościowy i ilościowy:** Każda tabletka powlekana zawiera 40 mg gilterytynibu (w postaci fumaranu). Pełny wykaz substancji pomocniczych, patrz punkt 6.1 Charakterystyki Produktu Leczniczego (ChPL). **Postać farmaceutyczna:** Tabletka powlekana (tabletka). **Wskazania do stosowania:** Produkt Xospata jest wskazany w monoterapii nawrotowej lub opornej na leczenie ostrej białaczki szpikowej (ang. acute myeloid leukaemia, AML) z mutacją FLT3 u dorosłych pacjentów (patrz punkty 4.2 i 5.1 ChPL). **Dawkowanie i sposób podawania:** Leczenie produktem Xospata powinien rozpocząć i nadzorować lekarz mający doświadczenie w stosowaniu terapii przeciwnowotworowych. Przed przyjęciem gilterytynibu u pacjentów z nawrotową lub oporną na leczenie AML należy potwierdzić mutację FMS-podobnej kinazy tyrozynowej 3 (ang. FMS-like tyrosine kinase 3, FLT3) [wewnątrztandemową duplikację genu (ang. internal tandem duplication, ITD) lub mutację w obrębie domeny kinazy tyrozynowej (ang. tyrosine kinase domain, TKD)] przy użyciu zwalidowanego testu. Podawanie produktu Xospata można wznowić u pacjentów po przeszczepieniu krwiotwórczych komórek macierzystych (ang. haematopoietic stem cell transplantation, HSCT). Dawkowanie: Zalecana dawka początkowa to 120 mg gilterytynibu (trzy tabletki po 40 mg) raz na dobę. Przed rozpoczęciem leczenia, w 15. dniu, a następnie co miesiąc przez cały czas trwania leczenia należy ocenić badania biochemiczne krwi, w tym aktywność fosfokinazy kreatynowej. Przed rozpoczęciem leczenia gilterytynibem, w 8. i 15. dniu pierwszego cyklu oraz przed rozpoczęciem każdego kolejnego miesiąca leczenia przez następne trzy miesiące należy wykonać badanie elektrokardiograficzne (EKG) (patrz punkty 4.4 i 4.8 ChPL). Leczenie należy kontynuować do czasu, kiedy pacjent nie będzie już odnosić korzyści klinicznych z leczenia produktem Xospata lub do momentu wystąpienia niedopuszczalnej toksyczności. Odpowiedź na leczenie może być opóźniona, dlatego należy rozważyć kontynuowanie stosowania zaleconej dawki do 6 miesięcy, aby zapewnić czas na odpowiedź kliniczną. W przypadku braku odpowiedzi [pacjent nie osiągnął złożonej całkowitej remisji (CRc)] po 4 tygodniach leczenia można zwiększyć dawkę do 200 mg (pięć tabletek po 40 mg) raz na dobę, jeżeli leczenie jest tolerowane lub uzasadnione klinicznie. Modyfikacje dawki: Zalecenia dotyczące przerwy w podawaniu, zmniejszenia dawki i zaprzestania podawania produktu Xospata u pacjentów z nawrotową lub oporną na leczenie AML: Zespół różnicowania: W przypadku podejrzenia zespołu różnicowania podać kortykosteroidy i rozpocząć monitorowanie hemodynamiczne (patrz punkt 4.4 ChPL). Przerwać stosowanie gilterytynibu, jeśli ciężkie objawy przedmiotowe i/lub podmiotowe utrzymują się przez ponad 48 godzin po rozpoczęciu stosowania kortykosteroidów. Wznowić leczenie gilterytynibem, podając taką samą dawkę, gdy nasilenie objawów przedmiotowych i podmiotowych zmniejszy się do stopnia 2[a] lub niższego. Zespół odwracalnej tylnej encefalopatii: Zaprzestać stosowania gilterytynibu. Odstęp QTcF > 500 ms: Przerwać stosowanie gilterytynibu. Wznowić leczenie gilterytynibem, podając zmniejszoną dawkę (80 mg lub 120 mg[b]), gdy odstęp QTcF powróci do wartości w zakresie 30 ms wartości początkowej lub ≤ 480 ms. Zwiększenie odstępu QTcF o więcej niż 30 ms w badaniu EKG w 8. dniu pierwszego cyklu: Potwierdzić w badaniu EKG w 9. dniu. W przypadku potwierdzenia należy rozważyć zmniejszenie dawki do 80 mg. Zapalenie trzustki: Przerwać podawanie gilterytynibu aż do ustąpienia zapalenia trzustki. Wznowić leczenie gilterytynibem, podając zmniejszoną dawkę (80 mg lub 120 mg[a]). Inna toksyczność stopnia 3[a] lub wyższego uznawana za związaną z leczeniem: Przerwać stosowanie gilterytynibu aż do ustąpienia toksyczności lub zmniejszenia jej nasilenia do stopnia 1[a]. Wznowić leczenie gilterytynibem, podając zmniejszoną dawkę (80 mg lub 120 mg[b]). Planowane HSCT: Przerwać stosowanie gilterytynibu na jeden tydzień przed zastosowaniem leczenia kondycjonującego w HSCT. Leczenie można wznowić 30 dni po HSCT, jeśli nastąpiło wszczepienie, u pacjenta nie wystąpiła ostra postać choroby przeszczep przeciw gospodarzowi (stopień ≥ 2) i znajdował się on w złożonej całkowitej remisji (CRc)[c]. a. Stopień 1. oznacza nasilenie „łagodne", stopień 2. oznacza nasilenie „umiarkowane", stopień 3. oznacza nasilenie „ciężkie", stopień 4. oznacza nasilenie „zagrażające życiu". b. Dawka dobowa może być zmniejszona ze 120 mg do 80 mg lub z 200 mg do 120 mg. c. CRc jest definiowana jako współczynnik remisji wszystkich całkowitych remisji (definicja całkowitej remisji, patrz punkt 5.1 ChPL), CRp [całkowita remisja z niepełną regeneracją płytek krwi (< 100 x 10^9/l)] i CRi (spełnione kryteria całkowitej remisji z wyjątkiem pełnej regeneracji hematologicznej, z utrzymującą się neutropenią < 1 x 10^9/l i całkowitą regeneracją płytek krwi lub bez niej). Produkt Xospata należy podawać mniej więcej o tej samej porze każdego dnia. Jeśli dawka zostanie pominięta lub nie zostanie przyjęta o zwykłej porze, należy podać dawkę jak najszybciej tego samego dnia, a pacjent powinien powrócić do zwykłego schematu dawkowania następnego dnia. Jeśli po podaniu dawki wystąpią wymioty, pacjenci nie powinni przyjmować kolejnej dawki, tylko powinni powrócić do zwykłego schematu dawkowania następnego dnia. Osoby w podeszłym wieku: Nie jest wymagane dostosowanie dawki u pacjentów w wieku ≥ 65 lat (patrz punkt 5.2 ChPL). Zaburzenia czynności wątroby: Nie ma konieczności dostosowania dawki u pacjentów z łagodnymi (klasa A wg skali Child-Pugh) lub umiarkowanymi (klasa B wg skali Child-Pugh) zaburzeniami czynności wątroby. Produktu Xospata nie zaleca się do stosowania u pacjentów z ciężkimi zaburzeniami czynności wątroby (klasa C wg skali Child-Pugh), ponieważ w tej populacji nie oceniano bezpieczeństwa stosowania i skuteczności (patrz punkt 5.2 ChPL). Zaburzenia czynności nerek: Nie ma konieczności dostosowania dawki u pacjentów z zaburzeniami czynności nerek o nasileniu łagodnym do umiarkowanego. Nie ma doświadczenia klinicznego u pacjentów z zaburzeniami czynności nerek o nasileniu ciężkim (patrz punkt 5.2 ChPL). Dzieci i młodzież: Nie określono dotychczas bezpieczeństwa stosowania ani skuteczności produktu leczniczego Xospata u dzieci w wieku poniżej 18 lat. Dane nie są dostępne. Ze względu na wiązanie się z receptorem $5HT_{2B}$ w warunkach in vitro (patrz punkt 4.5 ChPL) u pacjentów w wieku mniej niż 6 miesięcy istnieje możliwość oddziaływania na rozwój serca. Sposób podawania: Produkt Xospata jest przeznaczony do podania doustnego. Tabletki można przyjmować z posiłkiem lub bez. Należy je połykać w całości, popijając wodą i nie należy ich przełamywać ani rozkruszać. **Przeciwwskazania:** Nadwrażliwość na substancję czynną lub na którąkolwiek substancję pomocniczą wymienioną w punkcie 6.1 ChPL. **Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania:** Zespół różnicowania: Stosowanie gilterytynibu wiązało się z występowaniem zespołu różnicowania (patrz punkt 4.8 ChPL). Zespół różnicowania polega na szybkiej proliferacji i różnicowaniu komórek mieloidalnych. Nieleczony może zagrażać życiu lub prowadzić do zgonu. Objawy podmiotowe i stan kliniczny w zespole różnicowania obejmują gorączkę, duszność, wysięk opłucnowy, wysięk osierdziowy, obrzęk płuc, niedociśnienie tętnicze, szybki przyrost masy ciała, obrzęk obwodowy, wysypkę i zaburzenia czynności nerek. W przypadku podejrzewania zespołu różnicowania należy rozpocząć leczenie kortykosteroidami wraz z monitorowaniem hemodynamicznym aż do ustąpienia objawów podmiotowych. Jeśli ciężkie objawy przedmiotowe i/lub podmiotowe utrzymują się przez ponad 48 godzin po rozpoczęciu stosowania kortykosteroidów, należy przerwać stosowanie produktu Xospata do czasu ustąpienia ciężkiego nasilenia objawów przedmiotowych i podmiotowych (patrz punkty 4.2 i 4.8 ChPL). Dawkę kortykosteroidów można zmniejszyć po ustąpieniu objawów podmiotowych i podawać je przez minimum 3 dni. Przedwczesne zakończenie leczenia kortykosteroidami może spowodować nawrót podmiotowych objawów zespołu różnicowania; Zespół odwracalnej tylnej encefalopatii: Zgłaszano występowanie zespołu odwracalnej tylnej encefalopatii (ang. posterior reversible encephalopathy syndrome, PRES) u pacjentów otrzymujących produkt leczniczy Xospata (patrz punkt 4.8 ChPL). PRES to rzadkie, odwracalne zaburzenie neurologiczne, które może manifestować się szybko rozwijającymi się objawami podmiotowymi obejmującymi drgawki, ból głowy, stan splątania, zaburzenia widzenia i zaburzenia neurologiczne z towarzyszącym nadciśnieniem tętniczym i zaburzeniami stanu psychicznego lub bez nich. W przypadku podejrzewania PRES należy to potwierdzić radiologicznym badaniem obrazowym mózgu, najlepiej metodą rezonansu magnetycznego (ang. magnetic resonance imaging, MRI). Zaleca się zaprzestanie leczenia produktem Xospata u pacjentów, u których wystąpił PRES (patrz punkty 4.2 i 4.8 ChPL). Wydłużony odstęp QT: Stosowanie gilterytynibu wiązało się z wydłużeniem czasu repolaryzacji komór serca (odstęp QT) (patrz punkty 4.8 i 5.1 ChPL). Wydłużenie odstępu QT można zaobserwować w pierwszych trzech miesiącach leczenia gilterytynibem. Dlatego też przed rozpoczęciem leczenia, w 8. i 15. dniu pierwszego cyklu i przed rozpoczęciem każdego kolejnego miesiąca leczenia, przez następne trzy miesiące należy wykonać badanie elektrokardiograficzne (EKG). Należy zachować ostrożność u pacjentów z istotnym wywiadem kardiologicznym. Hipokaliemia lub hipomagnezemia mogą zwiększać ryzyko wydłużenia odstępu QT. Dlatego też przed rozpoczęciem leczenia produktem Xospata i w jego trakcie należy wyrównać hipokaliemię lub hipomagnezemię. Należy przerwać stosowanie produktu Xospata u pacjentów, u których stwierdzono odstęp QTcF > 500 ms (patrz punkt 4.2 ChPL). Decyzję o wznowieniu leczenia gilterytynibem po wystąpieniu wydłużenia odstępu QT należy podjąć po dokładnej ocenie korzyści i ryzyka. Jeżeli stosowanie produktu Xospata wznawia się w zmniejszonej dawce, po 15 dniach dawkowania i przed rozpoczęciem każdego kolejnego miesiąca leczenia, przez następne trzy miesiące należy wykonać badanie EKG. W badaniach klinicznych 12 pacjentów miało odstęp QTcF > 500 ms. Trzech pacjentów przerwało i ponownie rozpoczęło leczenie bez nawrotu wydłużenia odstępu QT. Zapalenie trzustki: Zgłaszano przypadki zapalenia trzustki. Należy badać i monitorować pacjentów, u których wystąpią objawy przedmiotowe i podmiotowe sugerujące zapalenie trzustki. Należy przerwać stosowanie produktu Xospata, ale można je wznowić, podając zmniejszoną dawkę, gdy ustąpią objawy przedmiotowe i podmiotowe zapalenia trzustki (patrz punkt 4.2 ChPL). Interakcje: Jednoczesne podawanie leków indukujących CYP3A/P-gp może prowadzić do zmniejszonej ekspozycji na gilterytynib i, w konsekwencji, ryzyka braku skuteczności. Dlatego należy unikać jednoczesnego stosowania gilterytynibu z silnymi induktorami CYP3A4/P-gp (patrz punkt 4.5 ChPL). Należy zachować ostrożność w przypadku jednoczesnego przepisywania gilterytynibu z produktami leczniczymi, które są silnymi inhibitorami CYP3A, P-gp i/lub białka oporności raka piersi (ang. breast cancer resistant protein, BCRP) (takimi jak między innymi: worykonazol, itrakonazol, pozakonazol i klarytromycyna), ponieważ mogą one zwiększać ekspozycję na gilterytynib. Należy rozważyć zastosowanie alternatywnych produktów leczniczych, które nie hamują silnie aktywności CYP3A, P-gp i/lub BCRP. W sytuacjach, gdy nie istnieją zadowalające alternatywy terapeutyczne, pacjentów należy ściśle monitorować pod kątem wystąpienia toksyczności w trakcie podawania gilterytynibu (patrz punkt 4.5 ChPL). Gilterytynib może zmniejszyć działanie produktów leczniczych, dla których receptorem docelowym jest receptor $5HT_{2B}$ lub nieswoiste receptory sigma. Dlatego też należy unikać równoczesnego stosowania gilterytynibu z tymi produktami, chyba że jego stosowanie uznaje się za niezbędne dla pacjenta (patrz punkt 4.5 ChPL). Działanie toksyczne na zarodek lub płód i antykoncepcja: Należy poinformować kobiety w ciąży o potencjalnym ryzyku dla płodu (patrz punkty 4.6 i 5.3 ChPL). Kobietom w wieku rozrodczym należy doradzić wykonanie testu ciążowego w ciągu siedmiu dni przed rozpoczęciem leczenia produktem Xospata i stosowanie skutecznej metody antykoncepcji w trakcie leczenia produktem Xospata i przez co najmniej 6 miesięcy od zakończenia leczenia. Kobiety stosujące antykoncepcję hormonalną powinny dodatkowo stosować antykoncepcję barierową. Mężczyznom, których partnerki są w wieku rozrodczym, należy doradzić stosowanie skutecznej metody antykoncepcji w trakcie leczenia i przez co najmniej 4 miesiące od przyjęcia ostatniej dawki produktu Xospata. **Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: Bezpieczeństwo produktu Xospata oceniono u 319 pacjentów z nawrotową lub oporną na leczenie AML, którzy otrzymali co najmniej jedną dawkę 120 mg gilterytynibu. Najczęstszymi działaniami niepożądanymi gilterytynibu były zwiększenie aktywności aminotransferazy alaninowej (ang. alanine aminotransferase, ALT) (82,1%), zwiększenie aktywności aminotransferazy asparaginianowej (ang. aspartate aminotransferase, AST) (80,6%), zwiększenie aktywności fosfatazy alkalicznej we krwi (68,7%), zwiększenie aktywności kinazy kreatynowej we krwi (53,9%), biegunka (35,1%), zmęczenie (30,4%), nudności (29,8%), zaparcia (28,2%), kaszel (28,2%), obrzęki obwodowe (24,1%), duszność (24,1%), zawroty głowy (20,4%), niedociśnienie tętnicze (17,2%), ból kończyny (14,7%), astenia (13,8%), ból stawów (12,5%) i ból mięśni (12,5%). Najczęstszymi ciężkimi działaniami niepożądanymi były ostre uszkodzenie nerek (6,6%), biegunka (4,7%), zwiększenie aktywności AlAT (4,1%), duszność (3,4%), zwiększenie aktywności AspAT (3,1%) i niedociśnienie tętnicze (2,8%). Inne, kliniczne istotne, ciężkie działania niepożądane obejmowały zespół różnicowania (2,2%), wydłużenie odstępu QT na elektrokardiogramie (0,9%) i zespół odwracalnej tylnej encefalopatii (0,6%). Działania niepożądane zaobserwowane w trakcie badań klinicznych wymieniono poniżej według kategorii częstości. Kategorie częstości są zdefiniowane następująco: bardzo często (≥ 1/10), często (≥ 1/100 do < 1/10), niezbyt często (≥ 1/1 000 do < 1/100), rzadko (≥ 1/10 000 do < 1/1000), bardzo rzadko (< 1/10 000) i częstość nieznana (nie może być określona na podstawie dostępnych danych). W obrębie każdej grupy częstości występowania działań niepożądanych przedstawiono według zmniejszającej się ciężkości.

Niepożądane działanie leku	Wszystkie stopnie (%)	Stopnie ≥ 3 (%)	Kategoria częstości
Zaburzenia układu immunologicznego			
Reakcja anafilaktyczna	1,3	1,3	Często
Zaburzenia układu nerwowego			
Zawroty głowy	20,4	0,3	Bardzo często
Zespół odwracalnej tylnej encefalopatii	0,6	0,6	Niezbyt często
Zaburzenia serca			
Wydłużenie odstępu QT w elektrokardiogramie	8,8	2,5	Często
Wysięk osierdziowy	4,1	0,9	Często
Zapalenie osierdzia	1,6	0	Często
Niewydolność serca	1,3	1,3	Często
Zaburzenia naczyniowe			
Niedociśnienie tętnicze	17,2	7,2	Bardzo często
Zaburzenia układu oddechowego, klatki piersiowej i śródpiersia			
Kaszel	28,2	0,3	Bardzo często
Duszność	24,1	4,4	Bardzo często
Zespół różnicowania	3,4	2,2	Często
Zaburzenia żołądka i jelit			
Biegunka	35,1	4,1	Bardzo często
Nudności	29,8	1,9	Bardzo często
Zaparcia	28,2	0,6	Bardzo często
Zaburzenia wątroby i dróg żółciowych			
Zwiększenie aktywności aminotransferazy alaninowej*	82,1	12,9	Bardzo często
Zwiększenie aktywności aminotransferazy asparaginianowej*	80,6	10,3	Bardzo często
Zaburzenia mięśniowo-szkieletowe i tkanki łącznej			
Zwiększenie aktywności fosfokinazy kreatynowej we krwi*	53,9	6,3	Bardzo często
Zwiększenie aktywności fosfatazy alkalicznej we krwi*	68,7	1,6	Bardzo często
Ból kończyny	14,7	0,6	Bardzo często
Ból stawów	12,5	1,3	Bardzo często
Ból mięśni	12,5	0,3	Bardzo często
Ból mięśniowo-szkieletowy	4,1	0,3	Często
Zaburzenia nerek i dróg moczowych			
Ostre uszkodzenie nerek	6,6	2,2	Często
Zaburzenia ogólne i stany w miejscu podania			
Zmęczenie	30,4	3,1	Bardzo często
Obrzęki obwodowe	24,1	0,3	Bardzo często
Astenia	13,8	2,5	Bardzo często
Złe samopoczucie	4,4	0	Często

* Częstość opiera się na wartościach laboratorium centralnego. Opis wybranych działań niepożądanych: Zespół różnicowania: W badaniach klinicznych z udziałem 319 pacjentów leczonych produktem Xospata u 11 (3%) wystąpił zespół różnicowania. Zespół różnicowania polega na szybkiej proliferacji i różnicowaniu komórek szpiku i nieleczony może zagrażać życiu lub prowadzić do zgonu. Objawy podmiotowe i stan kliniczny w zespole różnicowania u pacjentów leczonych produktem Xospata obejmowały gorączkę, duszność, wysięk opłucnowy, wysięk osierdziowy, obrzęk płuc, niedociśnienie tętnicze, szybki przyrost masy ciała, obrzęk obwodowy, wysypkę i zaburzenie czynności nerek. W niektórych przypadkach jednocześnie wystąpiła ostra gorączkowa dermatoza neutrofilowa. Zespół różnicowania wystąpił od jednego dnia (najwcześniej) do 82 dni od rozpoczęcia leczenia produktem Xospata i przebiegał ze współistniejącą leukocytozą lub bez niej. Z 11 pacjentów, u których wystąpił zespół różnicowania, 9 (82%) powróciło do zdrowia po okresie leczenia lub po przerwie w dawkowaniu produktu Xospata. Zalecenia w przypadku podejrzewanego zespołu różnicowania podano w punktach 4.2 i 4.4 ChPL. PRES: W badaniach klinicznych z udziałem 319 pacjentów leczonych produktem Xospata u 0,6% wystąpił zespół odwracalnej tylnej encefalopatii (PRES). PRES to rzadkie, przemijające zaburzenie neurologiczne, które może objawiać się szybko rozwijającymi się objawami podmiotowymi obejmującymi drgawki, ból głowy, stan splątania, zaburzenia widzenia i zaburzenia neurologiczne z towarzyszącym nadciśnieniem tętniczym lub bez niego. Objawy podmiotowe ustąpiły po zaprzestaniu leczenia (patrz punkty 4.2 i 4.4 ChPL). Wydłużenie odstępu QT: Spośród 317 pacjentów leczonych gilterytynibem w dawce 120 mg w badaniach klinicznych, którym zmierzono QTc po rozpoczęciu badania (ang. post-baseline), u 4 pacjentów (1%) stwierdzono odstęp QTcF > 500 ms. Ponadto, w zakresie wszystkich dawek, u 12 pacjentów (2,3%) z nawrotową lub oporną na leczenie AML maksymalna wartość odstępu QTcF po rozpoczęciu badania (ang. post-baseline) wynosiła > 500 ms (patrz punkty 4.2, 4.4 i 5.1 ChPL). Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, PL-02 222 Warszawa, tel.: +48 22 4921 301, faks: +48 22 4921 309, strona internetowa: https://smz.ezdrowie.gov.pl. **Podmiot odpowiedzialny:** Astellas Pharma Europe B.V., Sylviusweg 62, 2333 BE Leiden, Holandia. **Numery pozwoleń na dopuszczenie do obrotu:** EU/1/19/1399/001 – wydane przez Komisję Europejską. **Kategoria dostępności:** Produkt leczniczy wydawany na receptę do zastrzeżonego stosowania – Rpz.
Charakterystyka Produktu Leczniczego dostępna na stronie internetowej Europejskiej Agencji Leków http://www.ema.europa.eu lub na stronie www.astellas.com/pl/product-introductions/charakterystyki-produktow-leczniczych.

Na podstawie: 1. Charakterystyka Produktu Leczniczego Xospata. 2. Perl AE et al. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. N Engl J Med 2019; 381: 1728-1740.

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Dose adjusted R-EPOCH and other etoposide-containing regimens in first-line treatment of diffuse large B-cell lymphoma

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Abstract

Etoposide is a well-known cytotoxic agent effective in the treatment of B-large cell lymphoma (B-LCL). Currently, there is no consensus regarding the place of etoposide-containing regimens (R-CHOEP, R-ACVBP and DA-R-EPOCH) in front-line treatment of B-LCL. This paper summarizes published data and our own experience regarding the activity and toxicity of these regimens, especially DA-R-EPOCH. Most non-randomized, real-life and retrospective studies suggest that, compared to R-CHOP, DA-R-EPOCH, similarly to other etoposide-containing regimens, has superior antitumor efficacy but is also more toxic. The most important severe side-effects are hematological and infectious, making the regimen unfeasible in unfit patients. However, in fit patients with high-risk features, progression-free survival rates seem improved by 15–20% compared to R-CHOP. In our series of high-risk [age-adjusted International Prognostic Index (aaIPI) 2–3] fit [Eastern Cooperative Oncology Group (ECOG) performance status 0–2]) B-LCL patients older than 60 treated with DA-R-EPOCH, PFS at 2 years was 70%, while it was 53% in a comparable historical cohort treated with R-CHOP. DA-R-EPOCH resulted in more hematological and infectious toxicity, but no treatment-related mortality. In our opinion, DA-R-EPOCH should be considered in newly diagnosed, fit, high-risk patients with B-LCL who are older than 60, provided that there is adequate outpatient supervision, supportive care, and prompt hospital admittance in case of neutropenic fever or other severe toxicities.

Key words: diffuse large B-cell lymphoma, etoposide, elderly

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Introduction

B-large cell lymphoma (B-LCL) is the most common type of non-Hodgkin lymphoma (NHL); diffuse large B-cell lymphoma not otherwise specified (DLBCL NOS) is its most common variant, with an incidence of around 4.5 per 100,000 [1]. The disease occurs in all age groups, including children. Immunochemotherapy is the mainstay of front-line treatment, and trials with newer agents have so far failed [2, 3]. However, there is no consensus on the choice of optimal chemotherapy regimen. Too often in international meetings one hears it suggested that R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and steroids) is the standard front-line treatment for this disease. While this might be true according to the National Comprehensive Cancer Network (NCCN) guidelines [4], it is not the case according to European recommendations which list two additional options for younger, high-risk patients [age below 60, age-adjusted International Prognostic Index (aaIPI) 2–3]: R-CHOEP14 and R-ACVBP [5].

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Less than 60% of patients with high-risk disease, defined as aaIPI ≥2 or International Prognostic Index (IPI) 4–5, can be cured with R-CHOP. In contrast, multiple case series and phase II trials, as well as a phase III trial, have shown that R-CHOEP14 and R-ACVBP result in progression-free survival (PFS) above 75%, which seems to be 15–20% higher than what can be achieved with R-CHOP [6]. These etoposide-containing regimens result in more acute toxicity, but no treatment-related mortality, and also carry a somewhat increased risk of late toxicities, including secondary tumors and cardiac disease. Still, the net effect seems to be positive, at least in high-risk patients below the age of 60. For instance, our cohort of B-LCL patients with aaIPI ≥2 treated with R-CHOEP14 had 5-year OS of 90% and PFS of 87% which, to the best of our knowledge, has never been reported with R-CHOP [6]. The role of DA-R-EPOCH in this context is less well defined.

DA-R-EPOCH

DA-R-EPOCH is a regimen devised by the National Cancer Institute (NCI), consisting of the same drugs as CHOEP [7, 8]. Instead of administering cytotoxic agents in fixed dosed short infusions or as a bolus, etoposide, doxorubicin and vincristine are administered by continuous infusion over four days. The dose of cytotoxic agents is titrated according to hematologic toxicity, in order to achieve severe granulocytopenia lasting less than a week without severe thrombocytopenia. The acceptance of this regimen has been hindered by its complexity. In many countries, continuous 4-day infusion can be performed only in hospital. Patients need to check their blood counts at least twice a week and attending physicians need to be aware of these findings (and dose adjustment rules) when prescribing the next treatment cycle. On the other hand, continuous infusion of doxorubicin is less cardiotoxic than the standard way of administration, making this regimen feasible in fit patients with borderline cardiac function and those who have been pretreated with standard CHOP [7].

DA-R-EPOCH is more toxic than R-CHOP, resulting in significantly more grade 3–4 hematological and infectious side-effects, but also more mucositis and neuropathy. Prospective clinical trials usually show no difference in treatment-related mortality in fit patients.

NCCN lists DA-R-EPOCH as the recommended standard regimen for primary mediastinal B-cell lymphoma (PMBCL) based on a phase II study performed mainly in the NCI [9]. PMBCL is different from DLBCL NOS in some respects, but similarly responds to immunochemotherapy when adjusted for main prognostic factors, IPI and bulk; the results of NCI using DA-R-EPOCH in DLBCL-NOS were also excellent. One might therefore question whether treatment recommendations for immunochemotherapy for the two entities should really be different.

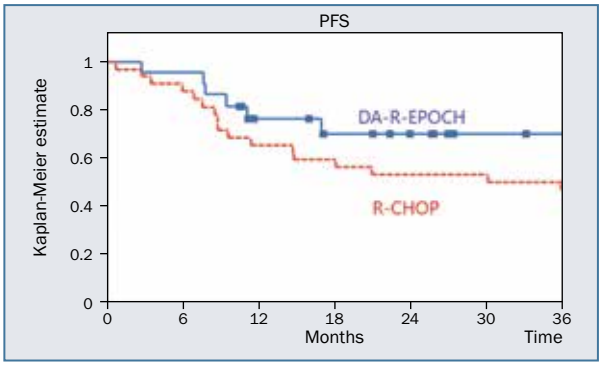

Figure 1. Progression-free survival (PFS) of high-risk elderly patients with Eastern Cooperative Oncology Group (ECOG) performance status 0–2 treated with R-CHOP (red line) or DA-R-EPOCH (blue line); p =0.2 [15]

Phase II studies of DA-R-EPOCH resulted in outcomes that seem superior to what can be achieved with R-CHOP [8, 10–13]. This was not borne out by a large randomized clinical trial performed in the USA comparing these two regimens [14]. PFS and OS were largely similar in the two treatment arms. However, PFS of the subgroup of patients with IPI 3–5 treated with DA-R-EPOCH was 15–20% better, a statistically significant and clinically meaningful difference. Additional analysis of non-randomized studies, comparing outcomes of DA-R-EPOCH and R-CHOP, suggest that the advantage of the former is mainly limited to high-risk fit patients.

Based on these findings, we started using DA-R-EPOCH in high-risk patients older than 60 [15]. In our experience, cytotoxic drug dose can be increased in only 35% of patients. We found the regimen to be unfeasible in patients with ECOG performance status of 3–4 who had an unacceptably high frequency of toxic deaths (5 out of 9). In contrast, in the 22 patients with ECOG performance status 0–2, the regimen had significant toxicity, but was feasible and so far without toxic deaths. In that group, granulocytopenia was universal by design, anemia grade 3–4 occurred in 23%, thrombocytopenia in 14%, and infections in 77%. 86% of fit patients responded to DA-R-EPOCH, and 64% achieved complete remission. After a median follow-up of 22 months, 2-year PFS was 70%, which compares favorably to outcomes of a comparable historical cohort treated with R-CHOP that had a 2-year PFS of 53%, but failed to reach statistical significance (Figure 1). KroHem, the Croatian Cooperative Group for Hematologic Disease, collected data on the outcomes of 103 newly diagnosed high-risk B-LCL patients older than 60 who were treated with DA-R-EPOCH in Croatian hospitals. The results were largely similar as in our single-center experience. 79% of patients responded: 2-year PFS of fit (ECOG performance status 0–2) patients was 67% and of unfit (ECOG performance status 3–4) patients was 44% [16].

Discussion

Discussions as to the role of etoposide in front-line treatment of DLBCL have continued for a number of years and remain unresolved. Medical evidence 'purists' point to the lack of randomized trials (usually neglecting the French trial comparing R-ACVBP to R-CHOP in younger intermediate risk patients). And while performing a randomized trial in the proper patient population might seem the best approach, current rules and regulations and the availability of commercially sponsored trials with new agents make this idea unrealistic. However, in our opinion, the preponderance of available data suggests that front-line regimens containing etoposide: R-CHOEP14, R-ACVBP and DA-R-EPOCH, have superior anti-tumor activity compared to standard R-CHOP in DLBCL. On the other hand, these regimens are also more toxic, making them unsuitable for unfit patients and those with low risk disease. In order to enjoy the benefit of increased efficacy without undue treatment-related mortality, more intensive supervision than is usual with R-CHOP during the whole treatment period, and the possibility of prompt admittance and in-hospital treatment of infectious, and to a lesser extent other, complications is of paramount importance.

Conclusions

A significant number of patients might benefit from the addition of this inexpensive agent. The magnitude of potential clinical benefit (15–20% increase in PFS and 10–15% in OS), is similar to, or higher than, that seen in phase II trials of new and expensive agents such as polatuzumab and venetoclax [17, 18].

These are valid arguments for at least considering etoposide-containing regimens in appropriate patients. Therefore, KroHem, the Croatian Cooperative Group for Hematologic Diseases, recommends treating fit patients with newly diagnosed high-risk DLBCL with R-CHOEP14 or DA-R-EPOCH.

Author's contributions

IA — sole author.

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Novel monoclonal antibodies for diffuse large B-cell lymphoma

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Abstract

Novel immunotherapeutic approaches to the treatment of diffuse large B-cell lymphoma (DLBCL), including recent approvals of chimeric antigen receptor T-cell therapy, the antibody-drug conjugates polatuzumab vedotin (PV) and loncastuximab tesirine-lpyl, and the anti-CD19 antibody tafasitamab, provide efficacious new treatment options for patients with relapsed and refractory disease.

PV was the first novel therapy approved in combination with bendamustine/rituximab (BR) for relapsed/refractory (r/r) DLBCL patients after two or more lines of treatment who are ineligible for high-dose chemotherapy and autologous hematopoietic cell transplantation. This approval was based on a randomized phase II study comparing PV-BR versus BR arms, resulting in significantly improved rates of complete metabolic response, progression-free survival, and overall response (OS). Remarkably, this was the first randomized study in DLBCL demonstrating OS benefit to an experimental arm to have been conducted in several years. The promising activity of PV-BR in rrDLBCL may be a result of the use of innovative target CD79b that enables the bypassing of resistance mechanisms related to the CD20 molecule.

Two other recently approved antibodies are directed to CD19 antigen, the other attractive alternative target in lymphoma. Although these agents are generally approved for use as third- or second-line therapy, studies are in progress exploring their value in earlier treatment lines including induction treatment.

While we still await the successful incorporation of other targeted agents into the treatment of DLBCL, R-CHOP prevails as the standard of care for DLBCL, regardless of immunohistochemical or molecular subtype at diagnosis.

Key words: diffuse large B-cell lymphoma, monoclonal antibodies, clinical trials

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Introduction

The monoclonal anti-CD20 antibody rituximab initiated the era of anti-cancer immunochemotherapy more than two decades ago, starting with R-CHOP for DLBCL and subsequently in other B-cell lymphomas. The cure rate and long-term disease-free survival increased markedly across the B-cell lymphoma entities, but DLBCL patients with recurrent or progressive disease were more difficult to treat due to reduced response rate and duration to second-line therapies [1].

Immunochemotherapy R-CHOP21 has been a standard of care for two decades, and results in long-term disease-free survival or cure of 60% of DLBCL patients. But efficacy in an individual patient depends on their age and on other International Prognostic Index (IPI) clinical risk factors, and ranges from 30% to 90%+. The National Comprehensive Cancer Network and the British Columbia Cancer Agency recently validated the prognostic value of the IPI in DLBCL patients treated with R-CHOP in 2000–2010 [2]. The prognostic value of all five factors: age, performance score, disease

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stage, elevation of LDH, and extranodal involvement, was confirmed. Age and LDH level were subdivided into ranges to account for a continuous negative influence of these variables on survival. In addition, there is a confirmed negative prognostic influence of particular extranodal sites (E) including the bone marrow, central nervous system, the GI tract, the liver, and the lungs, but not the E number itself.

Attempts to make R-CHOP better

There have been a number of attempts to improve the outcomes of initial immunochemotherapy, including modifications of the anti-CD20 antibody itself such as glycoengineering and fine tuning of the epitope specificity in obinutuzumab or modifying antigen complementarity and mechanism of action in ofatumumab. Disappointingly, substituting rituximab with the new compound has failed to improve treatment outcome in DLBCL [3, 4].

Obinutuzumab is a type II antibody with a glycosyl moiety engineered by means of fructose deletion that demonstrates increased ability to induce antibody-dependent cellular cytotoxicity (ADCC) and lyzosome-dependent cell death with attenuated activation of complement-dependent cytotoxicity (CDC).

The recently published GOYA randomized study [3] in patients with advanced DLBCL with two or more IPI risk factors and/or the presence of bulky disease, directly comparing PFS of patients treated with obinutuzumab or rituximab to both combined with CHOP showed no difference: 3-year PFS of 70% and 67% for G (obinutuzumab)-CHOP and R-CHOP, respectively.

Other attempts to improve the efficacy of R-CHOP have been based on the concept of targeted agents that were expected to switch off pathogenic signaling and increase the cytotoxic effect of standard immunochemotherapy. Prospective randomized trials were designed to provide a proof of concept including bortezomib, ibrutinib, and lenalidomide, but these studies failed to show a statistically significant improvement in outcome, even in patients selected on the base of detecting appropriate pathways (i.e. ABC or non-GCB-subtype) [1]. The immunomodulatory agent lenalidomide was tested in several B-cell lymphoma types based on a mechanistic rationale including reduction of IRF-4 (interferon regulating factor) needed for plasmablastic differentiation and cell survival as well as derepression of IL-2 (interleukin-2) synthesis.

In addition, some phase II data has suggested that lenalidomide may reverse the negative prognostic impact of the ABC phenotype in DLBCL. Although a randomized phase II trial evaluating the addition of lenalidomide to R-CHOP in unselected patients suggested an improvement in PFS and OS, a phase III trial involving patients with the ABC subtype of DLBCL identified with gene expression profiling showed no benefit to lenalidomide added to R-CHOP [5, 6].

CAR-T cell therapy

More success has been achieved with new approaches to recurrent DLBCL. Anti-CD19 CAR-T cell therapy is now approved, with three distinct second-generation products becoming commercially available: axicabtagene ciloleucel (axi-cel), tisagenlecleucel (t-cel), and lisocabtagene maraleucel (liso-cel). Overall and complete remission rates were within the ranges 52–82% and 40–54% respectively, despite most of the patients having refractory disease [1, 7–9]. Toxicity is substantial and careful supportive care is needed including ICU admission, tocilizumab administration, and short-term corticosteroids treatment. Recent updates on the registration study or standard-of-care use of axi-cel showed a sustained complete remission rate, after two years of follow up, of 37% [7, 10].

Approved indications for use of these CAR-T cell products include relapsed/refractory adult DLBCL after two or more lines of treatment. The product t-cel is also indicated for children and adults aged under 25 with refractory ALL relapsed after transplantation or in second or more relapse. The products axi-cel and liso-cel are also indicated in primary mediastinal large B-cell lymphoma and transformed follicular lymphoma.

The approvals of axi-cel, t-cel, and liso-cel were based on extremely encouraging phase I/II studies including ZUMA-1 [7, 10], JULIET [8], and TRANSCEND NHL 001 [9] that demonstrated significant improvements in outcomes, if not cures, in a proportion of patients compared to the results of conventional salvage therapy evaluated in the SCHOLAR-1 study [11]. Acute toxicity of CAR-T cell therapy including CRS (cytokine release syndrome) and ICANS (immune effector cell associated neurotoxicity syndrome) is of concern with grade 3/4 CRS and ICANS rate of 2–22% and 10-28% respectively, but treatment-related mortality is rare. This extremely beneficial therapeutic index applies to all CAR-T cell products even though they differ in costimulatory domains, method of lymphocyte procurement, wait time for cell infusion, permission for bridge therapy, cell doses, timing of adverse events, as well as cytotoxic potential.

Currently, accessibility to CAR-T therapy is limited due to toxicity, non-satisfactory activity of salvage/bridging therapy, rapid disease progression, and financial burden. In addition, most patients eventually progress [1].

Tafasitamab

An alternative promising approach to targeting CD19 is tafasitamab, a novel Fc-engineered, humanized monoclonal antibody. CD19 is an attractive target as it is not only upfront expressed on malignant B cells, but also remains present in the case of CD20 downregulation as a result of prior rituximab exposure. Fc domain engineering leads to decreased binding affinity to inhibitory receptor FcγRIIa and

increased binding to stimulatory FcγRIIIa on the effector cells, resulting in more potent ADCC (antibody dependent cell mediated cytotoxicity). A phase IIa trial of tafasitamab monotherapy in 35 patients with r/r DLBCL resulted in ORR of 26% and median duration of response (DOR) of 20 months in nine responders including five patients with a response sustained ≥12 months [12].

The combination of tafasitamab and lenalidomide is the first therapy approved by the FDA for second-line treatment of DLBCL based on the results of a phase II trial (L-MIND) in 80 patients with r/r DLBCL ineligible for aHCT [13]. ORR was 60% with CR of 43% and DOR of 21.7 months and median progression-free survival (PFS) of 12.1 months. Responses were seen across risk categories including cell of origin subtype, and refractory status. Toxicity was tolerable, and the most common adverse event was neutropenia or grade 1–2 diarrhea and rash. With additional follow up, median DOR was 34.6 months, and median overall survival (OS) 31.6 months demonstrating the durability of responses to this immunologic chemo-free combination [14].

Tafasitamab is currently undergoing a randomized study in combination with bendamustine compared to bendamustine plus rituximab in r/r DLBCL [15] and a phase I frontline study in combination with R-CHOP or R-CHOP plus lenalidomide [16].

Loncastuximab tesirine-lpyl

CD19 is also a target to the antibody-drug conjugate loncastuximab tesirine (lonca), a humanized anti-CD19 antibody conjugated to a pyrolobenzodiazepine dimer designed to bind irreversibly to DNA to create highly potent interstrand cross-links that block DNA strand separation, therefore disrupting essential DNA metabolic processes such as replication and ultimately resulting in cell death.

In a phase I study involving 183 patients, ORR was 45.6% and CR 26.7% with median DOR of 5.4 months. Adverse events were mostly hematologic plus fatigue [17]. The compound is undergoing further evaluation in phase II and III studies. Recently, the FDA granted accelerated approval to loncastuximab tesirine-lpyl as therapy for patients with relapsed or refractory large B-cell lymphoma following two or more prior lines of therapy, including DLBCL not otherwise specified, DLBCL arising from low-grade lymphoma, and high-grade B-cell lymphoma. The approval was based on data from the pivotal phase II multi-center, open-label, single-arm, LOTIS-2 clinical trial [18], evaluating the efficacy and safety of the antibody-drug conjugate in patients with relapsed or refractory DLBCL following two or more lines of prior therapy (n =145). Loncastuximab tesirine demonstrated an objective response rate (ORR) of 48.3% and a complete response rate of 24.1%. The median duration of response in 70 responders was 10.3 months, with a median time to response of 1.3 months.

Polatuzumab vedotin

Another new target proven to be successful in overcoming resistance to initial therapy of B-cell lymphomas is CD79b, an essential component of the B-cell receptor signaling pathway expressed on normal and lymphoma B cells, but not on hematopoietic stem cells.

Polatuzumab vedotin (PV) is a humanized anti-CD79b monoclonal antibody linked to a microtubule poison MMAE (monomethyl auristatin E). Linker chemistry was refined in this particular antibody to ensure a consistent drug-to-antibody ratio of 2:1 and resulting consistency in pharmacological properties. In addition to MMAE-mediated cell death, PV can induce target cell death by antibody-mediated opsonization, and antibody-dependent cellular cytotoxicity. PV was the first novel therapy approved in combination with BR (bendamustine/rituximab) for r/r DLBCL patients after two or more lines of treatment who are ineligible for aHCT [19]. This approval was based on a randomized phase II study enrolling 80 patients, 40 per arm (PV +BR vs. BR), resulting in significantly improved rates of complete metabolic response, PFS, and OS, compared to BR alone. End of treatment and best ORR was 45.0% vs. 17.5% and 62.5% vs. 25.0%, and CR was 40.0% vs. 15.5% and 50.0% vs. 22.5%, respectively. Median OS was 12.4 vs. 4.7 months, p =0.002. This was the first randomized study in DLBCL demonstrating OS benefit to experimental arm for several years. Efficacy was evident across risk groups independent of cell of origin, expresser status, IPI score, refractory status, and number of prior treatments. Responses were best in patients receiving PV+BR as second-line treatment and those with non-refractory disease. Toxicity was more pronounced in the PV arm with higher rates of grade 3–4 neutropenia without excess rate of infection. There was a grade 1–2 peripheral neuropathy in 44% of patients reversible in the majority of patients.

Updated results from this study including the phase Ib safety run-in cohort, phase II randomized arms, and results from an extension cohort (n =106), confirmed the response rates from the phase II PV+BR arm and sustained a significant survival benefit over a longer follow up [20]. 2-year PFS and OS was 28.4% and 38.25%, respectively, in the randomized PV+BR cohort. Ten patients (25%) from the randomized PV+BR cohort had an ongoing response of >25 months (range: 26–49). No new safety signals were identified with longer follow-up or in additional patients. Recently, a phase III trial evaluating PV in place of vincristine in R-CHOP in previously untreated DLBCL patients with an IPI score of 2–5 completed recruitment, with results pending.

Magrolimab

Another promising target for DLBCL patients is CD47, an anti-phagocytic protein with increased expression on lymphoma compared to normal B cells. Overexpression of

CD47 protects lymphoma cells from antitumor macrophages and has been shown to be an independent predictor of poor outcome in DLBCL. Anti-CD47 antibodies block the interaction between CD47 and its ligand signal-regulatory protein alpha (SIRPα) on macrophages and enhance recognition and phagocytosis of lymphoma cells. Magrolimab, a humanized anti-CD47 antibody, was tested in a phase Ib/II study and demonstrated an ORR of 50% and a CR rate of 36% in combination with rituximab in a heavily pretreated population of DLBCL and FL patients with no clinically significant safety event [21].

Bispecific antibodies

Bispecific antibodies are designed to target molecules on both tumor cells and T cells with the aim of inducing T-cell activation and cell mediated cytotoxicity. Blinatumumab, a bispecific construct directed against CD3 and CD19 approved for recurrent CD19 positive acute lymphoblastic leukemia, is also active in DLBCL but its use is problematic due to neurotoxicity and a need for continuous infusion schedule [22]. There are four different full-length bispecific antibodies targeting CD3 and CD20 in development that have a longer half-life and can be administered every 3–4 weeks.

A phase I/Ib study of mosunetuzumab showed promising results with durable responses in patients with r/r DLBCL including patients who progressed on CAR-T cell therapy [23]. Additional agents targeting CD3 and CD20 that showed preliminary efficacy are glofitamab, odronextamab, and epcoritamab. A potential adverse event with these agents is cytokine release syndrome [1, 24].

Conclusions

There are three recently approved monoclonal antibodies for relapsed/refractory DLBCL: two are conjugated with the toxin, and one is Fc-fragment engineered. They are labeled for use in third- or second-line treatment based on documented substantial clinical activity including improved overall survival in the case of polatuzumab vedotin, which is unusual for randomized trials in r/r DLBCL. It is likely that these agents may eventually find a role in earlier lines of treatment. Several other antibodies, including bispecific CD3/CD20 full length agent, are in advanced stages of clinical research.

Author's contributions

JW — sole author.

Conflict of interest

JW — advisory role: Roche, Takeda, Abbvie, Novartis, Gilead; research funding: Roche, GSK/Novartis; lecture honoraria: Roche, Takeda, Servier, Amgen, Abbvie, Gilead; conference travel support: Roche.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

VIA MEDICA

Treatment of peripheral T-cell lymphomas

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Abstract

Peripheral T-cell lymphomas (PTCLs) are rare neoplasms that recently have been the subject of much research into their complex pathophysiology. PTCLs are a heterogeneous group of tumors consisting of nodal and extranodal leukemic and cutaneous neoplasms. PTCLs are associated with complex biology and arduous pathology which is currently being studied. According to this research, the pathophysiology of PTCLs can be divided into intrinsic and extrinsic mechanisms. Among the intrinsic mechanisms, scientists have described JAK-STAT pathway deregulation, as well as different somatic mutations including *RB1, PTEN, TP53* and structural changes to the receptors. Also, there are scientific papers that correlate Epstein-Bárr virus or human T-cell lymphotropic virus type 1 infections with the occurrence of the neoplasm. PTCLs are most likely to develop in Asian and African populations. Due to poor clinical outcomes, PTCL treatment is the subject of intense clinical research. As a result of that, new drugs have been approved by the Food and Drug Administration for use among patients with refractory PTCL: pralatrexate, an antifolate drug; romidepsin, belinostat, an inhibitor for histone deacetylase, and brentuximab vedotin, a CD30 antibody. Also, clinical trials with mogamulizumab are being carried out for PTCL treatment. In addition to this, lenalidomide, as a substance that regulates the immune system and has shown antineoplastic effect in several hematological studies, could possibly be considered as treatment.

Key words: T-cell lymphoma, peripheral T-cell lymphoma, brentuximab vedotin, mogamulizumab

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Introduction

Peripheral T-cell lymphomas (PTCLs) are rare neoplasms that develop from mature-stage T-cells and natural killer cells (NK), presenting diverse clinical symptoms. A subtype of non-Hodgkin lymphoma, the heterogeneous group of PTCLs accounts for c.10–15% of tumors. The World Health Organization (WHO) categorizes PTCL into four, depending on localization: nodal, extranodal, leukemic, and cutaneous neoplasms. There are approximately 30 subtypes of PTCL, where the most prevalent nodal T-cell lymphomas are: peripheral T cell lymphoma not otherwise specified (PTCL-NOS) [1]; nodal T cell lymphoma with T follicular helper (TFH) phenotype which includes angioimmunoblastic T cell lymphoma (AITL); and systemic anaplastic large cell lymphoma (sALCL).

PTCLs are associated with complex biology and arduous pathology which is being studied. Among the causes of tumor pathogenesis are the abovementioned deregulation of the signaling pathways controlling T-cell development, differentiation and maturation; remodeling of the peritumor environment, and virus-mediated rewiring of T-cell biology [2]. Some studies have related Epstein-Bárr virus (EBV) or (HTLV-1, human T-cell lymphotropic virus type 1) infections with the occurrence of the neoplasm [3–5]. PTCLs are more likely to develop in Asian and African populations, and most frequently in people aged 60 and above, although a few cases of PTCL in childhood

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have been reported. They appear more often among males than females.

Pathogenesis

Many PTCLs remain unclassified and are known as peripheral T cell lymphoma not otherwise specified (PTCL-NOS). Even so, two subpopulations have been identified. One displays high expression of the GATA3 [6] transcription factor (described as master regulator for T-helper-2 cells). The other group features TBX21 exposition (master regulator for T-helper-1 cells and cytokine T cells) together with EOMES [7], and this results in a better prognosis of the clinical process. All of the above supports the assumption that these two subpopulations present unique cells of origin. Considering the very complex molecular genesis of PTCL, researchers have distinguished between intrinsic and extrinsic mechanisms that control T-cell transformation.

Intrinsic mechanisms

The first described intrinsic molecular event is JAK-STAT deregulation (performing a crucial role in cytokine signaling) that activates mutation which results in enhancing cell growth and main resistance to targeted therapy [8, 9]. Activation of the STAT precedes a unique transcriptional pattern which is characterized by the expression of genes that are responsible for growing, immune response, angiogenesis, and many metabolic pathways [10]. Moreover, alternations of the structure and somatic mutations are reported among intrinsic mechanisms. Studies have shown that loss of *PRDM1, CDKN2A, CDKN2B, RB1, PTEN, TP53* genes is observed in the GATA1-PTCL-NOS group of neoplasms, whereas mutations occurring in VAV1, ITK, SYK are present in PTCL-NOS and TFH-PTCL [11]. The next observable molecular mechanism that leads to the permanent activation or dysregulation of respective signaling pathways in PTCL are uncontrolled T-cell receptor (TCR) signaling trend [12, 13]. The loss of negative regulators of TCR, structural diversions as well as somatic mutations, constitute activation of the TCR signaling pathway leading to progression of the neoplasms [14]. Metabolic changes contributing to the expression of the PTCL-NOS often refer to the overexpression of the proteins linked to the lipid metabolism [15]. In addition to this, dysregulation of the choline metabolism has also been described in the pathophysiology of PTCLs, maintaining AKT and ERK phosphorylation, RAS activity and MYC oncoprotein expression [16]. Also, the phosphoinositide 3-kinase (PI3K) (maintaining the metabolism of glucose, glutamine, lipids and nucleotides) pathway deregulation affects the phenotype and the metabolism of T-cells neoplasms [17, 18]. Studies show that levels of AKT phosphorylation can correlate with an inferior outcome of PTCL [19] (Table I).

Table I. Intrinsic and extrinsic mechanisms of PTCL pathogenesis

Intrinsic mechanism	Extrinsic mechanism
JAK-STAT deregulation	Decreased immunogenicity of host
Enhanced cell growth and resistance to targeted therapy	Immunosuppression by Treg cells
Loss of PRDM1*, CDKN2A#, CDKN2B#, RB1&, PTEN#, TP53‡	Mutations in T- and NK-cells
Dysfunction of immune response, angiogenesis and enhanced cell growth	
Uncontrolled TCR‡‡ signaling trend	EBV infection
Permanent activation and dysregulation of signaling pathways	
Lipid metabolism	
Overexpression of proteins linking to expression of PTCL-NOS	HTLV-1 infection
Choline metabolism	Environment
AKT/ERK phosphorylation, RAS activity, MYC oncoprotein expression	More neoplasms in Asia
PI3K‡ deregulation	Production of VEGF
Glucose, glutamine, lipids and nucleotides metabolism	Level of VEGF correlates with progression of lymphoma

*Denotes protein BLIMP-1 that is crucial for most terminal effector cell differentiation in CD4 and CD8 T cells; #CDKN2A and PTEN deletions have emerged as most frequent aberration associated with poor outcomes in patients with peripheral T-cell lymphoma not otherwise specified (PTCL-NOS). PTEN acts as tumor suppressor gene through action of its phosphatase protein product; &RB1 retinoblastoma protein 1, a tumor suppressor protein; ‡together with damage to *TP53* gene, tumor suppression is severely compromised; ‡‡TCR is a protein complex found on surface of T cells, or T lymphocytes, that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC); it is responsible for developing uncontrolled signaling pathways; ‡PI3K as a phosphoinositide; 3-kinases are involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which in turn are involved in cancer; TCR — T-cell receptor; EBV — Epstein-Bárr virus; HTLV-1 — human T-cell lymphotropic virus type 1; PI3K — phosphoinositide 3-kinase; VEGF — vascular endothelial growth factor

Extrinsic mechanisms

Taking into account the extrinsic mechanisms, the tumor cells in PTCLs are remarkably dependent on the environment. As already mentioned, the activation of TCR combined with the cytokine signals are essential factors in PTCL occurrence [20]. Protumorigenic pathways, combined with decreased immunogenicity of the host, are major agents responsible for the successful growth and survival of neoplastic cells. Furthermore, mutations in T- and NK-cells diverge the microenvironment of cells and are responsible for the systemic symptoms that frequently

Table II. New medical agents in treatment of peripheral T-cell lymphoma

Substance	Receptor	Dosage	Survival
Brentuximab vedotin	CD30	1.8 mg/kg every 21 days	PFS 16.7 months
Ruxolitinib	JAK1, JAK2	20 mg (orally) twice daily	Not reported
Lenalidomide	Cereblon	Lenalidomide (25 mg per day for 14 days every 21 days) +CHOP (standard, every 21 days)	PFS 2 yrs 42%
Mogamulizumab	CCR4	Once a week for eight weeks by intravenous infusion at 1.0 mg/kg	PFS 3 months
Pembrolizumab	PD1, PD-L1	200 mg fixed dose every three weeks in combination with romidepsin	Overall response rate 44%

PFS — progression-free survival; CHOP — cyclophosphamide, doxorubicin, vincristine, and prednisone

occur with PTCLs. Immunosuppression by Treg cells as well as different models of immunoevasion in PTCL have also been noted. Among PTCL-NOS, there is loss of MHC class I proteins and CIITA, that is responsible for the MHC class II transactivator and *TP53* with CDKN2A alternations [21, 22]. Moreover, both tumor and endothelial cells can produce vascular endothelial growth factor (VEGF), the levels of which can correspond to the progression of lymphoma [23]. Moreover, PD1+ PTCL cells inhibit the immune response throughout the transmembrane protein, resulting in downmodulation on CD8+ T-cells [24]. Additionally, high levels of flice-like inhibitory protein (flip) can develop PTCL evasion [25].

As for the overexpression of CD47, this can inhibit antitumor macrophage activity and recently has been used in a targeted therapy using CD47 antibodies or CD47 receptor molecules [26]. As a prevention of the expansion of PTCL, a mechanism of host-suppression is being looked into as possible therapy maintenance. In accordance with the studies, PDL1 is highly expressed in ENKTCL176, ALK+ ALCL and among the subpopulation of PTCLs [27, 28]. Soluble factor (PDL1) plays a role as a main biomarker rather than predicting the clinical outcomes among patients with PTCL. There are reports regarding EBV correlation with PTCL occurrence: recent data shows that EBV may affect precursor lymphoid cells that can develop into T, B or NK cells. Recently, EBV-associated lymphoproliferative diseases have been described, in a broad range from highly aggressive PTCL to chronic active EBV infection without any presence of neoplasms.

There is a higher incidence of PTCL lymphomas in Asia, supposedly due to the clonal expansion of premalignant EBV-infected normal T-cells and NK cells. Therefore, insufficiency of the immune system to eliminate the EBV infections plays a crucial role in the development of these neoplasms. Another viral factor associated mainly with ATLL is HTLV-1, at which the risk of developing lymphoma is low (7% in males and 2% in females) [29]. Many reports show that HTLV-1 is clonally integrated with tumor cell genetic content [30].

Treatment

For most subtypes of PTCLs, initial treatment is a combination of a chemotherapy regimen based on either CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), or CHOEP (etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone), or another multidrug plan [31]. Considering the high risk of PTCL relapse, researchers recommend the implementation of high-dose chemotherapy followed by autologous stem cell transplantation. The Food and Drug Administration (FDA) has approved four substances for use in patients with refractory PTCL: pralatrexate, an antifolate drug; romidepsin, belinostat, an inhibitor for histone deacetylase (HDAC), and brentuximab vedotin, a CD30 antibody (Table II). For CD30-expressing PTCLs, brentuximab vedotin (BV), an antibody that can combine cytotoxic monomethylauristatin E (MMAE), a potent microtubule-disrupting substance, into anti-CD30+ lymphoma cells, is now approved for use in combination with cyclophosphamide, doxorubicin, and prednisone as an initial treatment [32, 33].

Considering the complex biology, the diversity in describing CD30 expression, and the differing mechanisms of management, the level of CD30 antibodies expression is not a predictive factor of the response to BV [34]. A study found that single-agent BV given at 1.8 mg/kg intravenously every three weeks for up to 16 cycles in 58 patients with relapsed or refractory (R/R) sALCL showed an overall response rate (ORR) of 86%, with a 57% complete response (CR) in a pivotal phase II study leading to approval in the USA, EU, and Japan for R/R sALCL. Responses in sALCL appear to be durable: 5-year overall survival (OS) and progression-free survival (PFS) were 79% and 57%, respectively, in patients who achieved a CR. In patients who did not achieve CR, 5-year OS was 25% [35].

Moreover, for many PTCLs, the JAK-STAT pathway is described as a potential target for the new therapies. Studies are currently testing monotherapy using the JAK inhibitor ruxolitinib in treatment-naive as well as relapsed PTCL [36]. Following the pathogenesis of SYK signaling trend, cerdulatinib, described as a dual SYK-JAK inhibitor, has shown

significant efficacy in a phase IIa clinical trial (43% of refractory PTCL with a partial or complete response, and 50% responses in patients with AITL) [37]. Among different immunotherapies, lenalidomide as an immunoregulator has shown antineoplastic effect in several hematological studies [38]. Maintaining binding to cereblon, suppressing the cell cycle by the degradation of cyclin-dependent kinases, lenalidomide presents an antiproliferative activity [39]. It also has immunomodulatory effects based on the increased levels of IL-2 present among T-cell and NK-cell activity. Lenalidomide has been studied as a single agent in recurrent PTCL, showing a response rate of 33%, and currently is being studied in combination with CHOP as first-line treatment among patients with AITL [40].

The next immunotherapy drug, mogamulizumab, a monoclonal antibody CCR4 that stimulates antibody-dependent cellular cytotoxicity has shown potential antitumor effect against PTCL cell lines and ATLL mouse models in research trials [41]. Mogamulizumab was approved by the Japan FDA in 2012 for the treatment of ATLL, based on a multicenter phase II study conducted in 28 relapsed patients. In 2018, it was approved for the treatment of recurrent mycosis fungoides and Sézary syndrome (~40% positive for CCR4) [42].

Studies show that many PTCLs constitutively express PD1 or PDL1, the blockage of which has been shown to be therapeutic in a variety of host intratumoral cells in non-Hodgkin and Hodgkin lymphoma. Pembrolizumab is described as efficient in relapsed NK-cell/T-cell lymphomas and also shows moderate activity in PTCL [43]. Moreover, rapid progression after a single infusion of nivolumab, a PD1 humanized antibody, was observed among patients with ATLL. The gene expression profile of tumor-associated Treg cells and ATLL cells after PD1 blockade was remarkably similar, suggesting a suppressive role of PD1 in indolent ATLL [44]. This is combined with the findings that PD1 suppresses oncogenic T cell signaling in a mouse model via PTEN and attenuates signaling by AKT and protein kinase C (PKC) in premalignant cells [45]. Additionally, the role of CAR (chimeric antigen receptor) T cell therapy is limited to those patients with relapsed PTCL. Research shows that CD30+ CAR-T cells present some effects in mouse models, but the reactivity against alloreactive T cells or Treg cells that express very high levels of CD30 remains unknown. CD30 CAR-T cells clinical trials have demonstrated either a stable disease or partial response [46]. Only one patient with ALCL in any of these clinical trials presented a complete remission that lasted nine months. Recently, CD4 CAR-T cells have shown cytotoxic efficacy both in PTCL cell lines and in mouse models, with some degree of antineoplastic effect and on-target/off-tumor effect leading to CD4+ T cell lymphopenia. Also, expressions of CD37 and CCR4 were recently shown to be targets for CAR-T cell therapy [47].

PTCL as a rare, very heterogeneous group of neoplasms, show advanced mechanisms of molecular biology, as well as distinct subgroups with defined clinical outcomes and responses to therapies. Improvements will be needed to achieve early detection and to anticipate recurrences. With the creation of research networks, the future will feature genomic studies as well as clinical trials testing new agents that could be used to tackle this rare neoplasm. Collecting more data should broaden the understanding of the molecular mechanisms that may lead to new rationally-based strategies of treatment.

The recent FDA approval of novel agents and their promising results, together with new drugs and immune-based therapies, are expected to improve clinical outcomes, although therapies using multiple targets might also be necessary to maintain therapeutic success.

Author's contributions

JK — preparatory work, collection of literature; GM — work concept, preparatory work, critical reviewing, data collection and interpretation, acceptance of final version for publication.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

VIA MEDICA

Primary central nervous system lymphoma: how to treat younger patients?

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Abstract

Primary central nervous system lymphoma (PCNSL) is a rare subtype of extranodal lymphoma which is associated with a relatively poor prognosis compared to other diffuse large B-cell lymphomas.

Population-based cancer registry data demonstrates that there has been a significant improvement in the survival of patients with PCNSL over the past two decades. This improvement likely reflects the introduction of high-intensity chemotherapy based on an induction regimen with high-dose methotrexate, and consolidation strategy including autologous stem cell transplantation. As a result, the improvement has been mainly observed in younger patients. New approaches such as Bruton tyrosine kinase inhibitor, immunomodulatory agents, immune checkpoint inhibition, and chimeric antigen receptor T-cell therapy are under investigation for PCNSL. In addition, trials combining novel agents in front-line induction treatment are ongoing.

Key words: primary central nervous system lymphoma, PCNSL, HD-MTX, methotrexate

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Introduction

Great progress has been made over the last 20 years in optimizing therapeutic platforms in primary central nervous system lymphoma (PCNSL), particularly in younger patients who can undergo optimal therapy based on an induction regimen and consolidation treatment.

In the context of optimal combination therapy, younger patients are usually defined as <65 years of age. In clinical practice, age, performance status (PS) and comorbidities are of fundamental importance for prognosis, as they all determine the possibilities of adequate therapy. Optimal induction treatment is possible in patients with PS 0–2/3, without significant age restrictions, but with adequate renal function (creatine clearance >50 mL/min) and heart ejection fraction, which will allow the administration of high-dose methotrexate (HD-MTX) and the use of adequate hydration (minimum 4–5 liters of infusion fluids per day).

The prognosis of younger patients with a worse general condition, which results directly from the presence of lymphoma, without significant disease burden (also those with a borderline creatine clearance, but ≥40 ml/min) should be carefully assessed, because the administration of HD-MTX can sometimes dramatically improve the patient's condition and kidney function. In this case, treatment can be started with lower doses of MTX and escalated in subsequent cycles of chemotherapy. Regardless of age, special attention should be paid to diabetic patients, in whom large fluctuations in glycemia can be expected and the risk of discovering renal failure is high. Performing optimal consolidation treatment is much more related to age, as high-dose chemotherapy with autologous stem cell transplantation (HD-ASCT) is usually proposed as safe for patients <60–65, while it is recommended to avoid radiotherapy in consolidation in patients >60 due to the risk of significant neurotoxicity [1–4].

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Induction treatment

The established standard in the treatment of PCNSL are multiagent regimens of chemotherapy based on the combination of HD-MTX with rituximab (an anti-CD20 monoclonal antibody). The optimal dose is MTX ≥3.5 g/m^2 in a rapid, 2–4-hour infusion, every 2–3 weeks (optimally every two weeks), repeated 4–8 times (optimally at least six cycles) [1, 2]. HD-MTX at a dose ≥3.5 g/m^2 achieves the therapeutic concentration in the cerebrospinal fluid (CSF), and therefore does not require additional drug administration by lumbar puncture. Methotrexate in lower doses, but >1 g/m^2, also penetrates the blood brain barrier (BBB), but does not reach the appropriate concentration in the CSF. In this case, additional intrathecal administration (12–15 mg it.) is recommended. HD-MTX is usually associated with rituximab (day 1 of the cycle) [1, 2]. It has been suggested to optimally use rituximab by administering the drug once a week, at the beginning of treatment (the first 6–8 weeks) i.e. in the period of the greatest damage to the BBB, which may favor better penetration for a large molecule such as anti-CD20 [5]. Rituximab (R) is currently included in most induction programs of chemotherapy, although there is still controversy about its role in the treatment of PCNSL [6–8]. The choice of other drugs in the regimens comes down to individual preferences and does not result from a direct comparison of regimens.

Currently, four induction regimens are considered to be equivalent: MATRix/IELSG-32 (R-HD-MTX, cytarabine, thiotepa) [9], R-MPV (R-HD-MTX, vincristine, procarbazine) [10, 11], MR-T (R-HD-MTX, temozolomide) [5] and R-MBVP (R-HD-MTX, etoposide, carmustine and prednisone) [12]. The expected remission rate (ORR) after induction treatment, as well as progression-free survival (PFS) and overall survival (OS) after consolidation, are in the range: 77–95% ORR, 2-year PFS 57–87% and 5-year OS 65––81% [5, 9–12]. The MATRix program was associated with a significant risk of grade 3 and 4 hematological toxicities. Based on real-world study data, the British Society of Hematology recommends for patients at higher risk (PS >2, age >65, significant comorbidities) a reduction of the cytarabine dose by 25% (with a possible 25% reduction in the dose of thiotepa), especially in the first cycle [13]. The R-MPV regimen is characterized by low toxicity and can be safely used in elderly patients or those in a worse general condition [10, 11]. The MT-R scheme with escalation of the MTX dose to 8 g/m^2/every two weeks, requires a dose reduction in 45% of cases [5].

In the opinion of most researchers, there are no rational grounds for escalating the MTX dose significantly >3.5 g/m^2. At the Department of Lymphoid Malignancies, Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw, Poland, in cooperation with the Polish Adult Lymphoma Group (PALG), a program has been developed by the clinic team (R-MIV-AT), based on a combination of HD-MTX at dose of 3.5 g/m^2 every two weeks with ifosfamide at a dose of 1.5/0.8 g/m^2/days 3–5 (age-dependent dose) and vincristine 1.4 mg/m^2/day 1 (six cycles in total). Rituximab 375 mg/m^2 is administered once weekly at the initiation of therapy for a total of six administrations. The induction phase completes one cycle with cytarabine at a dose dependent on age, 2/1 g/m^2/bid/days 1–2 (four doses per cycle), in combination with thiotepa 30 mg/m^2/ /day 3. Then depending on risk groups, patients are qualified for the consolidation stage with HD-ASCT (thiotepa, BCNU, etoposide) or whole-brain radiation therapy (WBRT) at a dose of 36 Gy (partial remission or stable remission after induction) or 24 Gy (complete remission after induction).

Consolidation therapy

Consolidation is integral to optimal therapy. Despite the high effectiveness of induction treatment, it is unlikely that long-term remission will be maintained without consolidation treatment. The goal of consolidation is to significantly improve progression-free survival and delay the time to relapse through the eradication of minimal persistent disease (potential highly resistant cell clones). For this purpose, WBRT, HD-ASCT and non-myeloablative chemotherapy may be considered [4].

Radiotherapy

The role of radiotherapy is uncertain. Despite its high effectiveness, recurrences and progressions are very common and occur shortly after the end of therapy. A study to compare chemotherapy with consolidation of WBRT (45 Gy) to a chemotherapy-only arm (G-PCNSL-SG1 study) did not provide conclusive answers. The benefit of adding WBRT was only the effect on PFS, but not OS, while late neurotoxicity was observed in the WBRT arm [14–16]. Standard doses of radiation therapy (43–36 Gy) are associated with a significant risk of early neurotoxicity, including life-threatening leukoencephalopathy, but also of late-delayed neurotoxicity complications such as dementia, gait ataxia and urinary incontinence, which significantly impair patient quality of life. In a retrospective analysis of PCNSL patients treated with HD-MTX chemotherapy followed by WBRT (45–36 Gy), 24% developed symptoms of rapidly progressive subcortical dementia (RTOG 93–10 study) within five years of follow-up [17]. These observations are confirmed by a large meta-analysis [18], supporting the recommendation to avoid standard doses of WBRT in first-line treatment, especially in patients older than 60 [16–18]. The risk of significant neurotoxicity after WBRT has recently been confirmed by two large randomized trials, IELSG-32 (36 Gy) [9] and PRECIS (40 Gy) [12], in which WBRT vs. HD-ASCT were compared directly in the consolidation. The neurological

status of HD-ASCT patients was consistently improved, in contrast to WBRT patients who continued to develop and worsen neurotoxicity symptoms. Nevertheless, WBRT has been shown to be an effective method of consolidation and produces PFS and OS comparable to the ASCT arm (although significant relapse rates were observed in the PRECIS study in the WBRT arm) [9, 12].

Since the possibilities of safe HD-ASCT implementation concern a limited, selected group of patients, new ways of optimizing the use of WBRT are being investigated. One of these was hyperfractionated WBRT, but several years of observations confirmed that this technique did not reduce neurotoxicity, but only delayed its effect over time even in relatively young patients [19, 20].

More promising seems to be the possibility of using reduced doses of WBRT (rdWBRT). In a phase II study, after R-MPV induction treatment (5–7 cycles of chemotherapy), rdWBRT at a dose of 23.4 Gy (13 fractions at 180 cGy) was used as a consolidation, with impressive results: 2-year PFS of 77% and 5-year OS of 80%. At the same time, no increase in neurotoxicity was observed during the 4-year follow-up. These results represent some of the best results in terms of OS and safety, but apply to a very small group of PCNSL patients and should be treated with caution [11]. Results from RTOG 1114 are awaited and should answer the question of whether rdWBRT plays a significant role in the consolidation of R-MPV/cytarabine chemotherapy compared to the cytarabine-only consolidation arm. In other words, is it safe to eliminate the WBRT from first-line treatment?

In summary, it can be stated that the use of WBRT in consolidation gives a potential advantage over chemotherapy alone, but one must take into account significant neurotoxicity and, compared to HD-ASCT, worsening of PFS. Standard doses of WBRT are generally not recommended for first-line treatment, especially for those over 60. Currently, in consolidation for patients who are not candidates for HD-ASCT, WBRT 36 Gy (20 fractions) or preferred 23.4–24 Gy (180 or 200 cGy per fraction) may be considered.

High-dose chemotherapy with autologous stem cell transplantation

HD-ASCT is usually recommended for consideration as a consolidation for first-line treatment in all patients for whom it is potentially safe [1, 2, 4]. This indication is supported by the recent results of two large randomized trials comparing WBRT to HD-ASCT in consolidation treatment.

In the IELSG32 study, WBRT (36 Gy) was used in one of the arms, and in the other HD-ASCT (conditioning: thiotepa/TT and carmustine/BCNU). There was no difference between the arms in either progression-free survival (2-year PFS 80% vs. 69%, respectively) or overall survival (2-year OS 85% vs. 71%, respectively) [9]. However, a consequent improvement of neurological status observed in HD-ASCT as opposed to an increase in neurotoxicity in the WBRT arm, made HD-ASCT the first-choice method in consolidating PCNSL treatment for patients who qualify for this procedure [10].

In the similar PRECIS study (WBRT 40 Gy versus HDC-ASCT with TBC conditioning: thiotepa, busulfan, cyclophosphamide), a trend was observed in the HD-ASCT arm towards improvement of progression-free survival (2-year PFS 86.8% vs. 63.2%, respectively) without impact on overall survival (2-year OS 75% vs. 66%, respectively) [12]. It should be remembered that HD-ASCT is associated with a significant toxicity of treatment and may apply to a selected group of patients. Conditioning with TT-BCNU compared to TBC is associated with lower treatment-related toxicity (TRM 1–3% [9, 21] vs. 11% [10, 12], respectively), however the results of the meta-analysis indicate a higher efficiency of TBC conditioning, with the possibility of plateauing in long-term relapse-free survival (5-year PFS 81% vs. 46%, respectively) [22]. Although there is no strict age limit, patients <60 years are usually eligible for HD-ASCT, although the 60–70 age group may also benefit.

Non-myeloablative chemotherapy

Consolidation of non-myeloablative chemotherapy is usually considered in elderly patients who are not eligible for HD-ASCT and who want to avoid WBRT-related neurotoxicity, but also for younger unfit patients. HD-ASCT is likely superior to non-myeloablative chemotherapy, but no randomized studies are available. Two multicenter, randomized trials are currently underway to answer the question of whether non-myeloablative chemotherapy can be an effective alternative to HD-ASCT. In the CALGB 51101/NCT01511562 study, the EA scheme (etoposide 40 mg/kg/96 hour continuous infusion plus cytarabine 2 g/m^2/bid/4 days), and in the IELSG 43/NCT02531841 study, the R-DeVIC scheme (rituximab, dexamethasone, etoposide, ifosfamide and carboplatin) are being compared to TT-BCNU conditioning [1, 2, 4].

Recent advances in targeted therapy

The use of novel agents has so far been limited to patients with recurrent or refractory PCNSL. Agents targeting B-cell receptor (BCR) and Toll-like receptor (TLR), Bruton tyrosine kinase (BTK) inhibitors, PI3K/mTOR targeted agents, immunomodulatory drugs (IMiDs), checkpoint inhibitors, and CD19 CAR T-cells therapy, despite high response rates, have a relatively short duration of response. Two agents, ibrutinib (BTK inhibitor) and lenalidomide (IMiD), based on reliable data from several studies have been included in the NCCN Guidelines for consideration as salvage therapies. Better outcomes are expected as a result of incorporating new agents into combination therapy, including chemotherapy.

The TEDDi-R regimen was the first to combine a novel agent with chemotherapy in PCNSL, but with high frequency of treatment-related adverse events [23], However, a combination of ibrutinib with HD-MTX ±rituximab in another study proved to be effective and safe [24].

In addition, trials combining novel agents in front-line treatment are ongoing. The LOC-R01 study is of particular interest here. The objective of this randomized phase II study is to improve first-line induction chemotherapy by combining either ibrutinib or lenalidomide with a conventional immuno-chemotherapy of R-MPV (R-HD-MTX, procarbazine, vincristine) [NCT04446962].

Author's contributions

BO — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Role of targeted therapy in central nervous system lymphoma

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Abstract

Longer life expectancy, better diagnostic measures and advances in neuro-imaging account for the increasing numbers of diagnosed cases of primary central nervous system (CNS) lymphoma (PCNSL). Unfortunately, PCNSL is usually diagnosed late and that leads to poor performance status of patients, reducing their chances of accurate and timely therapy. This accounts for significant differences between real-life treatment outcomes and clinical trials. Although PCNSL had long been considered incurable, rapidly evolving therapeutic paradigms have shown significant progress with an absolute necessity for efficient diagnosis, staging and initiation of therapy conducted at experienced centers. High-dose methotrexate combined with rituximab and high-dose cytarabine in younger patients, or alkylating agents and rituximab in older patients, still remains the standard of care as induction therapy, while relapsed/refractory disease is a challenge necessitating the search for new, safe and effective therapeutic approaches.

Thanks to the discovery of the crucial molecular pathways leading to lymphomagenesis, it is now possible to target points of deregulation of specific pathways and stop the cancerous process. The very recent developments of efficient therapies, including high-dose methotrexate-based chemotherapy and targeted therapies comprising the monoclonal antibody rituximab and the immune checkpoint inhibitors lenalidomide and ibrutinib, have brought about improved outcomes.

Such novel agents bring hope for better results and seem to hold great promise for the treatment of patients with relapsed/refractory PCNSL. The key to future approaches is to target different molecular pathways in order to overcome mechanisms of resistance.

Key words: diffuse large B-cell lymphoma, primary central nervous system lymphoma, targeted therapy

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Introduction

Primary central nervous system (CNS) lymphoma (PCNSL) is a rare type of aggressive non-Hodgkin lymphoma (NHL). It comprises about 1% of all NHL cases and 4–5% of all primary brain tumors [1]. PCNSL is defined as a malignancy confined exclusively to the central nervous system (CNS), i.e. the brain parenchyma, spinal cord, eyes, cranial nerves and/or meninges [2]. The incidence rate of PCNSL is significantly higher in immunocompromised patients such as people with human immunodeficiency virus (HIV) infection or solid organ transplant recipients [3]. Longer life expectancy, better diagnostic measures, and advances in neuroimaging account for the increasing numbers of diagnosed cases of PCNSL [4]. The latest developments of efficient therapies including high-dose methotrexate-based chemotherapy (MTX) and targeted therapies comprising rituximab and the immune checkpoint inhibitors lenalidomide and ibrutinib, have brought about outcome improvement. Unfortunately, PCNSL is usually diagnosed

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late, leading to poor performance status of patients, preventing them from getting accurate and timely therapy. This accounts for the significant differences between real-life treatment outcomes and clinical trials [5–7].

The etiology of PCNSL is still poorly understood. It is mainly associated with immunosuppression (chronic use of immunosuppressive agents, HIV/AIDS patients, organ transplant recipients), but it can also be found in immunocompetent patients.

PCNSL is a rare type of lymphoma. It accounts for 1% of all non-Hodgkin lymphoma cases and 4–5% of all primary brain tumors. Each year, 1,500 patients in the USA are diagnosed, mostly people aged 40–60. It is rare in the pediatric population, but there has been a significant increase in the incidence ratio in elderly people in recent years [1, 4].

PCNSLs share some common features with systemic diffused large B-cell lymphomas (DLBCLs). However, there are a few key characteristics that distinguish them. Histologically, mature non-Hodgkin B-cell lymphomas constitute c.95% of PCNSLs and are almost identical with DLBCLs of other organs. The most common markers of PCNSLs are B-cell markers such as CD20, CD19, CD22 and CD79a. Other prevalent markers of PCNSLs are BCL6 (60–80%), a marker of germinal-center (GC) B cells, and IRF4/MUM1 (90%), a marker of late GCB cells and plasma cells, with approximately 10% being CD10+ [2, 6, 8–10].

Although this is a disease long considered incurable, rapidly evolving therapeutic paradigms have shown significant progress in PCNSL, with an absolute necessity for efficient diagnosis, staging, and initiation of therapy conducted at experienced centers. High-dose methotrexate (HD-MTX) combined with rituximab and high-dose cytarabine in younger patients as alkylating agent, and rituximab in older patients, remains the standard of care as induction therapy [11–13]. Relapsed/refractory disease still remains a challenge, necessitating the search for new, safe and effective therapeutic approaches.

This review aims to highlight recent advances in PCNSL treatment options, placing the emphasis on targeted therapy.

Novel agents as treatment options

Establishing the crucial molecular pathways leading to lymphomagenesis has been a milestone in the development of new agents that can target points of deregulation of specific pathways and stop the cancerous process.

One of the first agents used in targeted therapies was rituximab. Rituximab is a monoclonal antibody targeting the CD20 cell surface protein. This protein is present on PCNSL cell surface. The antibody connects with the CD20 marker, leading to immune system activation and destroying marked cells. It has been established that the CHOP regimen incorporating rituximab has significantly improved the outcomes of patients suffering from systemic DLBCL. In PCNSL, the challenge comes with the blood–brain barrier (BBB). Rituximab is a significantly large particle (145 kD) and it is not clear whether it can pass the BBB. There is a suggestion that the BBB is generally disrupted by neoplastic process. This theory is partially backed by neuroimaging that shows homogenous enhancement with gadolinium contrast agent where the cancerous infiltration occurs. A study has shown that when active leptomeningeal involvement was present, the CSF concentration of rituximab was 3–4% of the serum concentration. This finding may suggest that there is a slight possibility of penetration through the BBB [14, 15]. There is a promising way of enhancing the permeability of the BBB with tumor necrosis factor alpha coupled with NGR (NGR-hTNF). NGR-hTNF is a particle that targets CD131 vessels that leads to better penetration through the endothelium, and that in turn improves tumor access of cytostatics. This method has been used to boost the uptake of rituximab combined with CHOP regimen (R-CHOP) and proved to be effective [16].

Despite several meta-analyses of studies on regimens containing rituximab, it is unclear whether this agent actually improves overall survival (OS) in PCNSL patients. There are discrepancies between age groups. It has been suggested that younger patients (under 60) may benefit more from regimens containing rituximab, whereas in older patients a higher risk of neurotoxicity has been shown. Moreover, it has been pointed out that an induction regimen comprising MTX with or without cytarabine with alkylating agent and rituximab in patients under the age of 70, followed by consolidation in the form of WBRT, autologous stem-cell transplant (ASCT), or non-myeloablative chemotherapy, has been associated with high response rates, long-term disease control, and minimal neurotoxicity in a few single-arm, phase II trials.

Unfortunately, it is difficult to draw conclusions regarding the effect of each drug individually in these trials. Although the overall evidence of benefits resulting from adding rituximab to chemotherapy schemes is slight, the low toxicity of this kind of treatment has resulted in the widespread use of such regimens in PCNSL [3, 12, 14, 15, 17].

Nuclear factor-κB (NF-κB) is a major pathway generally active in PCNSLs. Its increased activity shows in NF-κB-regulating genes, genes of the NF-κB complex, NF-κB target genes and the nuclear location of the p50 protein in tumor cells. Amplification of the *MALT1* gene (37%) and activating mutations of the *CARD11* gene (16%) in a part of PCNSL leads to this overactivity of NF-κB.

In spite of nodal DLBCLs showing inactivating mutations of TNFAIP 3, in PCNSLs this inactivation is not significant in activating the NF-κB pathway. This pathway can be targeted with ibrutinib, a Bruton's tyrosine kinase

(BTK) inhibitor, that, thanks to its small size (MW 5,440), provides promising CNS distribution, thus representing a potential treatment for PCNSL. It stops cell growth and induces apoptosis in DLBCL driven by active, chronic BCR signaling. Ibrutinib, a first class oral BTK inhibitor, has been investigated as a single agent and in combination with chemotherapy in CNS lymphoma. A large, multicenter, phase II French study investigated ibrutinib at a dose of 560 mg in 52 patients with relapsed/refractory PCNSL. This reported an overall response rate (ORR) of 50% after two months of treatment; 25% of patients experienced disease progression at two months, and 62% discontinued treatment at a median follow-up of nine months [18]. Ibrutinib showed a good tolerability at 560 mg and 840 mg a day doses, and its activity in the brain clinically, biologically and radiologically in PCNSL in a phase I study conducted by the National Cancer Institute (NCI) with 18 patients treated with single-agent ibrutinib for two weeks before the addition of chemotherapy (dose-adjusted temozolomide, etoposide, doxorubicin, dexamethasone, intrathecal cytarabine and rituximab); PR was noted in 83% of patients treated with single agent ibrutinib, and CR was assessed in 86% of patients treated with combination chemotherapy. This study included patients with newly diagnosed and relapsed/refractory PCNSL, with median progression-free survival (PFS) in patients with relapsed/refractory disease of 15.3 months [19].

The next disruption occurring in DLBCL and PCNSL is mutation in the gene *MUM1* which is responsible for pathogenesis of B-cell lymphomas through upregulating the transcription of *MYC* and other genes. Immunomodulatory drugs such as lenalidomide and pomalidomide can downregulate this path. There is evidence that lenalidomide can be used with good outcomes in treating relapsed systemic DLBCL and mantle cell lymphoma (MCL). Its role in managing PCNSL is yet to be tested, but there are ongoing clinical trials [5, 6, 18, 20]. In 2018, a phase I study of pomalidomide and dexamethasone for relapsed/refractory primary CNS or vitreoretinal lymphoma concluded that remission with this regimen is achievable, with good therapeutic activity [21].

A third-generation immunomodulatory drug, pomalidomide has shown promising efficacy in combination with dexamethasone in a phase I study at a dosage of 5 mg/day for 21 days of a 28-day cycle that was assessed to be the maximum tolerable dose; ORR was 40% and median PFS was 5.3 months [22]. Additionally, another clinical trial suggested that immunomodulatory therapy may be a good choice for people older than 60 and for those who do not qualify for WBRT as consolidation and maintenance process. Preliminary results of this study are promising. It is possible to achieve improved PFS and OS with low doses of MTX as induction treatment followed by low dose lenalidomide maintenance, and at the same time provide therapy that is well tolerated by older patients [23]. A phase II, multicenter, French LOC network study of rituximab and lenalidomide conducted in relapsed/refractory PCNSL and intraocular lymphoma demonstrated an ORR of 63% with a median PFS of 8.1 months; lenalidomide was administered at a dosage of 20–25 mg/d on days 1–21 of 28 in combination with rituximab per month as induction therapy for eight cycles followed by maintenance lenalidomide 10 mg/day [24]. Thanks to these findings, there is a need to further investigate the effect that immunomodulatory drugs have on PCNSL.

Another interesting target in treating PCNSL is PD-1. The cancerous process occurring in PCNSL leads to a high inflammation response mediated by T-cells and macrophages. Mutations in 9p24.1 *loci* are often seen in PCNSL. They lead to excessive expression of PD-1 ligands. This process can be stopped by targeting this path with the anti-PD-1 antibody nivolumab. Nivolumab has been used to treat other lymphomas (e.g. testicular lymphoma or Hodgkin lymphoma) with this genomic alteration with good effect, and thus there have been attempts to administer it in relapsed PCNSL. Data so far suggests that nivolumab is an excellent active agent in PCNSL and can lead to satisfactory responses [5, 22, 25].

Novel agents seem to hold promise for the treatment of patients with relapsed/refractory PCNSL. Most trials have comprised patients with refractory or relapsed disease, making it difficult to assess their prospects in treating newly diagnosed PCNSL. A major challenge remains the short durability of responses and mechanisms of resistance with worsening prognosis and limited therapeutic options. The key to future approaches is to target different molecular pathways to overcome these mechanisms of resistance.

Author's contributions

RK 50%, MD-D 50%.

Conflict of interest

RK — none. MD-D — Abbvie, Janssen, Roche, Servier.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Primary central nervous system lymphoma in neurosurgery

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Abstract

Primary central nervous system lymphoma (PCNSL) most commonly (>95%) constitutes a specific kind of diffuse large B-cell lymphoma, which shows expression of CD20, CD19 and CD79a antigens on its surface and belongs to non-germinal center B-cell-like type (non-GCB). This results from both its limited localization as well as immunophenotypic and molecular features. It frequently has an aggressive clinical course and its prognosis remains highly uncertain.

PCNSL's development in areas normally free from lymphoid tissue has not been adequately explained thus far. PCNSL is usually a solitary lesion (60–70%), with the majority (c.60%) occurring in supratentorial areas, and less frequently in telencephalic nuclei and periventricular areas, corpus callosum, infratentorial structures, spinal cord or orbital cavities. Lymphoid cells can occasionally create diffuse infiltration with no mass effect — a PCNSL variant known as lymphomatosis cerebri. Reported clinical symptoms depend on the localization of the tumor in central nervous system. The most common include: cognitive impairment, behavioral changes, focal neurological deficit and symptoms of increased intracranial pressure. A final diagnosis of PCNSL requires histopathological evaluation of tissue samples obtained usually during a stereotactic biopsy. Identifying lymphoid cells in cerebrospinal fluid may also be sufficient. Chemotherapy combined with radiotherapy is the standard treatment of PCNSL. For many years, surgical treatment has been controversial. This provides constant encouragement to explore effective treatment methods, with neurosurgical involvement waiting to be further defined.

Key words: primary central nervous lymphoma, PCNSL

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Introduction

Primary central nervous system lymphoma (PCNSL) is an uncommon extranodal group of non-Hodgkin lymphoma (NHL), with no systemic symptoms. PCNSL constitutes approximately 3% of primary central nervous system (CNS) tumors, and approximately 1% of all non-Hodgkin lymphoma [1, 2]. It is diagnosed at all ages, although it is most common in men aged 60–70 [3]. Histologically PCNSL belongs to a homogenous lymphoma group. Most commonly (>95%) it is a diffuse large B-cell lymphoma (DLBCL). The subtype shows expression of CD20, CD19 and CD79a antigens on its surface and belongs to non-germinal center B-cell-like type (non-GCB) [4–6].

Pathogenesis

PCNSL's development in areas where lymphoid tissue is normally absent has not been adequately explained until now. It has been studied by Deckert et al. Immunohistochemical examinations in PCNSL patients showed no expression of lymphatic vessel endothelial hyaluronan

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receptor 1 (Lyve-1), Prospero homeobox protein 1 (Prox-1) or podoplanin — a protein that shows its expression in lymphatic vessel endothelium and is a remarkable marker of lymphatic vessels to evaluate lymphangiogenesis, especially in neoplasms. The authors also suggest that no connection between PCNSL and the lymphatic system prevents the spread of lymphoma outside CNS [7]. Lymphatic cells tend to remain in CNS, thus lymphatic foci outside its primary location are remarkably infrequent [8]. One of the theories behind PCNSL's pathogenesis suggests the possibility of 'capturing' lymphocytes by central nervous system to inflammatory regions, where the lymphocytes undergo neoplastic transformations [9, 10].

Lu et al. described a 44-year-old female patient who was diagnosed with PCNSL in the region where an active inflammatory process had been first observed 30 months previously. The authors stated that neuroinfection may go ahead of or together with primary brain lymphoma. These can show some similarities (especially at an early stage) and can make diagnosis or treatment difficult. Inflammation typically causes demyelination or damages the nervous tissue and differs remarkably from PCNSL during histological examination. However, that finding does not eliminate the hypotheses indicating the significance of inflammatory foci as the primary 'immunological' reaction to developing neoplasms. Such suggestions require verification in further research [11]. The evidence confirms lymphocyte migration to nervous tissue, which depends on specific and selective interactions between lymphocyte adhesive molecules and endothelial cells in brain vessels [12, 13]. This constitutes a partial explanation for angiocentric growth of PCNSL. In some cases, infiltration of the small and medium blood vessel wall is noted and may result in blood-brain barrier damage. This anomaly allows visualization of the lesion with pathological contrast medium enhancement. Reactive small lymphocytes T and active macrophages are visible in the surrounding of a lymphoma tumor [8]. The possible prognostic importance of angiocentric growth in PCNSL and reactive perivascular lymphocyte T infiltration has been suggested. However, this requires a larger tissue sample than what can be obtained during stereotactic biopsies [14, 15].

Clinical features

PCNSL is usually a solitary lesion (60–70%), with c.60% occurring in supratentorial areas (frontal, temporal, parietal and occipital lobes), and less frequently in telencephalic nuclei and periventricular areas, corpus callosum, infratentorial structures, spinal cord or orbital cavities. In c.15–20% of cases meninges are involved, however the course is usually asymptomatic and diagnosed through anomalies in the cerebrospinal fluid (CSF) [16]. Lymphoid cells may sometimes create diffuse infiltration with no mass effect — a PCNSL variant known as lymphomatosis cerebri [17].

Reported clinical symptoms depend on the localization of the tumor in CNS. The most common include: cognitive impairment, behavioral changes, focal neurological deficit and symptoms of increased intracranial pressure [18].

PCNSL has an aggressive clinical course and is thus important to diagnose promptly. Plain computed tomography (CT) in patients with serious neurological symptoms shows hypodense lesions which may resemble ischemic foci. In order to reach the appropriate diagnosis, one should perform magnetic resonance imaging (MRI). Primary central nervous system lymphoma is either iso- or hypointensive in T1-weighted series and is hyperintensive in T2-weighted scans. Contrast medium shows homogenous enhancement, however hypointensive necrosis is occasionally seen. Further diagnostic means, like MRI spectroscopy (MRS), perfusion MRI, single-photon emission computed tomography (SPECT) or positron emission tomography (PET), should be considered in order to differentiate from infections, other brain tumors (primary, metastatic) or neurosarcoidosis [19]. These diagnostic tools may also help differentiate PCNSL from glioma — the most common central nervous system tumor. Comparing with glioma, PCNSL shows higher leakage coefficient, lower central blood volume (CBV), more vascular permeability, and less damage to the blood-brain barrier [20]. After microscopic studies, Lai et al. proved that MRI scans do not always evaluate the area of lymphoma infiltration in CNS perfectly, since no 'radiological' pathology may be observed, even in T2-weighted images [21]. Adachi et al. [22] suggested that the contrast enhanced regions may only represent a part of the neoplastic process.

The final diagnosis of primary central nervous system lymphoma is set after histopathological examination of tissue samples obtained most frequently during a stereotactic biopsy. Identifying lymphoid cells in cerebrospinal fluid (CSF) may also be sufficient. However, lumbar puncture is not always possible. CSF usually has higher protein concentrations and lymphoid cells are observed only in 10–16% of cases [18]. A recent report on the evaluation of miRNA from CSF has shown that they involve *mir-19bi, miR-21* and *miR-92a* with the specificity of 96.7% for PCNSL [23].

Treatment

Chemotherapy combined with radiotherapy is the standard treatment of PCNSL. For many years, surgical treatment has been controversial. Single examples of long overall survival in PCNSL patients after gross total tumor removal with short-lasting steroid therapy [24, 25] or with radio- and chemotherapy [26] have been reported in the literature. Supporters of surgical resection emphasize the possible cytoreductive effect and eradication of genetically unstable and resistant to cytostatic therapy lymphoid cells [27]. Cytoreduction as an important treatment modality is

applied in the therapy of malignant brain tumors, including gliomas. Apart from remission of the symptomatic mass effect, gross total resection contributes to better oncological control and prolongs overall survival (OS) in certain cases. Correlations between resection range and OS are known from observational studies [28–31]. In 2010, the German group G-PCNSL-SG-1 conducted a randomized phase III study which evaluated the efficacy of WBRT combined with high doses of Mtx in 526 patients with newly diagnosed PCNSL. Apart from the principal study aim, OS and progression-free survival (PFS) were significantly longer in patients who underwent gross total or non-total tumor resection in comparison to the group that only had a biopsy. There was no proof for a significant correlation between the site of lymphoma and PFS or OS [32]. Weller et al. observed similar positive effects of surgical treatment. The extent of procedure (gross total/partial resection) had no significance, however the number of lymphoma foci in CNS proved to be significant for prognosis [33]. Tumor resection combined with chemo- and radiotherapy were an effective treatment modality in PCNSL patients studied by Bellinzona et al. [34]. Unfortunately, those positive results have not been confirmed in our own studies (unpublished data) or in studies by Bataille et al. [35] or Jahr et al. [36]. The main argument against radical surgeries is that lymphoid changes in the central nervous system are multifocal and spread throughout deep brain structures. Autopsy studies showed that lymphoma has no capsule and neoplastic cells practically spread throughout the brain. Patients with recurrent central nervous system lymphoma in locations remote from the primary area have been described [37]. Researchers also indicate that it is possible for lymphoid cells to migrate to the subarachnoid space [38]. The risk of postoperative complications is also regarded as a reason to postpone chemotherapy [35]. However, such opinions are mainly based on data from decades before [39, 40]. Lately, the number of complications caused by neurosurgical or anaesthetic procedures has significantly decreased [41–43]. This is connected with a more frequent use of MRI and other modern technologies of visualizing tumors, as well as with better perioperative care [44, 45].

Cloney et al. [46] showed in their retrospective analysis that the number of complications in PCNSL patients after lymphoma resection was comparable to the number of complications in patients with tumors for which gross total resection is the first line treatment. At the same time, no statistically significant difference has been shown for the risk of complications dependant on the range of procedure (resection/biopsy). According to the authors, age and multiple *loci* mainly in deep brain areas should be indications for biopsy [46]. Partial or gross total resection of a lymphoma tumor seems to be beneficial in patients with symptoms of rapidly increasing intracranial pressure. With the use of modern operative techniques, the resection may contribute to the improvement of general condition, and therefore it may be beneficial for the course of disease and for the possibility of starting intensive chemotherapy. Currently, it is the patient's general condition and age that belong to prognostic factors independent from treatment modality in PCNSL patients [47].

The choice of surgery type is extremely complex. Usually, the choice is made for the patient: 'yes for brain tumour' where resection is the treatment of choice, and 'no for lymphoma', since the diagnosis is set only after histopathology. Potential pre- or intraoperative differentiation between primary lymphoma and other tumors (e.g. glioma) remains in relation to this important unsolved problem as to which surgery modality to choose, and can significantly influence the treatment. Intraoperative cytometric examination creates such possibilities of differentiation. A pioneering study was carried out by Koriyama et al. [48], who used differences in DNA histogram of both tumors.

Corticosteroids play an important role in the treatment of neoplasms in the lymphatic system. Their immunosuppressive and cytostatic effects on neoplastic cells is used. At the same time, controversy regarding their application in PCNSL patients remains. First effects are usually present after 2–3 days and include reduction of brain edema and it leads to temporary clinical stability. Rarely observed total or partial response of lymphoma may appear within a few hours [49].

Discontinuation of corticosteroid therapy is always connected with recurrence which may take place after various times. Herrlinger et al. reported on one of the longest remission times — 6.5 years [50]. Unfortunately, restarting the treatment does not guarantee successful effects. Even with permanent corticosteroid therapy, maintaining the obtained partial or complete remission is impossible. No response to treatment, or its considerable reduction, may be explained by clonal evolution of lymphoma whose cells become resistant to the drug. This resistance may be a result of either low expression of glucocorticoid receptors [51] or high expression of gene *Bcl-2* that plays a role in apoptosis processes [52]. Histopathology examinations of stereotactic biopsy samples in PCNSL patients after pre-therapy with corticosteroids were analyzed by Önder et al. [53] who concluded that reaching the diagnosis was trouble-free only in 48% of patients. In all the other cases, however, atypical changes of lymphoma cells were observed and caused problems in reaching the diagnosis. Histopathological images sometimes suggest an inflammation. Occasionally they show areas of demyelination and T-cell infiltrations [54]. That is why Patrick et al. [55] suggest discontinuing corticosteroids 7–10 days before the elective biopsy. Corticosteroids also decrease infiltration of cytostatic drugs to the brain tissue by 'tightening' the blood-brain barrier [54]. Some researchers have reported

on the possible prognostic significance of the original reaction to steroids. Regression of radiological changes and clinical recovery have a importantly beneficial influence on OS (median 17.9 vs. 5.5 months) according to a retrospective analysis of 57 patients with primary central nervous system lymphoma [56]. Adequate 'radiological' response to corticosteroid therapy-caused PCNSL lesions has led to them being called 'disappearing tumors' or 'ghost tumors'. According to Yamaguchi et al. [57], this phenomenon, alongside MRI and FDG-PET, can be applied as an alternative diagnostic means for PCNSL, especially when lymphoma foci are located in deep brain areas, normally connected with a high risk of complications after surgical treatment. However, one must bear in mind that 'disappearing tumor' is not always an accurate description of PCNSL. Bromberg et al. showed that of 12 such cases, PCNSL was diagnosed only in five. In the remaining cases, a demyelinating disease, stroke, sarcoidosis, or renal carcinoma metastasis were diagnosed [58].

In some patients, a Rickham's reservoir is placed. This gives an opportunity to give cytostatic injections intraventricularly, most commonly combined with systemic chemotherapy. The fact that cerebrospinal fluid in some PCNSL patients is probably a specific reservoir of lymphoid cells justifies such a procedure. In a multidrug Boston regimen, Pels et al. [59] used the possibility to concomitantly treat systematically and intraventricularly as first line therapy in PCNSL patients. Surprisingly good results were noted with response rate of 71% (61% CR and 10% PR). Median OS reached 34 months for patients older than 60 and was not reached in younger groups of patients. The fairly high percentage (19%) of infectious complications should be underscored. Infections were caused by immunodeficiencies due to steroid usage and myelosuppression due to cytostatic agents. Repetitive administration of drugs through the reservoir plays a significant role [59].

Recently, the efficacy of immune- and chemotherapy with rituximab and methotrexate administered intraventricularly has been shown in patients with drug-resistant or recurrent primary central nervous system lymphoma. After intravenous administration, the concentration of rituximab in CSF reaches only 1% of its serum concentration and is caused by high (146 kDa) molecular weight [60]. Rubenstein et al. [61] used intraventricular immunochemotherapy and reported on regression of lymphoid changes in basal ganglia and corpus callosum. In a significant percentage (75%) of cases, total elimination of lymphoid cells from cerebrospinal fluid was observed. Such a good treatment efficacy is explained by a beneficial pharmacokinetic profile with a slower elimination of monoclonal antibody from CSF when methotrexate is given at the same time. This probably plays an important role in decreasing the risk of drug resistance to rituximab [61].

Conclusion

Primary central nervous system lymphoma constitutes a significantly aggressive kind of lymphoma that needs aggressive treatment, with chemo- and radiotherapy still playing the lead role. Neurosurgical treatment in PCNSL patients is not routine; however, it seems that in some clinical conditions it should be considered as part of a broad treatment protocol.

Authors' contributions

All authors: writing manuscript, analysis, final approval.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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REVIEW ARTICLE

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Folliculotropic mycosis fungoides

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Abstract

Folliculotropic mycosis fungoides (FMF) is a variant of mycosis fungoides (MF) noted in the World Health Organization — European Organization of Research and Treatment of Cancer (WHO/EORTC) update of 2018. FMF is characterized as a subtype with a worse prognosis than classic MF.

The situation changed recently when authorities proposed dividing FMF into two prognostically different subtypes: indolent and aggressive. Indolent FMF allows 92% of patients to survive five years, and 72% 10 years. But only 55% and 28% of patients with aggressive FMF can survive respectively five and 10 years. FMF with internal organ involvement on the day of diagnosis shortens lives drastically (23% survive five years, only 2% survive 10 years).

There are many clinical subtypes (with plaques with follicular accentuation, alopecia, comedones, erythematous follicular papules, acneiform lesions mimicking rosacea, milia, 'spikes', and facial involvement known as leonine face), as well as histopathological variants (with pattern with intact hair follicles, folliculotropism with or without mucinosis, basaloid folliculolymphoid hyperplasia with folliculotropism, granulomatous dermatitis associated with folliculotropism, eosinophilic folliculitis, follicular cysts with folliculotropism) of FMF. This all makes diagnosis even more difficult. Combined topical and systemic treatment can be useful, with topical corticosteroids, phototherapy, radiotherapy, bexarotene, interferon, as well as with methotrexate and brentuximab vedotin. If the disease does not respond to these therapies, allogeneic hematopoietic stem cell transplantation (allo-HSCT) should be considered. Chemotherapy (gemcitabine, liposomal doxorubicin, polychemotherapy) is often associated with a merely temporary response, and that is why it should be employed only in non-responsive cases and/or as a bridge to allo-HSCT.

Key words: folliculotropic mycosis fungoides, variants, differential diagnosis, treatment

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Introduction

Folliculotropic or pilotropic mycosis fungoides (FMF), a variant of MF, is characterized by a broad spectrum of clinical symptoms and histological pictures. It was previously believed that FMF is associated with an unfavorable prognosis [1, 2]. However, data from Hodak et al. [3] and van Santen et al. [4] has revealed that FMF can be divided into two prognostically different subtypes [3, 4]. Giovannini [5], and Mitteldorf et al. [6] described alopecia in in a 13-year-old girl patient with MF. This was suggested later as the first case of FMF. Pinkus described deposits of mucin in hair follicles as alopecia mucinosa in 1957 [7]. Jabłońska named that condition follicular mucinosis in 1959 [8]. Follicular mucinosis was divided into two entities: one without symptoms and without association with lymphoma was named idiopathic follicular mucinosis (iFM); the second associated with cutaneous T-cell lymphoma as MF, lymphomatoid papulosis (LyP), Sézary syndrome (SS) or adult T-cell leukemia/lymphoma (ATLL). Willemze et al. [1] established FMF as a distinct variant of MF in 2005 in the WHO/EORTC classification. Male predominance is observed in FMF

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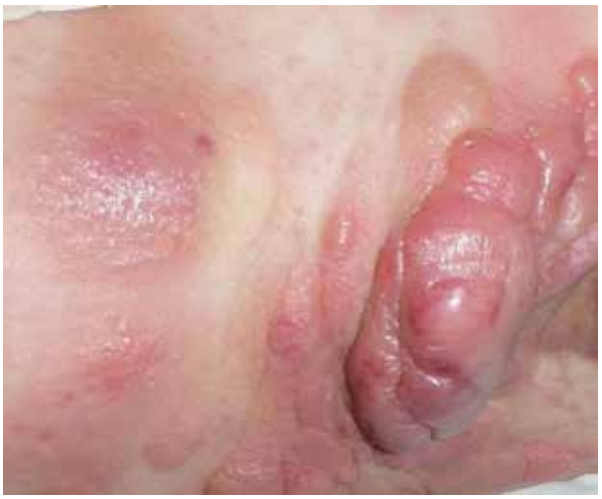

Figure 1. Follicular accentuation in folliculotropic or pilotropic mycosis fungoides (FMF), tumors arising from plaques

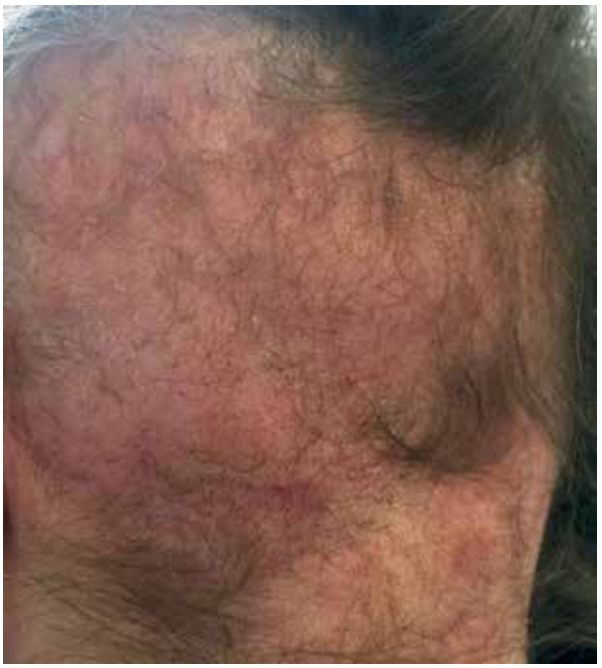

Figure 2. Alopecia in folliculotropic or pilotropic mycosis fungoides (FMF)

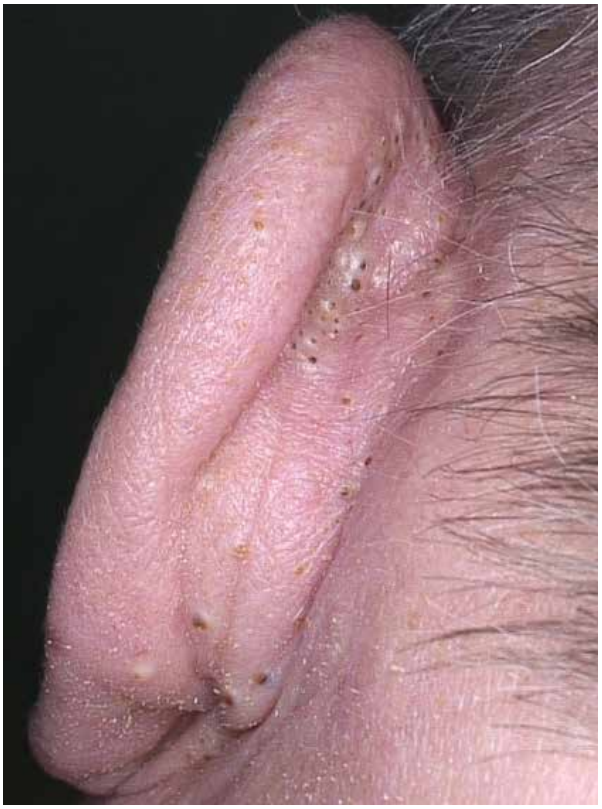

Figure 3. Comedones in course of folliculotropic or pilotropic mycosis fungoides (FMF)

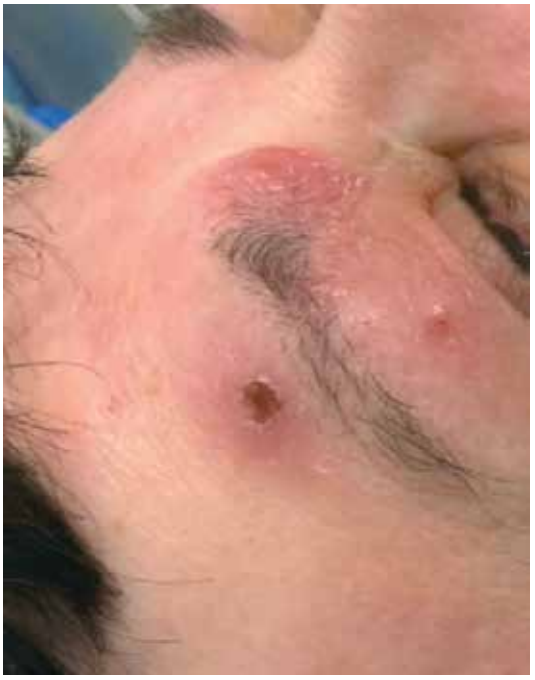

Figure 4. Folliculotropic lymphomatoid papulosis (FLyP)

(M:F ratio 2–5:1) with mean age at onset 46–59 years [4, 9–13], although it can occur in childhood [14, 15].

Clinical symptoms

The head and neck are commonly involved in FMF, but more than 70% of patients display lesions on the trunk and extremities. Unilesional FMF is rare, and most patients present multiple lesions. Patches, plaques and tumors typical for classic MF are commonly associated with FMF, but follicular accentuation is observed (Figure 1). These plaques are frequently associated with alopecia (Figure 2) typical for 81% of patients, comedones (Figure 3), and erythematous follicular papules which can cause difficulties in differentiation from follicular lymphomatoid papulosis (Figure 4). Acneiform lesions mimicking rosacea can

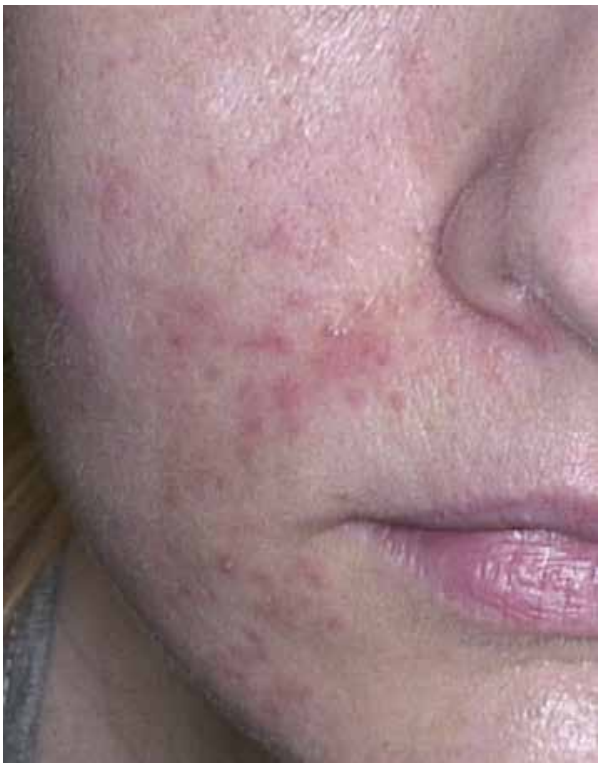

Figure 5. Folliculotropic or pilotropic mycosis fungoides (FMF) mimicking rosacea

be a symptom of FMF in one in three patients (Figure 5), as well as milia on the face or trunk [4]. The eyebrows are involved very often in the early stages of the disease. Follicular hyperkeratosis can appear, known as 'spikes'. Severe face involvement can lead to a leonine face. Less than 10% of patients present erythroderma. Pruritus is common, and severe in adults, but mild in children [4, 9, 16]. Yildizhan et al. [17] found a correlation between the presence of pruritus and disease progression in contrast to van Santen, who reported no effect of pruritus on survival or disease progression. Keratosis pilaris-like lesions are met quite often in children (although MF and FMF are extremely rare in children), mostly with a mild course of the disease [14]. It is worth mentioning that MF and FMF can appear as posttransplant lymphoproliferative disorder, with skin symptoms mostly on the trunk and a favorable prognosis [18].

Differential diagnosis

Depending on type of skin lesion, MF must be differentiated from many dermatoses [6]:

I. Patches and plaques:
   a) with classic MF, in which we do not observe follicular accentuation; histopathological characteristics reveal in MF epidermotropic infiltrate of atypical T-lymphocytes with Pautrier's microabscesses:
      - it is important to take skin biopsy from area with hair follicles if possible so as to not misdiagnose FMF;
   b) psoriasis — scaling is typical; localization: extensor of arms, belly button, auditory canal, nail changes (pitting, oil drop, onycholysis, leukonychia); joints can be involved; histopathologically: acanthosis, hypergranulosis, parakeratosis, neutrophilic abscesses;
   c) lichen planopilaris — Wickham striae visible on polygonal papules; histopathologically — lichenoid interface dermatitis, wedge-shaped hypergreanulosis, follicular involvement possible.

II. Nodules and tumors:
   a) classic MF — clinicopathological correlation necessary;
   b) other lymphomas — clinicopathological correlation e.g. anaplastic large cell lymphoma (ALCL) — no patches and plaques typical for MF; cohesive clusters of anaplastic CD30+ lymphocytes (CD30+ on 75% of neoplastic lymphocytes);
   c) other neoplastic tumors (e.g. Merkel cell carcinoma, basal cell carcinoma) — mostly on sun-exposed areas, no follicular accentuation, pathologist resolves problem.

III. Comedones, acneiform lesions, cysts:
   a) acne — typically on face and trunk, comedones, pustules, papules and cysts, biopsy unnecessary in most cases (if taken – mixed cellular infiltrate, no atypia of lymphocytes);
   b) nevus comedonicus — present since birth/before age of 12 months, circumscribed area with comedones; no inflammation around comedo-like dilatation of follicular infundibula in histopathological examination;
   c) lupus comedonicus — mostly on face — circumscribed lesion; comedo-like dilatation of follicular infundibula with lichenoid interface dermatitis, sometimes interstitial mucin deposits.

IV. 'Spikes':
   a) lichen spinulosus — follicular papules with spiny hyperkeratosis, histopathologically — without inflammatory infiltrate;
   b) lichen planopilaris — as above, mucin deposits possible in histopathological examination, V-shaped fibrosis;
   c) pityriasis rubra pilaris — red-orange follicular papules initially, extensive erythema with islands of sparing skin, palmar and plantar hyperkeratosis; erythroderma is possible; histopathologically — uniform epidermal acanthosis, chessboard-like alternation between ortho- and hyperkeratosis;
   d) atopic dermatitis — history, other atopic disorders (asthma, conjunctivitis, hay fever) but clinicopathologic correlation necessary in some cases: broad-based acanthosis, spongiosis, focal parakeratosis,

superficial lymphohistiocytic infiltrate with eosinophils;

e) drug eruption — history: relation to drug use, neutrophils and eosinophils often found in histopathology.

V. Leonine face:

a) actinic reticuloid — with chronic eczematous skin lesions, particularly on face and neck (sun-exposed area), leonine face in severe cases is possible; histopathologically: exematous lesions, sometimes mimicking lymphoma — difficult to distinguish [clinicopathological correlation — exacerbation after sun exposure, T-cell receptor (TCR) rearrangement molecular tests reveals polyclonality];

b) SS — hematological diagnostic criteria plus clinical findings (erythroderma and lymphadenopathy); skin histopathological examination rarely diagnostic (T-lymphocytic infiltrate in upper dermis with moderate atypia, mild pleomorphism), lymph node excisional biopsy necessary;

c) leukemia — hematologic diagnostic criteria.

VI. Alopecia

a) *Lichen planopilaris* — scaly perifollicular collar; histopathological examination as above;

b) chronic discoid lupus — cicatrical alopecia sometimes with scaling and obliteration of follicular ostia; histopathologically: epidermal atrophy with vacuolar interface dermatitis, apoptotic keratinocytes, involvement of follicular structures, interstitial mucin deposits.

Mitteldorf et al. [6] proposed five histomorphological patterns of FMF (modified after Gerami and Guitart) [19]):

- pattern with intact hair follicles, folliculotropism with or without mucinosis;
- basaloid folliculolymphoid hyperplasia with folliculotropism;
- granulomatous dermatitis associated with folliculotropism;
- eosinophilic folliculitis;
- follicular cysts with folliculotropism.

Histopathological examinations with correlation to clinical symptoms is necessary also in differentiating FMF from pseudolymphomatous folliculitis (S-100 positive and CD1a-positive cells as well as B cells are admixed in folliculotropic predominantly T-cells infiltrate) and follicular lymphomatoid papulosis (papules are waning and waxing spontaneously, but FMF and LyP can overlap with identical TCR rearrangement pattern) [6]. There is also discussion concerning the distinctiveness of iFM from FMF with mucinosis, because monoclonal rearrangement can be found in more than 50% of cases of iFM (compared to FMF-associated mucinosis) [20, 21].

The multitude of dermatoses from which to differentiate FMF, as well as several histopathological patterns, illustrate why the diagnosis is often made late: both the dermatologist and the pathologist can encounter difficulties. The diagnosis of FMF is established usually 18–48 months after the onset of skin symptoms [10, 12]. Immunohistochemistry can be helpful, but not in all cases thanks to antigen loss of CD2, CD3, CD4 and CD4:CD8 ratio shift mostly 6–10:1.

But we must underscore that CD4:CD8 shift is related not only to T cell but also Langerhans cells in some cases. CD30 can be expressed, and this is sometimes related to large cell transformation [6]. Mucin deposits can be found in 75% of skin biopsies in FMF [4, 11, 12]. Periecrine infiltrates are observed (this is called syringotropism) in 4–33% of cases [22].

Prognosis

Not all patients with FMF have as unfavorable a prognosis as was thought 20 years ago (5-year survival rate has been established as 66–80%) [1]. Hodak et al. [3], and van Santen et al. [4] have revealed that FMF can be divided into indolent and aggressive variants. Indolent (early) FMF allows 92% of patients to survive five years, and 72% 10 years. But only 55% and 28% of patients with aggressive (advanced) FMF survive respectively five and 10 years. FMF with internal organ involvement on the day of diagnosis shortens lives drastically (23% survive five years, only 2% 10 years). Skin symptoms distribution is different: 100% of indolent FMF affects the trunk and extremities, only 37% the head, as opposed to the aggressive variant, where the head is affected in 100%, but the trunk only in 20%, of cases. Pruritus is more often met in the aggressive variant (80% vs. 47% indolent). Syringotropism is more often met in the aggressive variant, as well as higher density of infiltrate, deeper infiltrate, higher eosinophilia and the discovery of more plasma cells in skin biopsy, which make a diagnosis even more difficult in the context of inflammatory dermatoses mimicking FMF [3, 4].

It is important to note that all patients with infiltrated plaques in the study by Hodak et al. were upstaged and considered to have tumor-stage disease [3]. Van Santen evaluated 40 FMF patients with plaques, dividing them into early-plaque and advanced-plaque, and reported disease progression in 50% over a median follow-up of 80 months [4]. Similar observations by Kalay et al. suggest that the increased density and depth of perifollicular infiltrates in advanced plaque FMF lesions is a marker for progression [17].

Both studies also suggested factors that might impact disease progression and death in FMF: clinical stage, large cell transformation (LCT), increased LDH level (in Kalay et al. [17]), age over 60 years, and the presence of extensive secondary bacterial infections at the time of first presentation (in Van Santen et al. [4]). Wieser et al. reported age over 65, leucocytosis and advanced stages to be associated with an increased risk of death in FMF [23].

Treatment

Combination therapy of topical and systemic treatment is useful. Topical corticosteroid, bexarotene gel (no refund in Poland), mechlerotamine (no refund in Poland), imiquimod (not registered for cutaneous T-cell lymphoma (CTCL), no refund in Poland), and resiquimod (in clinical trials) are among the topical methods of treatment. Radiation therapy can be useful locally in unilesional FMF, and total skin electron beam therapy (TSEB) can be considered in widespread patches and plaques. Phototherapy alone can be inadequate, and especially UVB311 is not recommended. PUVA therapy can be ordered in monotherapy or combined with systemic treatment (bexarotene, IFN alpha). Bexarotene and IFN alpha can be ordered as single treatments, as well as methotrexate (MTX).

If the disease does not respond to those therapies, extracorporeal photopheresis (ECP) should be considered, as well as allo-hematopoietic stem cell transplantation (allo-HSCT). But no studies have shown that ECP is effective in FMF. Yildizhan et al. concluded that ECP is not an effective option in FMF [17]. Allo-HSCT is suggested to be performed before LCT, but in advanced stages there must be awareness that almost 50% of transplanted patients relapse within the first 12 months after transplantation. A lower risk of relapse is observed in patients with a lower tumor burden, so the question as to when to transplant, due to the risk of allo-HSCT, remains open [17–24]. Therapy with brentuximab vedotin in CD30+ MF is approved and is now refunded in Poland (program B.66) [6, 23–27]. Chemotherapy (gemcitabine, liposomal doxorubicin, polychemotherapy) is often associated with only a temporary response, which is why it should be employed only in non-responsive cases and/or as a bridge to allo-HSCT. Pralatrexate and alemtuzumab are not available in Poland for MF/FMF patients [6, 23–27].

To avoid chemotherapy, a combination of different methods should be considered. Del Guzzo et al. proposed a therapeutic regimen combining IFN gamma, isotretinoin in a low dose (available but not registered for CTCL and not refunded in Poland) and/or topical carmustine (not available, and not refunded in Poland) for refractory advanced stage FMF [28].

Of six patients with FMF at stage IB-IIB without blood involvement and one patient in stage IIIB for whom prior therapies had failed (topical steroids, nitrogen mustard as single treatment, UVB311, PUVA, IFN alpha, bexarotene, TSEB, romidepsin (not available in Poland), acitretin, and local radiotherapy), four experienced a complete response (CR) and two a nearly CR. Local electron beam radiation was added in three cases, imiquimod in three cases (and in one case both methods were applied, and that patient was treated also with tretinoin); one patient was additionally treated with imiquimod and with PUVA. Time to response was 2–23 months with different terms of CR. Treatment was stopped not at any particular time but because of arthralgia on IFN gamma in one case and prohibitive costs of IFN gamma in a second case. The authors mentioned that IFN gamma can be more effective than IFN alpha in FMF. Higher efficacy is suggested for 0.04% carmustine ointment than topical nitrogen mustard ointment. With regards to conventional concentrations of both compounds currently in use in the USA in CTCL (nitrogen mustard is registered in Europe, but not refunded in Poland), measurable metabolites of carmustine can be found in the blood and urine of CTCL patients compared to metabolites of nitrogen mustard ointment (no metabolites can be found), which suggests deeper penetration to follicles by carmustine ointment. An isotretinoin mechanism of action in FMF is only suspected: probably the possibility of induction of apoptosis of sebaceous gland within the follicular unit can lead to atrophy of the sebaceous gland, which can affect the chemothaxix and recruitment of malignant T-cells into the follicle in FMF. Isotretinoin may synergize with Th1 cytokines to enhance CD8+ T-cells [28, 29].

Because we now understand the existence of indolent FMF, it is important to not overtreat those patients with aggressive methods of treatment: topical treatment and phototherapy can be a good therapeutic approach [6, 23–27].

Conclusions

FMF has many clinical and histopathological faces, which makes diagnosis difficult and delayed. But knowledge concerning the possible indolent course of some patients with FMF is useful in treatment decisions. All therapies recommended in classical MF find their place in FMF treatment, but not all of them seem good therapeutic options (e.g. ECP). Allo-HSCT seems to be an option in FMF; not for indolent FMF, but for aggressive FMF with a low tumor burden. New multimodality therapies could be useful in advanced FMF, provided there is awareness of off-label use, the agreement of ethics committees, and close cooperation between dermatologists, oncologists and hematologists.

Author's contributions

MS-W: 100% from concept to realization.

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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The importance of cytogenetic and molecular aberrations in multiple myeloma

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Abstract

Multiple myeloma (MM) is a heterogeneous clonal malignancy of plasma cells characterized by cytogenetic and molecular abnormalities. Chromosomal abnormalities are present at diagnosis and can evolve during the progression of MM. Metaphase karyotyping and fluorescence *in situ* hybridization are considered the standard diagnostic procedures performed in clinical practice. These test results are required to determine the Revised International Staging System classification, treatment algorithms, and short- and long-term prognoses.

Given the dynamic development of cytogenetic and molecular research, we should expect further progress in better understanding the biology of MM and changes to patient care in the coming years.

Key words: cytogenetic abnormalities, multiple myeloma, prognosis, risk classifications

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Introduction

Multiple myeloma (MM) is a heterogeneous clonal malignancy of plasma cells (PCs) that accounts for 1.8% of all cancers, and about 10–15% of all hematological malignancies [1]. The incidence in Europe is 4.5–6.0/100,000/year. The median age at diagnosis is 70 years, and 35% of patients are older than 75 [1, 2]. According to Narodowy Fundusz Zdrowia data, in 2016 there were nearly 2,600 new MM cases in Poland [3].

Multiple myeloma is characterized by chromosomal instability and cytogenetic abnormalities (CA) with significant impacts on prognosis [4–6]. Using current technology, abnormal karyotypes are found in c.20–30% of MM cases, and more often in advanced stages of MM [7, 8]. The use of the fluorescence *in situ* hybridization (FISH) technique reveals chromosomal aberrations in over 80% of cases [9].

Based on multicenter studies, the most common, clinically significant, CA detected in neoplastic plasma cells have been determined, and this is reflected in the new risk-stratification algorithm of MM, the Revised International Staging System (R-ISS), which considers the presence of the most common unfavorable CA (Table I) [10]. Less common CAs, such as t(14;20) and gain of chromosome 1q are not included in the R-ISS. This makes other staging systems like the Mayo Clinic Risk Stratification for Multiple Myeloma mSMART 3.0 (Table II) more appropriate in the presence of these CAs [11, 12].

Pathogenesis

The current hypothesis for the development of MM is the evolution of monoclonal gammopathy of undetermined

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Table I. Revised International Staging System (R-ISS) for multiple myeloma [10]

Stage	Criteria
I	$Beta_2$-microglobulin <3.5 mg/dL and albumin ≥3.5 g/dL, and
	Standard-risk CA by iFISH, and
	Normal LDH (defined as lower than ULN)
II	Not R-ISS stage I or III
III	$Beta_2$-microglobulin ≥5.5 mg/dL, and
	Either high-risk CA by iFISH [del(17p) and/or t(4;14) and/or t(14;16)] or
	High LDH (defined as higher than ULN)

CA — chromosomal abnormalities; del — deletion; iFISH — interphase fluorescence *in situ* hybridization; LDH — lactate dehydrogenase; t — translocation; ULN — upper limit of normal

significance (MGUS), which then progresses to smoldering and symptomatic MM. In general, MGUS develops with signs of primary CA. Symptomatic MM then develops as a result of secondary, random CA. The final stage of evolution in genetic changes is extramedullary MM/plasma cell leukemia (PCL) (Figure 1) [13–17].

Diagnostic methods of cytogenetic abnormalities

We recommend that all genetic analyses in MM should be preferentially performed in plasma cells-enriched samples, typically CD38+ and CD138+. Otherwise, samples may be impossible to interpret or give false negatives due to decreased sensitivity [18].

Conventional karyotyping

Karyotyping reveals CA in 20–30% of patients. This method fails to detect several translocations, including t(4;14). Normal karyotype in patients with low proliferation index corresponds to the kinetics of normal BM cells. The use of more sensitive techniques reveals CA in almost all MM [19].

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization is currently the standard technique for CA analysis, and is a practical cytogenetic tool to detect genomic aberration *in situ* and enumerate the percentage of cells harboring such abnormalities. It does not detect single-nucleotide variants [17]. Fluorescence *in situ* hybridization testing includes gain of (1q), del(1p), t(4;14)(p16;q32), t(14;16)(q32;q23), del(17p13), t(14;20) and a marker for aneuploidy.

There are three distinct groups of patients with *TP53* dysregulation: monoallelic deletion as part of deletion

Table II. Cytogenetic risk group in multiple myeloma according to International Myeloma Working Group [10, 19] and Mayo Clinic Risk Stratification for Multiple Myeloma [12]

International Myeloma Working Group	Mayo Clinic Risk Stratification for Multiple Myeloma (mSMART)
High-risk	
t(4;14)	t(4;14)
t(14;16)	t(14;16)
t(14;20)	t(14;20)
del17p	del17p
p53 mutation	*p53* mutation
amp1q	Gain 1q
del13p	Double hit MM: any two high-risk genetic abnormalities
Non-hyperdiploidy	Triple hit MM: three or more high-risk genetic abnormalities
Standard-risk	
Others including:	All others including:
• t(11;14)	• trisomies
• t(6;14)	• t(11;14)
	• t(6;14)

t — translocation; amp — amplification; del — deletion; MM — multiple myeloma

of chromosome 17p (~8%); monoallelic mutation (~6%); and biallelic inactivation (~4%). While deletion and biallelic inactivation have poor prognoses, the role of monoallelic mutation is unclear [20]. Table III presents the frequencies of the different abnormalities. For routine diagnosis, testing of t(4;14) and del(17p13) suffices [19].

Singe-nucleotide polymorphism-based mapping arrays

High-resolution genome-wide analysis (GWAS) of single-nucleotide polymorphisms (SNP) detect regions with loss of heterozygosity and numerical abnormalities. SNP mapping arrays identify copy number variations (CNV). Translocations are not usually detected and will require additional FISH. Comparative genomic hybridization is a tool for genome-wide classification of CNVs and detects numerical abnormalities [19].

Gene expression profiling

Gene expression profiling (GEP) is a technique to identify the expression of genes and pathways. Based on RNA expression using microarrays, subgroups of patients are recognized with a unique GEP phenotype that partly corresponds to the TC classification [21]. Developed GEPs

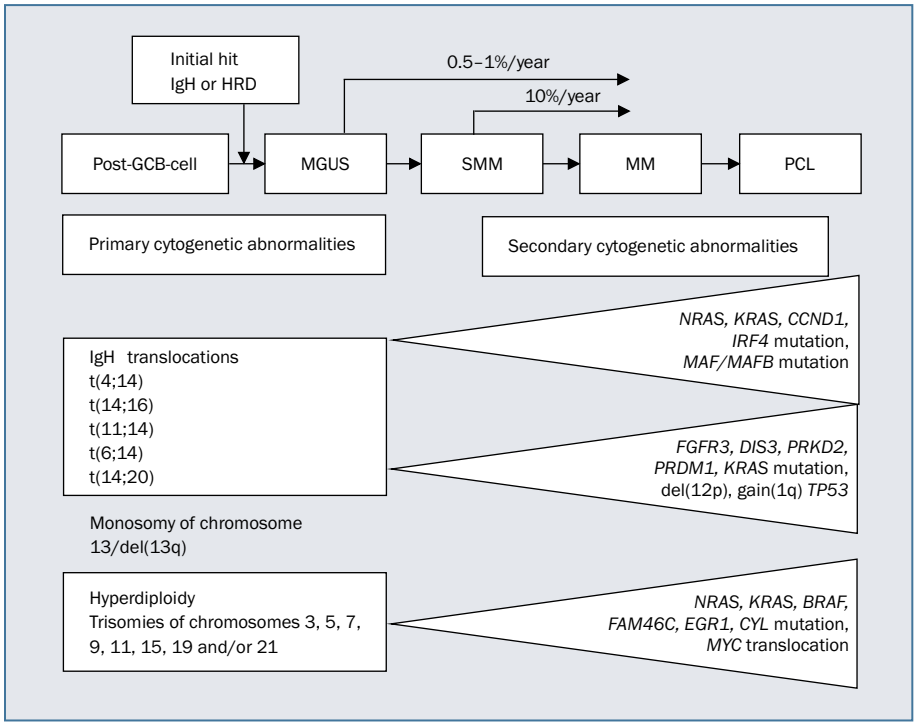

Figure 1. Pathogenesis of multiple myeloma (MM). Primary and secondary cytogenetic abnormalities associated with progression from precursor disease entities such as monoclonal gammopathy of undefined importance (MGUS) and smoldering multiple myeloma (SMM) to MM and plasma cell leukemia (PCL) (modified from Chesi et al. [16] and Manier et al. [17]); IgH — immunoglobulin heavy-chain; HRD — hyperdiploidy; GCB — germinal center B-cell-like; t — translocation; del — deletion

Table III. The most important cytogenetic abnormalities in multiple myeloma, and their prognostic impacts

IgH translocations	Gene(s)	Frequency [%]	Prognostic impact
Primary chromosomal abnormalities			
t(4:14)(p16;q32)	*FGFR3/MMSET*	10–15	Median OS: 5 years
t(6;14)(p21;q32)	*CCND3*	2	Median OS: 7–10 years
t(11;14)(q13;q32)	*CCND1*	15–20	Median OS: 7–10 years
t(14;16)	*C-MAF*	2–5	Median OS: 5 years
t(14;20)(q32;q12)	*MAFB*	1	Median OS: 5 years
Trisomies		40–50	Median OS: 7–10 years
Trisomies plus any one IgH translocation		15	May neutralize HR IgH and del 17p translocations
Hypodiploidy		13–20	Unfavorable prognosis, HR of progression
Deletion/isolated monosomy 13	*RB1, DIS3*	45–50	Effect on prognosis is unclear
Secondary chromosomal abnormalities			
17p deletion	*TP53*	10	Median OS: 5 years
1q21 gain	*CKS1B, ANP32E*	35–40	Median OS: 5 years

IgH — immunoglobulin heavy chain; t — translocation; OS — overall survival; HR — high-risk

highlight an important molecular heterogeneity in multiple myeloma. GEP70 and EMC-92-gene signature have been proved to be useful in risk assessment in clinical trials, and could provide a tool for treatment decision in high-risk MM [22, 23]. High-risk GEP signature is recognized in the mSMART 3.0 risk classification [12].

Cytogenetic risk classifications

According to the International Myeloma Working Group (IMWG), cytogenetic high-risk (HR) MM is identified when there is at least one of the following CA in the FISH test: del17p, t(4;14) or t(14;16) [19]. To the above-mentioned CA

representing a HR MM, researchers from Mayo Clinic have added hypodiploidy and t(14;20). Additionally, ultra-HR, which is defined when >3 CA is found (2%; median overall survival [OS] nine months, Table II), has been identified [24]. These classifications are subject to change as and when new treatments are introduced.

Cytogenetic abnormalities

The CA in MM and their prognostic effects are summarized in Table III [25, 26].

Hyperdiploidy

Depending on the number of chromosomes in the karyotype test, patients with MM can be divided into non-hyperdiploid (NH) and hyperdiploid types. The NH type is characterized by immunoglobulin heavy chain (IgH) translocation associated with a more aggressive MM and shorter survival. The hyperdiploid type is recognized when the number of chromosomes is greater than 46 [27, 28]. The mechanism of hyperdiploidy is not understood. The extra chromosomes are believed to occur in one catastrophic mitosis rather than a gradual increment of chromosomes [17].

The hyperdiploid group accounts for more than half of all MM cases, and the most common evidence is the presence of odd chromosome trisomy [28].

Hyperdiploidy is characterized by increased chromosomal gains, mainly trisomy of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, and is found in approximately 50% of NDMM patients [29–31]. Hyperdiploidy is defined as the primary CA in MM, and is associated with a favorable outcome. However, the coexistence of hyperdiploidy with unfavorable CA (such as del(17p), t(4;14), and increment 1q) is a negative prognosis factor [32].

Non-hyperdiploidy

The non-hyperdiploid MM group includes hypodiploid (up to 44/45 chromosomes), pseudodiploid (44/45 to 46/47), and near-tetraploid (more than 74) cases. Patients with hypodiploid karyotype present with shorter overall survival (OS). Abnormal clones include hypodiploid, pseudodiploid, or quasi-tetraploid variants, while common translocations are t(11;14) and t(4;14) [14, 33]. Loss of chromosomes 13, 14, 16, and 22 are common in NH MM [29, 32].

IgH translocations

The translocations involving immunoglobulin genes in MM most often concern the heavy chain gene (IgH, 14q32) and are found in 55–80% of patients with MM [24]. IgH translocations are considered to be primary mutations and occur in 50% of patients. They mainly consist of five chromosomal *loci*, 11q13 (15–20% patients), 6p21 (<5% patients), 4p16 (12–15% patients), 16q23 (3% patients), and 20q11 (1% patients), respectively, which contain *CCND1, CCND3, FGFR3/NSD2, MAF* and *MAFB*, respectively [15, 29]. *MYC* translocation is seen in c.15–20% of patients with newly diagnosed (ND) MM, and is considered to be a secondary mutation [30]. Approximately 20% of MM cases harbor mutation in *KRAS* [34].

Translocation t(4;14)(q16;q32)

MM-specific t(4;14)(p16;q32) translocation is detected by FISH or PCR using reverse transcription polymerase chain reaction (RT-PCR) in approximately 10–15% of patients with MM [35]. This translocation increases the expression of two genes: fibroblast growth factor receptor 3 (FGFR3) in 100% of cases; and MM SET (MMSET) in 100% of cases domain genes. In almost 25% of cases, the translocation is unbalanced due to frequent loss of derivative chromosome del(14) and lack of FGFR3 expression [36]. This change is often accompanied by deletion or monosomy of chromosome 13.

The presence of t(4;14) correlates with aggressive course of MM. The IMWG and R-ISS defined t(4;14) as a HR CA [10, 37].

Translocation t(14;16)(q32;q23) and t(14;20)(q32:q12)

Translocation (14;16) is found in 2–5%, and t(14;20) in less than 2%, of patients with MM. These translocations are difficult to detect by conventional cytogenetics techniques [38], and lead to deregulation of *MAF* and *MAFB* genes. Increased MAF levels accelerate DNA division and synthesis in clonal plasmocytes [39]. In turn, overexpression of MAFB increases proliferation and drug resistance of clonal plasmocytes and is a high MM risk marker [40]. t(14;16) may be associated with lack of CD56 expression, contributing to high proliferative activity and worsening patient prognosis [41]. In patients with t(14;20), renal impairment is more common [42].

Translocation t(11;14) and t(6;14)

Translocation (11;14) is found in 15–20% of patients with MM and is the most frequently found translocation. Translocations t(11;14) and t(6;14) juxtapose the IgH enhancer with CCND1 (15–20%) and CCND3 (1–4%). Patients with t(11;14) show increased expression of cyclin D1 [43]. Patients with NDMM with isolated t(11;14) are classified as standard risk [4, 44]. Translocation (11;14) is more common in lymphoplasmacytic lymphoma, IgM monoclonal protein secretion, non-secreting MM, plasma cell leukemia, and AL amyloidosis.

Translocation (6;14) is found in a relatively small percentage (<2%) of all MM cases. High levels of cyclin D3 mRNA can be found in this abnormality [45]. When this translocation occurs, a dysregulation of the proto-oncogene MUM1, which can be identified by immunohistochemistry, is observed, providing a marker for identifying a positive conversion of BCL6 and its expression of CD138 [46, 47]. Patients with t(6;14) are also included in the standard-risk group [10].

Deletion of 1p/1q21 gain

1q21 gain is detected in 35–40% of patients with NDMM and in almost 68% of patients with RRMM, and is associated with a poor prognosis [30, 48–50]. It is represented by 1q chromosome duplication, unbalanced 1q arm translocation, isochromosomes, or step translocation. The frequency of chromosome 1q21 gain increases as MM progresses. Overexpression of the *CKS1B* gene, located in 1q21, is associated with drug resistance [51–53].

The deletion of 1p is less common than gain of 1q, but both share a poor prognosis. Most frequently deleted regions are 1p32 (CDKN2C), 1p22, and 1p12. The 1p deletion seems to worsen treatment outcomes [38, 54].

Deletion of 13q/monosomy of chromosome 13

Deletion of 13q occurs in approximately 45–50% of patients with MM, including monosomy 13 and interstitial deletions in up to 85% of cases [55, 56]. Patients with del(13q) are included in the HR group [10]. It often coexists with other HR CA, including t(4;14) [57]. Patients with a del(13q14) are more likely to have advanced disease, in addition to high serum levels of β_2-microglobulin, and a higher percentage of PCs in the BM.

Deletion 17/17p

One of the most important CA is deletion of 17p13, del(17p) [38, 58, 59]. It is observed in 5–12% of patients with NDMM and increases with disease progression, reaching 75% in relapsed/refractory (RR) MM [60–63]. TP53 deletion in MM is an HR factor and is associated with an unfavorable prognosis. This CA results in the loss of the *TP53* gene [64]. A mutation in the *TP53* gene occurs in c.50% of patients with del(17p). The presence of del(17p) is associated with an increased incidence of hypercalcemia, extramedullary forms of MM, including the central nervous system's involvement, and transformation into PCL. It seems that bi-allelic del(17p) worsens prognosis more than does the monoallelic [65]. The bi-allelic inactivation of *TP53* due to the presence of a mutation in one allele, and deletion in the other, is considered an ultra-HR factor.

Management of patients with cytogenetic high-risk MM

Proteasome inhibitor protocols (bortezomib, carfilzomib, ixazomib), IMiDs (thalidomide, lenalidomide, pomalidomide), dexamethasone, and anti-CD38 antibody (daratumumab [Dara]) are recommended for induction treatment patients with HR NDMM eligible for ASCT; however, not all of them are approved for use in the first line. Following complete remission (CR) treatment, >50% of patients had negative minimal residual disease [MRD(–)] [66–68].

The achievement of MRD(–) after daratumumab, bortezomib, thalidomide, dexamethasone (Dara-VTd) induction treatment has been shown to prolong PFS [55, 68]. The use of high doses of melphalan (HDMel) and ASCT remains the standard of care in young patients with MM, including HR MM [56]. Compared to carfilzomib, lenalidomide, dexamethasone (KRd) treatment (12 cycles), ASCT results in a higher rate of MRD(–) (90% vs. 72%) [66].

The EMN02 study showed that the use of tandem ASCT overcomes the unfavorable prognosis of cytogenetic risk (3-year PFS: 76% vs. 69%; p =0.48) [69]. This result was confirmed in the STaMINA study, which found benefit in PFS after tandem ASCT [70].

Lenalidomide maintenance treatment in patients with cytogenetic HR MM did not prolong PFS and OS compared to standard cytogenetic risk patients [71]. On the other hand, the use of bortezomib as maintenance therapy in HR patients is compelling. The use of bortezomib in induction and maintenance therapy improved the prognosis in this group of NDMM patients with CAs [65]. These results contributed to studies being conducted with other PIs such as carfilzomib and ixazomib. Carfilzomib was better than bortezomib, but it did not significantly improve the poor prognosis associated with del(17p) [72, 73].

Conversely, the use of ixazomib combined with lenalidomide and dexamethasone prolonged PFS in patients with del(17p) compared to the use of lenalidomide with dexamethasone (Rd) [74]. The use of ixazomib in maintenance therapy showed similar results in high- and standard-risk patients, with a median improvement in PFS of five months [75]. Promising results in patients with RRMM with del(17p) were obtained using pomalidomide combined with dexamethasone (Pd). The achieved PFS in patients with del(17p) was comparable to the standard-risk patients [76].

In the Forte trial, carfilzomib, lenalidomide, dexamethasone (KRd)-ASCT-KRd and 12 months KRd induced high quality responses, with good MRD(–) rates, and ASCT showed additional benefit in the HR population [66]. The use of novel drug-based chemotherapy protocols (VMP, VMP/VTP with VT maintenance or Rd)

Table IV. Treatments for patients with high cytogenetic risk multiple myeloma

NDMM — patients eligible for ASCT								
HR patients	VRd [84]	FORTE [66] KRd12 vs. KRd-T vs. KCd			CASSIOPEIA [55] Dara-VTd vs. VTd		GRIFFIN [67] Dara-VRd vs. VRd	
Post-consol CR rate [%]	34.8	49	51	–	37	33	NR	NR
NDMM — patients not eligible for ASCT								
HR patients	SWOG [85] VRd vs. Rd		ALCYONE [78] Dara-VMP vs. VMP		MAIA [79] Dara-Rd vs. Rd			
PFS, m	38	16	NR		NR		29.6	
HR (95% CI)	*p* =0.19		0.78 (0.43–1.43)		0.57 (0.32–1.04)			
Relapsed/refractory multiple myeloma								
HR patients	POLLUX [81] Dara-Rd vs. Rd		ASPIRE [73] KRd vs. Rd		CASTOR [86] Dara-Vd vs. Vd		OPTIMISMM [82] VPd vs. Vd	
ORR rate [%]	89	68	79.2	59.6	85	56	NR	NR
PFS, m	26.8	8.3	23.1	13.9	12.6	6.2	8.44	5.32
HR (95% CI)	0.37 (0.18–0.76)		0.7 (0.42–1.16)		0.41 (0.21–0.83)		0.56 (0.35–0.9)	

NDMM — newly diagnosed multiple myeloma; ASCT — autologous stem-cell transplantation; HR — high-risk; VRd — bortezomib, lenalidomide, dexamethasone; KRd12 — carfilzomib, lenalidomide, dexamethasone 12 cycles; KRd-T — carfilzomib, lenalidomide, dexamethasone, thalidomide; KCd — carfilzomib, cyclophosphamide, dexamethasone; Dara-VTd — daratumumab, bortezomib, thalidomide, dexamethasone; VTd — bortezomib, thalidomide, dexamethasone; Dara-VRd — daratumumab, bortezomib, lenalidomide, dexamethasone; post-consol — post consolidation; NR — [explanation?]; CR — complete response; Rd — carfilzomib, lenalidomide, dexamethasone; Dara-VMP — daratumumab, bortezomib, melphalan, prednisone; VMP — bortezomib, melphalan, prednisone; Dara-Rd — daratumumab, lenalidomide, dexamethasone; PFS — progression free survival; HR — hazard ratio; CI — confidence interval; Dara-Rd — daratumumab, lenalidomide dexamethasone; KRd — carfilzomib, lenalidomide, dexamethasone; Dara-Vd — daratumumab, bortezomib, dexamethasone; VPd — bortezomib, pomalidomide, dexamethasone; Vd — bortezomib, dexamethasone; ORR — overall response rate

in cytogenetic HR MM patients not eligible for ASCT improved the response rate compared to patients at standard cytogenetic risk, even though survival times remain much lower.

The IFM study showed that the application of the VRD protocol did not overcome the unfavorable prognosis in a group of elderly patients with MM with HR cytogenetics [77]. However, the use of daratumumab (Dara-VMP, Dara-Rd and Dara-KRd) improved PFS in patients with HR, although it was still shorter than in patients with standard cytogenetic risk [78–80]. In the treatment of cytogenetic HR MM recurrence in untreated or lenalidomide-sensitive patients, the longest PFS (26.8 months) was achieved with Dara-Rd (POLLUX) [81]. The combination of Rd with other drugs such as carfilzomib, ixazomib, and elotuzumab results in a shorter PFS, but the differences between patients with HR and SR are less significant than in the POLLUX study.

On the other hand, the use of the Pd protocol has a beneficial effect in patients with RRMM and del(17p) [76]. The use of other pomalidomide-based protocols (bortezomib–Pd and Isatuximab–Pd) has resulted in benefits in HR, and may overcome the unfavorable prognosis in HR cytogenetics [82, 83]. Table IV summarizes the treatments for patients with cytogenetic HR MM.

Conclusions

The introduction of genetic and molecular tests to MM diagnosis has resulted in a much better understanding of this disease's biology and has allowed a more accurate prognosis. Identifying HR CA has changed the staging system of MM and the method of treating patients with MM. Undoubtedly, further development of cytogenetic and molecular research should be expected in the coming years.

Author's contributions

GC, AJ, AS, DHV — wrote and critically revised manuscript.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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New opportunities in immunotherapy in multiple myeloma

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Abstract

Immunotherapy is a rapidly developing field of multiple myeloma, a disease which despite the development of new drugs remains incurable. There are several antigens that are being assessed in preclinical and clinical settings, but the most studied of all is B-cell maturation antigen (BCMA). BCMA is a target for antibody conjugated with toxins (ADCs), bispecific engagers (BITEs), and modified autologous lymphocytes (CAR-T, chimeric antigen receptor T cells). Belantamab mafodotin (ADC, registered in the European Union), teclistamab (BITE) and two CAR-Ts: ide-cel (Food and Drug Administation-approved) and cilta-cel are the most studied therapies in myeloma. Immunotherapy will definitely change the treatment strategy of multiple myeloma and accelerate the fight against myeloma.

Key words: immunotherapy, CAR-T, bispecific antibodies

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Mutliple myeloma, despite the introduction of new drugs, remains incurable mostly due to its acquired resistance to all existing therapies. The mechanism of resistance is multifactorial and still not fully understood, but one major factor is immunological escape. Such escape is related to genetic alterations leading to loss of antigens and the development of resistance to immune effector responses. The immunosuppression seen in myeloma is a result of T cell exhaustion, tolerance by tumor-associated antigen-presenting cells, alterations in cytokine production, and accumulation of myeloid-derived suppressor cells and suppressive tumor-associated macrophages [1, 2]. Therefore, the search for therapies that are focused on activation of the immune system seems a promising direction for improvements in treatment.

Immunotherapy is currently the most promising area of the development of myeloma therapy. There are several different approaches to activating the immune system in the fight against myeloma, including immunomodulatory drugs (IMiDs).

IMiDs act not only by direct myeloma cell killing, but also by stimulation of T and NK cells which is an effect of increased production of IL (interleukin)-2 and IFNγ and reduction of IL-10 production in both CD4+ and CD8+ T [3]. IMiDs, due to their good toxicity profile, can be combined with other immunostimulatory drugs such as elotuzumab, an IgG monoclonal antibody against SMALF7 (previously CCS1). The cumulative immunostimulatory effect was very visible in the Eloquent 2 and 3 studies, where elotuzumab was added to either lenalidomide (Eloquent 2) or pomalidomide (Eloquent 3) and dexamethasone for patients with refractory myeloma. Elotuzumab has a weak direct impact on myeloma cells (no significant responses were seen when used as a single agent), but by activation of NK cells through both CD16-mediated antibody dependent cellular cytotoxicity (ADCC) and direct co-stimulation via engagement with SLAMF7 and promoting antibody dependent cellular phagocytosis (ADCP) by macrophages, it engages the immune system against myeloma. In Eloquent 2, the addition of elotuzumab to lenalidomide

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results in a 30% reduction of progression or death compared to lenalidomide and dexamethasone, with a median progression-free survival (PFS) of 19.4 months compared to 14.9 months in the control arm [4]. In Eloquent 3, the effect was even more visible: a combination of elotuzumab and pomalidomide resulted in a 47% reduction in risk of death or progression compared to pomalidomide, with median PFS of 10.9 and 4.7 months respectively [5]. Both combinations are already registered by the European Medicines Agency (EMA).

However, the most widely studied antigen in myeloma is B cell maturation antigen (BCMA). BCMA is present only in late memory B cells and plasma cells, and is more expressed on myeloma cells compared to healthy plasma cells. The development of immunotherapy that is focused on BCMA includes monoclonal antibodies conjugated with cellular toxins (ADCs), bispecific T cell engagers (BiTEs), and therapy with modified human lymphocytes (CAR-T, chimeric antigen receptor T cells).

There are several ADCs under development in the treatment of multiple myeloma: belantamab mafodotin, CC-99712 and MEDI2228. Belantamab mafodotin (Blenrep) is the most advanced of these. It is an IgG kappa monoclonal antibody directed against BCMA antigen conjugated with the cellular toxin auristatin F. There have been promising results in the DREAMM1 study, where clinical efficacy [at least a partial response (PR)] was demonstrated in 60% of patients, paving the way towards the phase II study DREAMM2, which assessed the efficacy and safety of two doses of belantamab: 2.5 and 3.4 mg/kg. That study included patients with very advanced disease (median number of lines of therapy for dose of 2.5 mg/kg was 7, and for dose of 3.4 mg/kg was 6). The vast majority of patients were refractory to daratumumab (92–100%), pomalidomide (78–87%) and carfilzomib (58–65%). The study showed a median PFS of 2.9 months for the lower dose and 4.9 months for the higher dose.

Among adverse events that are seen in patients in such advanced myeloma, one is new and definitely will impact patients treated with belantamab. Keratopathy, an effect of auristatin F, was seen in more than 70% of patients in DREAMM2. Keratopathy grade 3 or higher was observed in 46% treated with the lower dose and in 42% of those treated with the higher dose. This complication is temporary and usually resolves after dose modification and symptomatic treatment. However, it is very troublesome due to its affecting vision. In subsequent studies, the percentage of these side effects was slightly lower, which results from procedures being developed which more carefully qualify patients for belantamab therapy, introducing prophylactic masks to cool the eye area to reduce perfusion in the eyeball, a routine ophthalmological assessment, and the use of protective, moisturizing and anti-inflammatory drops. Belantamab is currently being explored in different phases of myeloma and will surely soon complement the drugs available to combat myeloma. This drug was approved in 2020 by the EMA for patients with refractory multiple myeloma who had received at least four lines of treatment including an immunomodulatory drug, a proteasome inhibitor, and a monoclonal antibody [6].

Bispecific antibodies that bridge T cells (typically via CD3) and tumor-specific antigens (in myeloma mostly BCMA) are more advanced forms of immunotherapy. The most common formulations are bispecific T cell engagers (BiTEs), which only comprise the variable heavy and light chain regions. This allows for T cell engagement and activation after tumor antigen recognition that is independent of T cell receptor (TCR) specificity. It is important to note that BiTEs rely on the presence of a functional T cell response, and this therapy is likely to be most efficacious early in the disease course. Several molecules are currently being investigated in different phases of clinical trials, including AMG 420, teclistamab, AMG 701, CC-93269, PF-06863135, and REGN5458. One of the first molecules used in humans was AMG 420, a bispecific antibody against CD3 antigen and BCMA. This molecule is composed of two light chains, which is associated with a very short half-life and the use of continuous, multi-day intravenous infusion. A study that enrolled 42 patients with very advanced disease (median number of previous treatment lines 7) showed very promising efficacy with an overall response rate reaching 70% depending on the dose of the drug with a good toxicity profile: no severe symptoms of cytokine release syndrome (CRS) and no neurological complications (ICANS, immune effector cell-associated neurotoxicity syndrome) were observed. The most important issue was infections, which occurred in 20/42 patients and resulted in the death of two of them. Despite quite optimistic results, this drug is no longer being developed due to the serious logistical difficulties resulting from the need to hospitalize patients for many weeks to allow continuous drug infusion [7].

A molecule with a similar concept of action against myeloma is teclistamab. This is an antibody similarly to AMG 420 directed against CD3 and BCMA antigens. It has been constructed as a complete immunoglobulin, which gives it a much longer half-life and the possibility of subcutaneous administration once a week. This drug is currently being evaluated at varying levels in clinical trials with very promising results. Efficacy was demonstrated in a phase I study involving 72 patients. The best results were achieved at a dose of 270 μg/kg: overall response rate (ORR) was 67% including very good partial response (VGPR) in 50%, although the number of patients was very small at 12. Importantly, the treatment was well tolerated except for hematological and infectious complications observed at a similar percentage and severity as in other studies

enrolling patients with very advanced myeloma; 44 patients had cytokine release syndrome, although no grade 3 or higher was found [8]. In the context of cell therapies and related logistic complications, teclistamab will clearly become an attractive alternative to CAR-T.

The therapy with the most spectacular results, and so the one with which the greatest hopes are associated, is therapy with modified human lymphocytes. CAR-T cells have been revolutionary in the treatment of patients with B cell malignancies, in which CD19 serves as an ideal target. There are several constructs of CAR-T currently under development in clinical and preclinical settings in myeloma that are directed against BCMA: ide-cel (called Abecma in the USA), cilta-cel, ALLO-715, bb21217, LCAR-B38M, orvacabtagene autoleucel, and P-BCMA-101. The first two are the closest to achieving EMA registration. Ide-cel, previously named bb2121, was registered by the Food and Drug Administation (FDA) based on the KarMMA study in March 2021. In the study, three different doses of 'the drug' were evaluated: 150 $\times 10^6$, 300 $\times 10^6$, and 450 $\times 10^6$ cells per kg. The study was performed on 128 patients with refractory and relapsed multiple myeloma with a median number of treatment lines of 6. The study showed an ORR of 73% in the entire population, including complete response (CR) of 31%. In the group of patients treated with the highest dose this was 82% and 35%, respectively. The median PFS of the whole population of the study was 10.6 months, while for the more effective higher dose it was 11.3 months. Severe neurological complications and CRS were observed only in 5% and 3%, respectively.

Based on the KarMMA study, ide-cel has been approved in the USA for patients previously treated with at least four lines of therapy including an immunomodulatory drug, a proteasome inhibitor, and an anti-CD38 monoclonal antibody [9]. This CAR-T construct is also being developed with a manufacturing modification (bb21217) which involves incubation of the cells with a phosphatidylinositol 3-kinase (PI3K) inhibitor. That process aims to increase the subpopulation of memory cells and eventually prolong the period of CAR-T anti-myeloma activity. In a phase I study which included 69 patients, very high efficacy was demonstrated: ORR reached 83% and CR 42%, with median PFS of 17 months. As in the KarMMa study, only isolated cases of severe CRS and ICANS syndromes were observed.

The second very advanced CAR-T construct studied in multiple myeloma is cilta-cel. This drug, like ide-cel, targets the BCMA antigen, although each receptor has two recognition domains, whereas ide-cel has only one. The Cartitude 1 study results, presented at American Society of Hematology (ASH) 2020, were very promising: in 97 patients with a similar population of myeloma patients as in the KarMMa study (median number of treatment lines of 6), 97% of patients achieved at least PR, including VGPR in 92.8% and CR in 67%.

Considering the challenging patient population that was included in Cartitude 1, these results are spectacular. Symptoms of cytokine release syndrome of at least grade 3 were found in 4% of patients, and ICANS in 2%. One patient died of hemophagocytic syndrome as a consequence of the CRS. Interestingly, late neurological complications other than ICANS of at least grade 3 were observed in 12% of patients [10].

CAR-T therapy based on the modification of autologous lymphocytes, despite its undeniable effectiveness, is associated with a number of logistical issues. CAR-Ts need to be produced and transported; this requires time and is very expensive and the process is not always successful. Modified allogeneic lymphocytes are an extremely attractive alternative, which as universal cells can wait for the patient, just like an ordinary medicine in a pharmacy. Research on such a design of allogeneic CAR-T 'off-shelf' is already underway and the results are very promising. The construction of ALLO715, where, in addition to the modified anti-BCMA receptor, lymphocytes lacked the TCR, was evaluated in a phase I study involving 36 patients with refractory multiple myeloma. ORR was observed in 30% of patients, but a much more important observation was the virtual absence of severe forms of CRS, ICANS and, above all, graft-versus-host disease (GvHD) [11].

BCMA is only one of a number of targets being explored as immunotherapeutic targets. An example is talquetamab, a BiTe specific to CD3 and GPRC5D. This antibody was successfully assessed in a phase I study, and is now being studied in a phase II [12].

Immunotherapy is a vigorous and dynamically developing field of oncology which we have high hopes for, and likewise in multiple myeloma. Advanced antibodies and cell therapies are becoming vital tools in the fight against cancer, and will remodel the treatment strategy of multiple myeloma over the next few years.

Author's contributions

DD — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Peripheral neuropathy in patients with multiple myeloma: molecular effects of bortezomib

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Abstract

Multiple myeloma (MM) is a B cell neoplasm characterized by uncontrolled growth of malignant plasma cells within the bone marrow. The introduction of new treatment regimens and medicinal substances, particularly proteasome inhibitors (e.g. bortezomib or carfilzomib) and immunomodulatory drugs (e.g. lenalidomide, pomalidomide, and monoclonal antibodies), have radically changed MM therapy by improving the response rate and progression-free survival. However, these potentially effective drugs are associated with a number of side effects, the most serious of which include peripheral neuropathy, which appears in 40% of MM patients with bortezomib treatment and up to 70% with thalidomide treatment during long-term exposure. Usually, symptoms of neuropathy disappear after drug discontinuation or dose reduction. However, as a result, the effectiveness of the treatment is lowered and survival time is reduced. The pathogenesis of chemotherapy-induced peripheral neuropathy is not fully understood. Current research focuses on areas such as the change in the expression of genes responsible for the proper functioning of the nervous system, neuroprotective protein factors, oxidative stress, pro-inflammatory factors and epigenetic changes (miRNA, DNA methylation or histone acetylation). Thoroughly elucidating the mechanisms responsible for the development of chemotherapy-induced peripheral neuropathy will allow us to reduce/eliminate this side effect and improve quality of life for patients.

Key words: bortezomib-induced peripheral neuropathy, multiple myeloma

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Introduction

Multiple myeloma (MM) is a B cell neoplasm characterized by uncontrolled growth of malignant plasma cells within the bone marrow (BM). These cells are ordinarily able to produce monoclonal proteins. MM constitutes 1% of all neoplasms and 10% of all hematological malignancies [1]. The American Cancer Society estimates that 34,920 new cases of MM and 12,410 MM-related deaths will occur in 2021. MM is one of the most intractable malignancies and is characterized by the infiltration and growth of malignant plasma cells in the BM [2].

Despite the application of high-dose chemotherapy followed by autologous stem cell transplantation (SCT) and novel therapeutic agents, the prognosis for patients with MM is still unsatisfactory [3]. Specific genetic abnormalities are present in MM, including immunoglobulin heavy chain (*IGH*) translocations, RB1 deletion, 1q gain, hyperdiploidy or RAS gene mutations [4]. The first developmental step is the occurrence of recurrent chromosomal translocations involving the *IGH* locus at 14q32 [5]. The most frequent translocation is t(11;14)(q13;q32), which is observed in 15–20% of MM cases [6], followed by t(4;14)(p16;q32), with a 12–15% prevalence [7]. Other translocations, including t(14;16)(q32;q23), t(14;20)(q32;q11), and t(6;14)(p21;q32), are less frequently detected (<5%) [8]. The t(4;14)(p16;q32) translocation causes deregulation of histone methyltransferase

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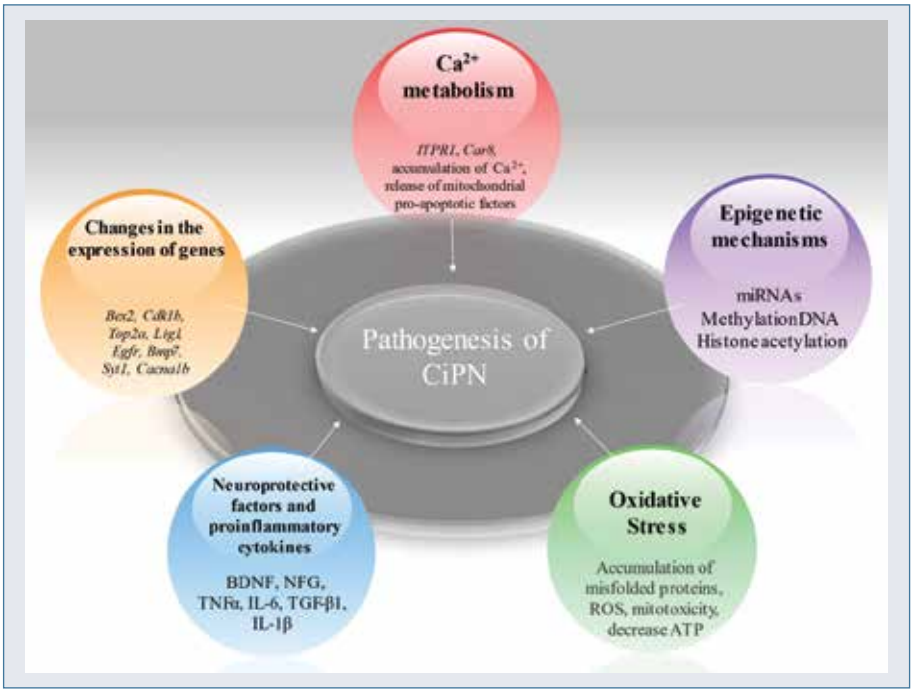

Figure 1. Pathogenesis of chemotherapy-induced peripheral neuropathy (CiPN); Ca^{2+} — calcium ions; BDNF — brain-derived neurotrophic factor; NFG — nerve growth factor; TNFα — tumor necrosis factor alpha; IL-6 — interleukin 6; TGF-β1 — transforming growth factor β1; IL-1β — interleukin 1β; ROS —reactive oxygen species; ATP — adenosine triphosphate

(MMSET), which promotes a decrease in H3K27me3 and H3K36me2 levels along the entire genome, and these changes cause the derangement of several genes, including cyclin D2 [9].

The second mechanism underlying the malignant transformation of plasma cells is hyperdiploidy, which is observed in approximately 55% of MM patients. For unknown reasons, odd-numbered chromosomes, such as 3, 5, 7, 9, 11, 15, 19 and 21 are increased in hyperdiploidy. The most prevalent hyperdiploidy (c.30%) is trisomy 11, which may cause cyclin D1 overexpression due to an increase in gene dosage [10]. The pathogenesis and survival time of patients is very heterogeneous. Introducing new treatment regimens and medicinal substances, particularly proteasome inhibitors [e.g. bortezomib (BTZ) or carfilzomib] and immunomodulatory drugs (e.g. lenalidomide and pomalidomide, and monoclonal antibodies), have radically changed MM therapy by improving the response rate and progression-free survival. State-of-the-art chimeric antigen receptor (CAR) T-cell immunotherapy uses mechanisms other than basic MM therapies. The CAR-T method involves the modification of patient or donor T cells to target specific cell surface antigens. The results of the latest clinical trials with anti-BCMA CAR-T lymphocytes have shown that patients with relapsed and/or refractory MM can achieve an objective response [11].

Unfortunately, similarly to the vast majority of drugs, those used in the treatment of MM also have a specific spectrum of side effects. One of the most important clinical problems seems to be chemotherapy-induced peripheral neuropathy (CiPN), which is mainly due to the symptom frequency, inconvenience for patients and dose-limiting effects [12]. CiPN occurs at varying severities during therapy, and its symptoms are observed in c.40% of MM patients with BTZ treatment [13] and up to 70% with long-term thalidomide treatment [14]. The incidence of CiPN depends on the dose, schedule and method of administration [15, 16].

Bortezomib-induced peripheral neuropathy (BiPN) is characterized by symmetrical distal sensory neuropathy with dominant pain symptoms. Subcutaneous administration of BTZ lowers neuropathy (38% vs. 53%) compared to former intravenous administration [17].

The degree of neuropathy is determined according to various scales. However, the most common scale is the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) [18]. This scale includes three types of neuropathy: a) sensory, b) autonomic-sensory, and c) sensorimotor. Moreover, in each type of neuropathy, its degree can be determined depending on the severity of symptoms (where 0 means no symptoms and 4 means permanent functional impairment) [19].

To elucidate the pathogenesis of CiPN, global research has focused on several areas, as shown in Figure 1.

This review focuses on the pathophysiology of CiPN based on the latest scientific data and our own research.

Pathophysiology of BiPN

Bortezomib is a boron-containing organic compound that specifically and reversibly inhibits the chymotrypsin-like activity of the 26S proteasome. Inhibition of proteasome activity by BTZ disrupts the processes necessary for proper functioning, which consequently leads to cell death [20]. The mechanism of action of BZT is disruption of the cell cycle, induction of apoptosis, disturbance of bone marrow microenvironment, and inhibition of nuclear factor kappa B (NFκB) [21].

One of the first studies on the mechanisms of bortezomib-induced neurotoxicity was conducted by Cavaletti et al. in 2007 [22] using a rat model. Studies have shown that BTZ causes disturbances in satellite cells and Schwann cells of the sensory nerves. Meregalli et al. [23] proved that the drug also affects synapses and causes unmyelinated C-fiber axonopathy. BTZ cytotoxicity is also attributed to disturbances in cellular calcium homeostasis as a consequence of abnormal mitochondrial function [24]. The accumulation of Ca^{2+} ions in mitochondria causes rupture of the outer membrane and then the release of mitochondrial proapoptotic factors into the cytosol [25, 26]. In addition, the downregulation of genes responsible for calcium metabolism, such as ITPR1 and Car8, may have a significant impact on the functioning of the nervous system, including the excitability of neurons, the growth of neurites and the release of neurotransmitters [27]. Protein neuroprotective factors, especially neurotrophins (NTs), play a special role in the context of nerve cell homeostasis. The family of classic neurotrophins includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/neurotrophin 5 (NT-4/5). These proteins are synthesized and released mainly by nerve cells [28, 29] but also by muscle [30], endothelium [31, 32], spleen, adipose tissue, liver, lung and hematopoietic cells [33–35]. Neurotrophins influence the proliferation, differentiation, viability and death of neuronal and non-neuronal cells. Due to the significant influence of NTs on the nervous system, lowering their concentration in tissue may contribute to the development of neuropathy [36, 37]. This hypothesis seems to be confirmed by Azoulay et al. [38], who described decreased BDNF concentrations in the plasma of patients with MM and symptoms of BiPN relative to patients treated with the same regimen but without symptoms of BiPN.

Peripheral neuropathy is associated with an increase in reactive oxygen species and a decrease in endogenous antioxidants [39]. BTZ inhibits the actions of the proteasome, which causes the accumulation of misfolded proteins that would be degraded under physiological conditions. Consequently, subsequent protein folding attempts generate high levels of reactive oxygen species (ROS) [40]. Thus, the development of BiPN may be related to mitotoxicity in primary axons (PNSAs) resulting from reduced mitochondrial bioenergetics. This association is confirmed by the fact that the development of mechano-hypersensitivity induced by BTZ is prevented by the administration of MnTE-2-PyP(5+), which belongs to the group of peroxynitrite decomposition catalysts (PNDCs, i.e. compounds with redox activity that detoxify peroxynitrite by catalyzing its isomerization or reduction to nitrates or nitrites). In addition, the action of BTZ is related to the nitration and inactivation of superoxide dismutase in the mitochondria and a meaningful decrease in adenosine triphosphate (ATP) production [41].

BTZ also causes higher proteotoxic stress associated with increased expression of heat shock proteins, reduced membrane potential of mitochondria, and ubiquitination of protein K48. Furthermore, BTZ downregulates the content of mitochondrial oxidative phosphorylation complexes, thereby decoupling protein 2 (UCP2) and voltage-dependent anion channel 1 (VDAC1) [42].

Proinflammatory cytokines are another area of research that may bring us closer to solving the problem of the pathomechanism of BiPN [43]. One of the most extensively studied proinflammatory factors is tumor necrosis factor alpha (TNFα). Zhao et al. [44] showed that during the administration of BTZ to rats, the expression of TNFα was significantly increased. Another study confirmed that the expression of TNFα was upregulated in the dorsal root ganglia after treatment with BTZ in a mouse model [45]. Furthermore, the same study showed increased expression of other proinflammatory cytokines, such as interleukin (IL) 6, transforming growth factor β1 (TGF-β1) and IL-1β, in the dorsal root ganglia, which was directly related to the administration of BTZ [45].

An increasing number of reports have focused the influence of BTZ on gene expression and epigenetic mechanisms. Although BTZ contributes to the inhibition of tumor progression, it also causes disturbances in cells that lead to the development of BiPN and other side effects such as thrombocytopenia, neutropenia or anemia. The activity profile of BTZ includes damage to DNA strands and inhibition of repair and replication processes and the cell cycle [46].

Epigenetics describes inherited gene expression mechanisms that are not dependent on changes in DNA sequences and provide diversity in the functioning of cells based on identical genetic materials. Epigenetic mechanisms include histone modification, DNA methylation, miRNA-based gene regulation, and monoallelic gene expression (parental imprinting, inactivation of the X chromosome) [47]. Fernández de Larrea et al. [48] demonstrated a relationship between the degree of total DNA methylation and the survival time of patients with relapsed MM who received treatment regimens based on BTZ. Patients with total DNA methylation >3.95% achieved longer overall survival (OS). In addition, patients with a relatively low percentage of methylation (<1.07%) of the *NFKB1* gene also showed a longer overall survival after BTZ therapy [48]. Epigenetic mechanisms

include the regulation of gene expression with small single-stranded noncoding microRNAs (miRNAs). During BTZ therapy, a decreased level of Let-7f has been observed, which promotes vascular neoplastic processes by lowering the expression of genes responsible for antiangiogenic effects [49]. Administering anti-Let-7f enhances apoptosis and reduces the proliferation rate of established MM cell lines [50].

Moreover, BTZ induces changes in the expression of miRNA molecules whose genes are involved in inhibiting the development of cancer cells or in the functioning of the nervous system. For example, miRNA-181, miRNA-20a, miR-342-3p, miR-128, miR-17-92 and miR-29b regulate genes involved in the process of neurogenesis and neuronal differentiation, and their plasma concentration is significantly lowered during BTZ therapy while the level of miRNA-34a is then elevated, which results in inhibition of BDNF expression and activation of the neuronal apoptosis process [51].

Currently, our research group is focused on gene expression and epigenetic changes that may influence the development of BiPN, which has not been well explored. We have shown changes at the molecular level that may contribute to inhibiting the development of both cancer [52] and neuropathy [53]. Two representative established cell lines, a) SH-SY5Y neuroblastoma and b) a PC12-derived nerve cell line, were used in these studies. Cells were treated with BTZ (50 nM/L) for 24 h, and global gene expression and miRNA expression were analyzed using genome-wide RNA and miRNA microarray technologies. Studies have shown that BTZ might exert toxic effects on both neuroblastoma cancer and PC12 nerve cells and regulate miRNA/mRNA interactions that affect important cellular functions. BTZ has been shown to exert a meaningful inhibitory effect on the proliferation (*TFAP2B*, *PEG10*) and apoptosis (*HSPA1B*, *CLU, HMOX1*) of human neuroblastoma cells. These mechanisms could be responsible for the advantages of using BTZ for cancer treatment. In contrast, in nerve cells, BTZ primarily inhibits the cell cycle (*Bex2, Cdk1b, Lin9*), DNA repair processes (*Top2a, TopBP1, Lig1*, *Ercc6*), neuronal morphogenesis (*Egfr, Bmp7, Ilk*), and neurotransmitter secretion (*Syt1, Cacna1b, Lin7a*). The obtained outcomes show differences in the major metabolic pathways and biological processes that are disturbed as a result of the action of BTZ in cancer and nerve cells.

An important observation from the conducted research was the mRNA/miRNA relationship. The most interesting correlation between miRNA and target genes in neuronal cells differentiated from PC12 cells was the decreased levels of miR-130a-3p and miR-152-3p, which resulted in an increase in *Gadd45* gene expression. This gene belongs to a group of genes whose expression at the transcript level is enhanced under stress conditions of growth arrest and exposure to DNA damaging agents, such as drugs or mutagens. In contrast, cancer cells showed changes in the correlation between mRNA and target genes, which influenced various processes such as a) promoting apoptosis (↓ hsa-miR-330-3p — ↑ *MDM2*; ↓ hsa-miR-124-3p — ↑ *SNAI2*; ↓ hsa-miR-503-5p — ↑ *DYNLL2*), b) regulating neurogenesis (↑ hsa-miR-21-3p — ↓ *NCAM2*; ↑ hsa-miR-335-5p — ↓ *SOX4*; ↑ hsa-miR-34a-5p — ↓ *CDK6*), and c) activating processes of neuronal death (↓ hsa-miR-6880-5p — ↑ *C12orf5*; ↓ hsa-miR-26b-5p — ↑ *DDIT4*; ↓ hsa-miR-26b-5p — ↑ *HSPD1*) (↑ miRNA/target gene expression; ↓ miRNA/target gene expression).

In subsequent studies, we revealed a significant effect of the immune response in myeloma patients on the development of CiPN. We observed increases in the levels of proinflammatory cytokines (CCL2, IL-1β, IFN gamma, properdin) and complement proteins (complement 9, factor D) at both the transcript and protein levels. In addition to understanding the pathogenesis of BiPN, an important goal is identifying biomarkers for faster diagnosis of neuropathy. Our recent studies have identified miR-22-5p as a potential marker of CiPN in patients with MM.

Resistance to bortezomib in multiple myeloma

Resistance to BTZ development in MM patients is a serious therapeutic problem. Current scientific reports show the involvement of PSMB5 mutations and proteasome subunit upregulation, changes in protein and gene expression in response to cell survival, stress, and antiapoptotic pathways in the development of resistance to BTZ [54, 55]. The epigenetic changes triggered by BTZ may contribute to the development of resistance. Class I histone deacetylases (HDACs) determine the sensitivity of proteasome inhibitors, and histone methyltransferase (EZH2) alters the transcription of antiapoptotic genes during the acquisition of cell adhesion-mediated drug resistance (CAM-DR) by myeloma cells. In addition, histone methyltransferase (MMSET) has been shown to confer drug resistance to myeloma cells, thereby facilitating DNA repair [56].

Additional research by our group in this area focused on analyzing the methylation profile following exposure of neuroblastoma cells to BTZ. The study consisted of treating neuroblastoma cells with BTZ for 24 hours and then leaving them for 12 days (in medium without BTZ) to examine the methylation profile in the daughter cells and assess the extent of proliferation after subsequent doses of BTZ. The obtained results showed that BTZ induced marked genome-wide methylation changes in cells. The obtained results showed a significantly altered global methylation profile after treatment of the cells with BTZ, manifested by hypermethylation of genes which were hypomethylated in control cells and a decrease in the

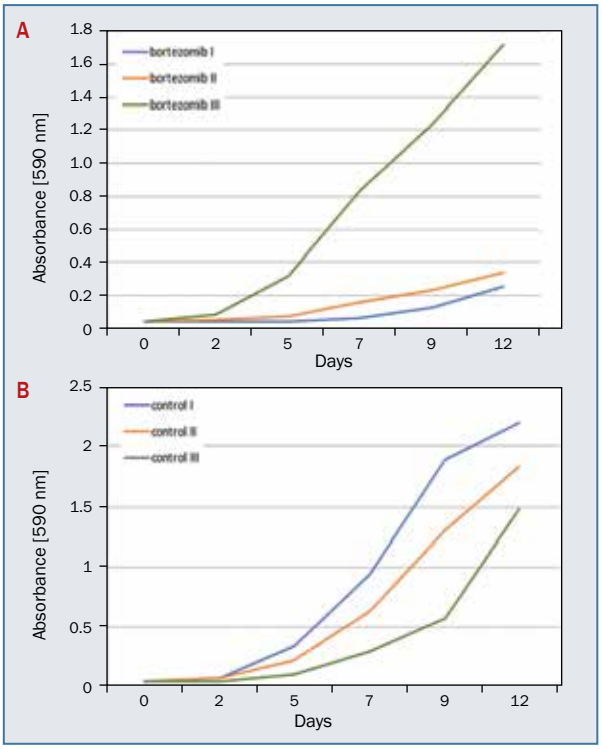

Figure 2A, B. MTT test results showing induction of unusual cell proliferation potential that increased with subsequent treatments

degree of methylation in hypermethylated genes. The observed changes mainly concerned the pathology of cancer pathways.

The consequence of these changes may be to bypass the primary antitumor activity of BTZ and develop a treatment-resistant phenotype. To investigate the acquisition of a proliferative phenotype, cells that had recovered after the first round of BTZ treatment were treated three times. Repeated treatment led to the induction of an unusual cell proliferation potential that increased with subsequent treatments (Figure 2) [57].

Conclusion

The pathogenesis of BiPN is still extremely unclear, and its development involves many molecular mechanisms; therefore. A relatively new area of research in this field is focused on the epigenetic mechanisms that may constitute the basis for the development of PN due to the global regulation of gene expression in many processes. Thorough elucidation of the mechanisms responsible for the development of BiPN will allow us to reduce/eliminate this side effect and improve the quality of life of patients.

Author's contributions

KŁ wrote a draft of the manuscript and prepared the figures, BM reviewed and edited the manuscript.

Conflict of interest

The author declares no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Monoclonal gammopathy of clinical significance (MGCS): when monoclonal gammopathy of undetermined significance (MGUS) is no longer undetermined

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Abstract

Monoclonal gammopathy of undetermined significance (MGUS) is a condition characterized by the presence of a monoclonal immunoglobulin (mIg) without its organ- or tissue-damaging effect. In recent years, attention has been paid to patients who show a MGUS-like condition, but at the same time present damage to the kidneys, peripheral nerves, or skin, resulting from the deposit of mIg. These disorders do not meet the criteria for smoldering myeloma or multiple myeloma. In 2018, the term 'monoclonal gammopathy of clinical significance' (MGCS) was introduced for this group of patients.

The dysfunction associated with MGCS is the result of the toxic activity of a monoclonal protein produced by dangerous, small clones of B cells and plasmocytes. Taking this into account, the term 'MGUS' should be limited to those cases where no association with mIg organ or tissue damage can be demonstrated, whereas the term 'MGCS' (monoclonal gammopathy of clinical significance) should be used in patients in whom the monoclonal protein plays a direct role in damage, especially to the kidneys, skin, and nervous system. This article summarizes the current state of knowledge of the main syndromes and symptoms of MGCS.

Key words: monoclonal gammopathy of undetermined significance (MGUS), monoclonal gammopathy of clinical significance (MGCS), monoclonal gammopathy of renal significance (MGRS), neurological MGCS, cutaneous MGCS

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Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is a condition characterized by the presence of a monoclonal gammopathy (MG), but without end organ damage [1]. The diagnosis of MGUS requires the serum monoclonal (M) protein and bone marrow plasma cells to be below 3.0 g/dL and 10%, respectively. As MGUS progresses to multiple myeloma (MM) or Waldenstrom's macroglobulinemia slowly, treatment is not usually initiated until the diagnosis of these malignant conditions. At the beginning of this century, researchers' attention was drawn to the increasing variety of pathological kidney conditions in patients with MGUS. As these patients did not meet the criteria for multiple myeloma (MM) or even smoldering myeloma (SMM), they were misdiagnosed as MGUS with coexisting renal disorder, for example "glomerulonephritis with MGUS" [2]. Unfortunately, MGUS was misrepresented in this context, as monoclonal gammopathy did not in fact have 'undetermined significance' in these patients. Despite their nonmalignant nature, these diseases were associated with high morbidity and mortality [3]. Therefore, in 2012 the term "monoclonal gammopathy of renal significance" (MGRS) was introduced in order to distinguish the

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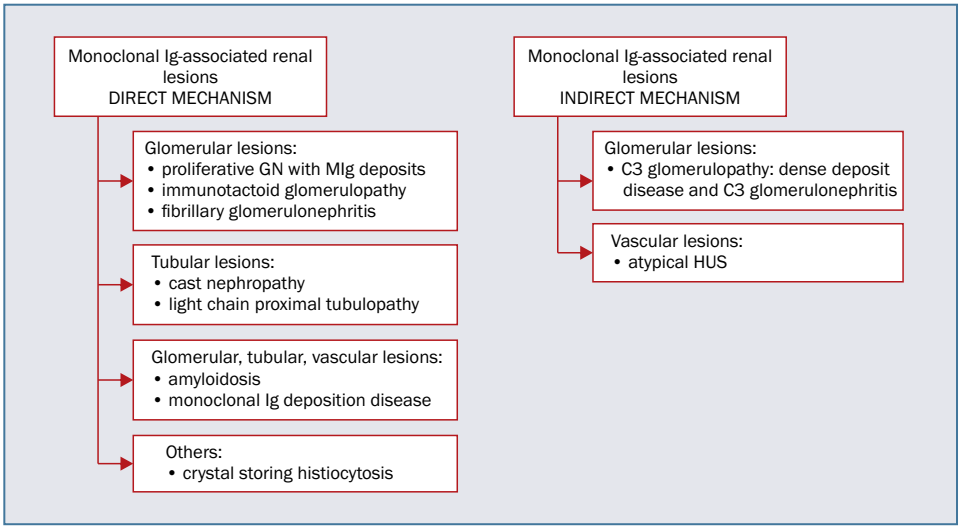

Figure 1. Renal lesions associated with monoclonal gammopathy of renal significance (MGRS) according to ultrastructural characteristics of deposits in kidney (acc. to [9])

nephropathic nature of these diseases from the truly benign monoclonal gammopathy of undetermined significance.

The goal was to segregate patients with MGUS, who have no evidence of end-organ damage (and a relatively good prognosis), from those with MGRS, who are at risk of developing progressive kidney disease (with a possibly fatal outcome) [4].

It became increasingly apparent that another term was required for patients with a small clone of B-cells producing monoclonal proteins that caused serious, potentially life-threatening disease. In 2018, the term "monoclonal gammopathy of clinical significance" (MGCS) was introduced. MGCS is a monoclonal gammopathy characterized by two main features: a quiescent underlying clone and symptoms associated with the monoclonal immunoglobulin [5]. MGCSs can be divided according to the different systems affected, the most common of which are the kidneys, nervous system, and skin. It must be emphasized however that there is an overlap in some cases, due to a systemic, multiorgan presentation and disease course.

Monoclonal gammopathy of renal significance (MGRS)

Monoclonal gammopathy of renal significance (MGRS) is a group of disorders in which a monoclonal immunoglobulin secreted by a nonmalignant or premalignant B cell or plasma cell clone causes renal damage [4]. These disorders do not meet the diagnostic criteria for overt, symptomatic MM or other lymphoproliferative diseases. It must be underscored that MGRS can also be associated with other hematological disorders, such as SMM, smoldering Waldenstrom's macroglobulinemia, and monoclonal B cell lymphocytosis (MB) [6–8].

The renal lesions in MGRS are primarily due to the abnormal deposition or activity of monoclonal proteins (light chains, heavy chains, or intact immunoglobulins) within the kidneys, specifically within the glomeruli, tubules, vessels, and interstitium that depends on the specific biochemical characteristics of the involved pathogenic protein.

Renal lesions that are associated with MGRS can be categorized according to the ultrastructural characteristics of the deposits in the kidney, if present (Figure 1) [9]. These deposits are divided into organized (with substructure) and nonorganized (without substructure, granular). In some cases of MGRS, including thrombotic microangiopathy associated with monoclonal gammopathy (i.e. in POEMS syndrome [**p**olyneuropathy, **o**rganomegaly, **e**ndocrinopathy, **m**onoclonal gammopathy, and **s**kin changes]), deposits within the kidney are not visible [10].

The mechanism of renal injury in MGRS can be direct with the deposition of the mIg, but in a few cases the mechanism is indirect, with renal lesions as the result of dysregulation of the complement pathway by the mIg (Figure 2) [11].

Clinical manifestation and diagnosis

A diagnosis of MGRS should be suspected in the following:

1. Patients with a nonmalignant or premalignant monoclonal gammopathy [e.g. MGUS, SMM, smoldering Waldenström's macroglobulinemia, or monoclonal B cell lymphocytosis (MBL)] who present with unexplained renal impairment and/or proteinuria.
2. Patients who present with unexplained renal impairment and/or proteinuria, and in whom during the evaluation of renal disease are found to have a monoclonal

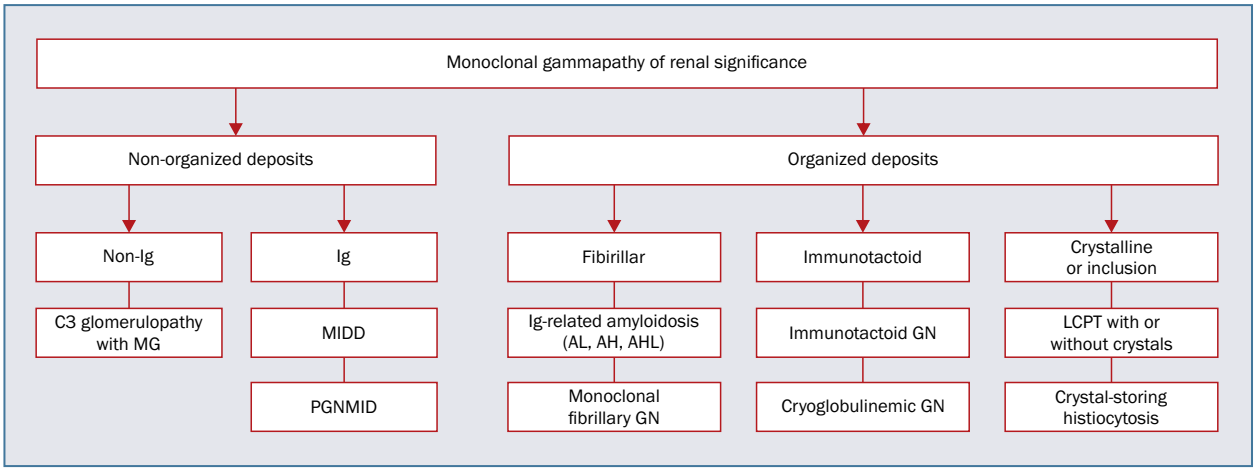

Figure 2. Renal lesions associated with monoclonal gammopathy of renal significance (MGRS) according to mechanisms of renal injury (acc. to [11])

gammopathy e.g. by serum or urine protein electrophoresis or immunofixation or by serum free light chain assay. Urine free light chain assay does not have any known diagnostic value with regard to these disorders.

A kidney biopsy must be performed in patients suspected of having MGRS, unless contraindicated. The presence of monoclonal immunoglobulin deposits in the kidney confirms the diagnosis of MGRS. For unknown reasons, a large majority (70–80%) of patients with proliferative glomerulonephritis with monoclonal immunoglobulin deposits (PGNMID) do not have a detectable circulating monoclonal gammopathy, both by serum and by urine monoclonal protein testing. Moreover, plasma cell or B cell clones on bone marrow aspirate and biopsy are not detectable. The monoclonal protein is only found in the kidney in patients with PGNMID, therefore the diagnosis of MGRS in these patients is usually established following kidney biopsy for the evaluation of unexplained renal insufficiency and/or proteinuria or renal allograft dysfunction.

The treatment of monoclonal gammopathy-associated renal lesions aims to eliminate the underlying clone of plasma cell population in order to decrease or stop the production of the harmful M protein. The most efficient treatment is to use the chemotherapy regimens that have been developed for the treatment of multiple myeloma and AL amyloidosis.

Unfortunately, MGRS recurs frequently and rapidly after kidney transplantation, therefore achieving complete hematological remission prior to transplantation is essential [12, 13].

The treatment options according to Leung [14] are presented in Figure 3.

Cutaneous monoclonal gammopathies of clinical significance (cutaneous MGCS)

Cutaneous MGCSs include scleromyxedema, Schnitzler syndrome, necrobiotic xanthogranuloma (NXG), TEMPI syndrome, cryoglobulinemia, and systemic capillary leak syndrome (SCLS).

Scleromyxedema is a rare systemic mucinosis characterized by generalized papular and sclerodermoid cutaneous eruptions. It is usually associated with monoclonal gammopathy involving an immunoglobulin G (mIgG) isotype with slow electrophoretic mobility [15]. Usually, scleromyxedema is a result of a small clone of secretory plasma cell (commonly referred to as MGUS) that may be associated with severe organ damage and could be a part of the MGCS [16]. Various types of extracutaneous involvement have been described in scleromyxedema, in particular neurological, gastrointestinal, cardiovascular, and joint impairments. The high mortality of scleromyxedema is primarily a result of treatment toxicity and dermatoneuro syndrome (DNS), as well as severe acute encephalopathy, usually manifested by epileptic seizures and/or coma. The efficacy of high-dose intravenous immunoglobulin (HDIViv) in the treatment of cutaneous symptoms of scleromyxedema has been described, and in 2020 Mahevas et al. presented a therapeutic algorithm for the treatment of MG-associated scleromyxedema [17, 18].

Schnitzler syndrome is another exceedingly rare, probably autoinflammatory, adult-onset disease. Since its first description in 1972, only 300 or so cases have been reported. The disease hallmark is the presence of a monoclonal IgM-κ protein in the vast majority of reported cases (classical type), although monoclonal IgG has been identified in a minority (variant type) [19]. Clinical phenotype with a chronic urticaria-like rash and a monoclonal IgM or IgG paraprotein is obligatory. Interleukin (IL)-1β plays a key role in this disease. The efficacy of novel anti-IL-1 antibodies such as rilonacept and canakinumab in the treatment has been proven [20]. Careful tracking of C-reactive protein level may be helpful in the monitoring of this disease [21].

Necrobiotic xanthogranuloma (NXG) is a non-Langerhans cell histiocytosis classically associated with paraproteinemia

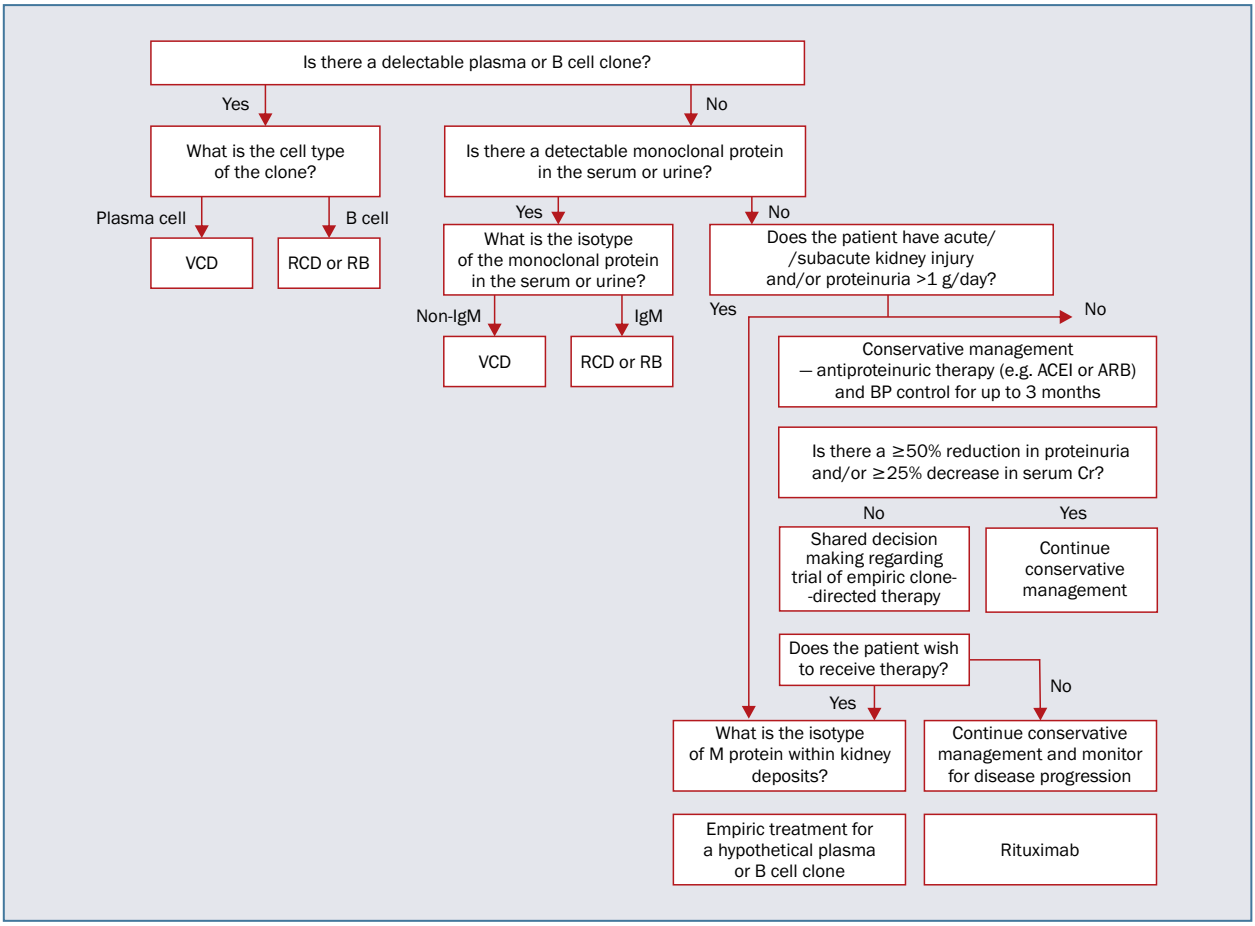

Figure 3. Algorithm of treatment of glomerulonephritis with monoclonal Ig deposits (acc. to [14])

attributable to plasma-cell dyscrasias or lymphoproliferative disorders, first described in 1980 [22]. The pathogenesis of NXG remains unclear; the paraprotein-lipoprotein interaction has been studied [23]. NXG is considered to be a skin manifestation of systemic disease. Extracutaneous involvement including the eyes, heart, gastrointestinal tract, liver, and lungs can result in organ dysfunction and death [22]. Clinically, yellow-to-orange papules, plaques, and/or nodules in a periorbital distribution are classic.

The diagnostic criteria for necrobiotic xanthogranuloma are below.

Major criteria:

1. Cutaneous papules, plaques, and/or nodules, most often yellow or orange.
2. Histopathological features demonstrating palisading granulomas with lymphoplasmacytic infiltrate and zones of necrobiosis. Characteristic features, that are variably present, include cholesterol clefts and/or giant cells (Touton or foreign body).

Minor criteria:

1. Paraproteinemia, most often IgG-κ, plasma-cell dyscrasia, and/or other associated lymphoproliferative disorder.
2. Periorbital distribution of cutaneous lesions.

Both of the major criteria, and at least one minor criterion, are required for diagnosis, applicable only in the absence of foreign body, infection, or other identifiable cause [24]. In a multicenter cohort, intravenous immunoglobulin had the best response rate (100%), followed by antimalarial drugs (80%), intralesional triamcinolone (75%), surgery (75%), chemotherapy (67%), and lenalidomide or thalidomide (63%) [25].

TEMPI syndrome is a rare and acquired disorder characterized by five features: telangiectasias; elevated erythropoietin and erythrocytosis; monoclonal gammopathy; perinephric fluid collections; and intrapulmonary shunting [26]. TEMPI syndrome generally manifests in the fourth or fifth decade of life, in both men and women, and without any discernable ethnic or geographical predisposition. Patients firstly present with erythrocytosis and telangiectasias, and many have been erroneously diagnosed with polycythemia vera and initiated on programs of therapeutic phlebotomy. In all patients, laboratory values are notable for an elevated serum erythropoietin and the lack of a JAK2 mutation. In those patients who have been tested, hemoglobin electrophoresis and hemoglobin oxygen affinity testing have been

normal. Telangiectasias are seen most prominently on the face, upper back and chest. The hands are also commonly affected, whereas the lower extremities seem to be spared of telangiectasias. The characteristic feature of TEMPI syndrome is a monoclonal gammopathy. Serum erythropoietin measurements can be extremely high: >5,000 mIU/mL (normal range 3–19 mIU/mL), driving a predictable abnormalities syndrome in which the monoclonal antibody is almost always restricted [10].

The diagnostic criteria for TEMPI syndrome are:

I. Major criteria:

1. Telangiectasis.
2. Elevated erythropoietin and erythrocytosis.
3. Monoclonal gammopathy.

II. Minor criteria:

1. Perinephric fluid.
2. Intrapulmonary shunting.

Other: venous thrombosis.

Complete resolution of symptoms following treatment with plasma cell-directed therapy supports the hypothesis that the monoclonal antibody is causal and pathogenic [26].

Neutrophilic dermatoses associated with monoclonal gammopathy refer to a group of cutaneous inflammatory disorders characterized by neutrophilic infiltration of the skin. This has been reported in association with various conditions including autoimmune diseases, inflammatory bowel diseases, myeloproliferative disorders, and (most frequently) monoclonal gammopathy [27]. Analysis has revealed that patients with neutrophilic dermatoses share a particular cytokinic pattern, with increased rate of IL-6, vascular endothelial growth factor, intercellular adhesion molecule-1, and granulocyte colony-stimulating factor (G-CSF), but not granulocyte-macrophage colony-stimulating factor (GM-CSF).

The data highlights a strong association between IgA isotype and neutrophilic dermatoses, and the existence of a specific inflammatory profile of cytokine. Although neutrophilic dermatoses do not appear to be directly related to the mIg, and can be treated by anti-inflammatory or immunosuppressive drugs, in the era of new antimyeloma drugs the role of plasma cells and neutrophil function should be further investigated.

Idiopathic systemic capillary leak syndrome (SCLS; Clarkson's disease), is characterized by a capillary leak resulting in sudden-onset shock and anasarca caused by plasma extravasation (up to 70% of total plasma volume). The diagnostic triad is composed of the so-called 'three Hs' which occur in the absence of secondary causes of these findings: hypotension, hemoconcentration, and hypoalbuminemia. Sixty eight percent of adult patients with SCLS have monoclonal proteins, most commonly IgG-κ. The differential diagnosis for an acute attack includes sepsis, anaphylaxis, and hereditary angioedema. Treatment at the time of an acute attack is supportive, with fluid resuscitation until flare subsides, which typically occurs over the course of a few days. Empiric prophylaxis with IVIG is recommended [28].

Monoclonal gammopathy keratopathy

Corneal and conjunctival immunoglobulin deposition is rare, and its discovery is nearly always indicative of a systemic paraproteinemia. In 2005, Garibaldi et al. [29] noted ultrastructural structure similarity and a comparable to immunotactoid glomerulopathy. They coined the term 'immunotactoid keratopathy'. Immunoproteinemia has been found to be present in 98% of reported corneal cases; it was monoclonal in 57% of cases and associated with plasma cell myeloma in the other 43% [29]. Early recognition of corneal immunoglobulin deposition in patients without a known history of paraproteinemia is essential. The optimal management of corneal immunoglobulin deposition is controversial. According to the experience of Milman and several case reports, a more aggressive systemic intervention can modify corneal findings favorably and may improve visual status, but as yet the data remains scant and inconclusive [30].

Neurological MGCS

Neurological MGCS includes light-chain amyloidosis (AL), POEMS syndrome, cryoglobulinemia, CANOMAD (chronic ataxic neuropathy, ophthalmoplegia, M-protein, cold agglutinins and disialosylated antibodies), and DADS-neuropathy-distal demyelic neuropathy formerly known as "MGUS-related peripheral neuropathy". Itis important to distinguish peripheral neuropathy associated with monoclonal gammapathy from two other well-known diseases with specific criteria of diagnosis, i.e. immunoglobulin light chain (AL) amyloidosis, and neuropathy associated with osteosclerotic myeloma (POEMS syndrome) [31]. In both AL amyloid neuropathy and POEMS, the link between the neurological process and the M protein is well documented, and therapy is targeted at the underlying condition [32]. Peripheral neuropathy is more frequently observed with monoclonal IgM proteins than with IgG or IgA M proteins [32]. There are some differences in the clinical presentation of neuropathic IgM M proteins compared to IgG or IgA M proteins [33].

Overall, peripheral neuropathy associated with monoclonal IgM gammopathy presents itself as distal, acquired, demyelinating, symmetric M-protein neuropathy (DADS-M) [34]. It is usually diagnosed in males between the ages of six and nine as a distal symmetrical neuropathy causing sensory ataxia due to affection of large fibers of the sensory nerves. Motor involvement can occur, but is usually mild and distal, and cranial nerve involvement is rare. Anti-MAG antibodies are present in approximately

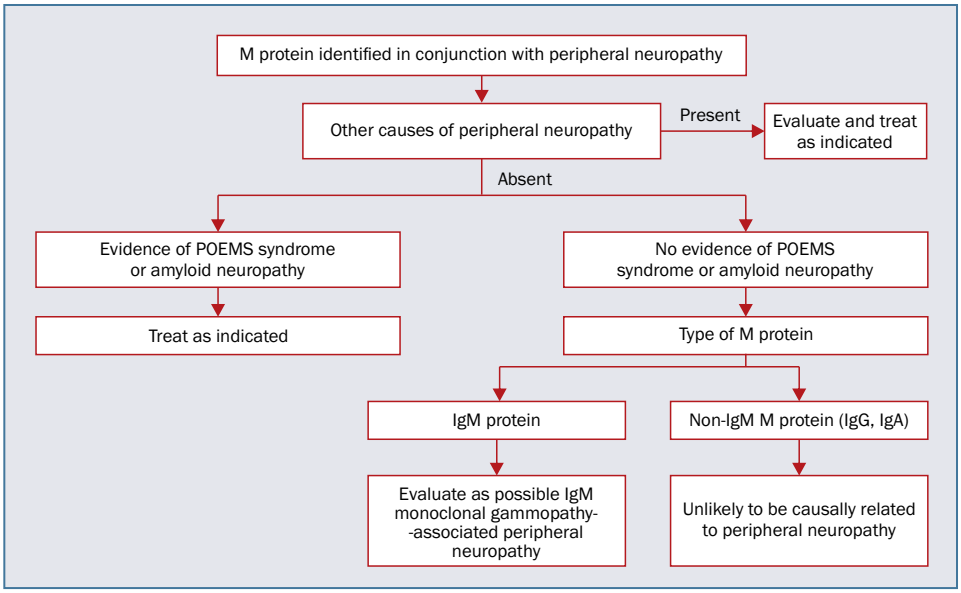

Figure 4. Algorithm for evaluation of patients with a monoclonal immunoglobulin with peripheral neuropathy (acc. to [31])

50% of patients; however, there is no difference in the severity or type of neuropathy with or without anti-MAG antibodies. Treatment includes immunoglobulin IV (IVIG) and rituximab [35].

Monoclonal proteins other than IgM can be observed in the full spectrum of neuropathic phenotypes, from the more common length-dependent axonal sensorimotor neuropathy, to chronic inflammatory demyelinating polyneuropathy (CIDP), which is mainly motor with proximal and distal involvement [36]. A Mayo Clinic study of 65 MGUS patients with peripheral neuropathy showed no significant clinical differences between IgG MGUS and IgA MGUS patients. Patients with IgG MGUS may have antibodies to nerve antigens, even in the absence of clinical neuropathy. Moreover, in CIDP, patients with and without paraprotein respond similarly to treatment.

An algorithm devised by Chaudhry for the evaluation of patients with a monoclonal protein identified in conjunction with peripheral neuropathy is presented in Figure 4 [31].

Summary

MGCSs are a constellation of diseases associated with clonal, nonmalignant B cells or plasma cells that produce monoclonal proteins and a pathology through diverse, ill-defined mechanisms.

The organs most affected among patients with MGCS are the kidneys, nerves, and skin. Some MGCSs predominantly involve only one organ, while others are systemic diseases that alter multiple organs. Diagnoses and assessment of the severity of the symptoms must be considered in order to institute the appropriate therapy.

The term 'MGUS' should be limited to cases where an association with end-organ damage cannot be demonstrated. Meanwhile, the term 'MGCS' should be used when the monoclonal protein plays a direct role in the pathomechanism of the kidneys, skin or central nervous system disorder.

Hopefully, this distinction will alert physicians to the seriousness of these conditions, and clarify the role of chemotherapy.

Author's contributions

LU-Z — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

FISH diagnostics in plasma cell myeloma: recommendations and our own experience

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Abstract

Plasma cell myeloma (PCM) is disease with heterogeneous clinical outcomes. It is increasingly evident that the genetic features of the tumor cells largely dictate the clinical heterogeneity of PCM. Primary chromosomal alterations in myeloma can be divided into hyperdiploid and non-hyperdiploid subtypes. Secondary chromosomal changes occur during progression of disease. Cytogenetic abnormalities are important prognostic markers in PCM and some of them were incorporated into the current prognostic staging system of PCM. The presence of t(4;14), t(14;16), t(14;20), gain of 1q or *TP53* deletion is considered to be high-risk myeloma. Detection of these alterations can be performed by interphase fluorescence *in situ* hybridization (FISH) after separation or identification of the plasma cells. The proper FISH examination in myeloma has to meet further requirements regarding aspirating and timing of samples, probe selection and their cut-off levels, the criteria of accurate analysis and reporting. Based on the literature, we here present technical recommendations regarding FISH in PCM. Furthermore, we share our own experience in FISH diagnostics acquired over 12 years. In this period, we have performed nearly 2,050 FISH tests in 603 myeloma patients and used two different methods of myeloma FISH: FISH on immunolabeled plasma cells, and target FISH with the BioView system.

Key words: myeloma, plasmacytoma, FISH, c-IG, target FISH, BioView

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Introduction

Plasma cell myeloma (PCM) is the most common malignant gammopathy and accounts for 10% of all hematological neoplasms [1]. The disease virtually always starts with a premalignant monoclonal gammopathy of undetermined significance (MGUS) that variably progresses to symptomatic PCM within months or years [2]. PCM is a heterogeneous disease with variable courses, responses to therapy, and survival outcomes that range from less than one year to more than 10 years. This clinical variety reflects the biological diversity driven by genetic abnormalities. Much has been learned regarding these genetic abnormalities. For instance, the translocations affecting immunoglobulin heavy chain (*IGH*) locus are essential in the pathogenesis of PCM in nearly 50% of patients. Most of the remaining patients have hyperdiploidy (trisomies of odd-numbered chromosomes) as the hallmark of the disease [1, 3]. In addition to these primary genetic events, presentation of myeloma is frequently accompanied by secondary chromosome abnormalities including deletion of chromosome 13q, gain of chromosome 1q [gain(1q)], and deletion of chromosome 1p [4]. Cytogenetic diagnosis constitutes an important part of the risk stratification of PCM and genetic diagnostic recommendations are constantly being updated. It must be underscored that the cytogenetic analysis of PCM can be challenging. Due to low proliferating features of malignant plasma cells and multiple marrow infiltrates, karyotyping is not recommended. Moreover, some chromosome aberrations such as t(4;14)(p16;q32)

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Table I. Cytogenetic abnormalities in newly diagnosed plasma cell myeloma (modified from [1, 2, 4, 6–9])

Type of genetic event	Cytogenetic abnormalities	Gene affected	Frequency [%]
Primary	Trisomies of odd-numbered chromosomes with the exception of chromosomes 1, 13		42–60
	IGH translocations:		30
	• t(11;14)(q13;q32)	*CCND1*	15–20
	• t(4;14)(p16;q32)	*MMSET, FGFR3*	6–15
	• t(14;16)(q32;q23)	*MAF*	2–7
	• t(14;20)(q32;q11)	*MAFB*	1
	• t(6;14)(p21;q32)	*CCND3*	1–4
	• t(12;14)(p13;q32)	*CCND2*	~1
	IGH translocations and trisomies of odd-numbered chromosomes		15
	Deletion of whole *IGH*	*?TRAF3*	4,5–45
Secondary	17p deletion	*TP53*	5–15
	13q deletion/monosomy of chromosome 13	*RB1, DIS3,DLEU2*, miR-15a, miR-16-1	50
	1q21 gain	*CKS1B*	34–40
	1p32 deletion, 1p22 deletion	*CDKN2C*	7–17
	Translocation of 8q24/other aberration of 8q24	*MYC*	15–35

are karyotypically cryptic. For these reasons, interphase fluorescence *in situ* hybridization (FISH) is the most useful cytogenetic method. Unlike with other hematological malignancies, in PCM, FISH should not be performed directly on bone marrow. Due to frequent low plasma cell percentage, plasma cell selection must be carried out. There are two popular methods of plasma cell enrichment: labeling of the cytoplasmic immunoglobulin light chains (c-IG), and plasma cell sorting.

The alternative method of plasma cell identification is target FISH. In this method, automated image analysis system combines the images of May-Grünwald-Giemsa (MGG) staining and FISH study on the same plasma cell for analysis [5].

Herein, we present international recommendations for FISH in PCM, together with own experience of c-IG FISH and target FISH.

Chromosomal abnormalities in PCM

Current understanding regarding the chromosomal abnormalities in PCM and the association of these genetic events with clinico-pathological features has enabled the creation of a biological genetic classification of PCM [3, 4, 6]. This classification denotes primary and secondary abnormalities (Table I) [1, 2, 4, 6–9]. Primary alterations divide myeloma into hyperdiploid and non-hyperdiploid subtypes. The first is characterized by trisomies of odd-numbered chromosomes: 3, 5, 7, 9, 11, 15, 19, 21 and is associated with a more indolent form of the disease. However, not all trisomies have the same prognostic impact: trisomy 21 impairs, while trisomies 3 and 5 actually improve, survival [7]. The non-hyperdiploid subtype is characterized by the *IGH* translocations and is associated with a more aggressive course. Primary *IGH* translocations with oncogenes include (in descending order of frequency): t(11;14)(q13;q32) (*CCND1*), t(4;14)(p16;q32) (*MMSET, FGFR3*), t(14;16)(q32;q23) (*MAF*), t(14;20)(q32;q11) (*MAFB*), t(6;14)(p21;q32) (*CCND3*), t(12;14)(p13;q32) (*CCND2*). Hyperdiploidy is almost mutually exclusive with *IGH* translocations, but in very rare cases, both trisomies and *IGH* translocations can be present. Hyperdiploidy and *IGH* translocations are present in all stages of gammopathy, suggesting that primary alterations initiate preneoplastic MGUS, but are not sufficient to cause the progression to PCM [4].

Besides trisomies and *IGH* translocations, the monosomy of *IGH* is considered as another primary chromosomal aberration [1]. Recent studies suggest that deletion of the whole *IGH* is an early event in the pathogenesis of myeloma [8, 9]. In addition to early (primary) chromosomal events, the presentation of myeloma is accompanied by acquisition of secondary chromosomal alterations. These secondary abnormalities are generally associated with a poor prognosis. The major secondary changes are: chromosome 13 deletion/monosomy, which co-occurs with t(4;14) and t(4;16), 1q21 gain and 1p32 deletion, which are closely related and chromosome 17p (*TP53*) deletion [del(17p)]. Other frequent secondary genetic events are alterations of the *MYC* involving not only translocations

Table II. Cytogenetic risk stratification of newly diagnosed plasma cell myeloma patients (acc. to International Myeloma Working Group classification [6] and Mayo Clinic classification [1])

High risk factors	Standard risk factors
t(4;14)	All other including:
t(14;16)	• t(11;14)
t(14;20)	• t(6;14)
del(17p)	• trisomies of odd-numbered chromosomes
gain(1q)	
'Double hit': two high risk factors	
'Triple hit': three or more high risk factors	

but also amplifications, duplications and inversions [3]. Deletion of *TP53* is a particularly poor prognostic factor, and is unresolved even by modern therapies or allogeneic stem cell transplantation [4, 6].

Cytogenetic risk stratification

Identifying high-risk patients and treating them properly is essential to improve outcomes in PCM [2]. Chromosomal abnormalities have important prognostic value for PCM, especially in identifying high-risk patients (Table II). According to classifications of the International Myeloma Working Group and Mayo Clinic, the high risk cytogenetic abnormalities are: t(4;14), t(14;16), t(14;20), del(17p), gain(1q). All other changes including: t(11;14), t(6;14), trisomies of odd-numbered chromosomes (hyperdiploidy) are considered as standard risk factors [1, 6, 10]. Compared to the previous risk classification, this stratification has incorporated new risk factors, 'double hit' and 'triple hit'. Recently it has been reported that patients with 'double hit' defined by the co-occurrence of at least two high risk alterations have an especially poor prognosis [11].

The cytogenetic risk stratification may change with treatment modalities. At present, the improved current prognostic staging system of PCM (The Revised International Staging System for Myeloma) incorporates the presence of three high risk abnormalities: t(14;16), t(14;20), del(17p) for the better stratifications of PCM patients [12].

Recommendations for FISH in PCM

Compared to other hematological neoplasms, an accurate FISH analysis in PCM is more complicated and more time-consuming. Practical guidelines for FISH testing have been developed by the European Myeloma Network and the European Cytogeneticists Association [3, 13, 14]:

- Morphological assessment of bone marrow cannot be used to decide whether or not to carry out FISH.
- Material should be a part of the first draw of aspirate, and the needle must be repositioned for further aspiration.
- The aspirate should be sent at a suitable time, because laboratory PCM processing is time-consuming.
- It is very important to purify or to identify the plasma cells (PC), but the method used should be chosen by the laboratory.
- It is strongly advised that cut-off levels for a positive result should be relatively conservative: 10% for dual fusion or break-apart probes, and 20% for single fusion probes and numerical abnormalities. These recommendations are subject to controversy and some laboratories may want to use their own threshold. Therefore, it should be pointed out that in purified or identified PCs, the vast majority of I PCs are expected to have primary changes. However, secondary abnormalities can only exist in a part of the PC population.
- Minimal panel of probes at the time of diagnosis should detect *FGFR3/IGH*, *MAF/IGH* and deletion of *TP53*.
- It is recommended that 100 cells be scored wherever possible. However, if high purity/identified PC samples were being analyzed, 50 cells should be sufficient for a normal result of primary abnormality. In exceptional circumstances, an abnormal result in as few as 10–20 PCs can be reported, but all doubts should be stated in the report.
- It is considered that a single experienced analyst is sufficient to examine the FISH specimens. However, cases with a low proportion of cells with alteration or a low level of plasma cells have to be analyzed by a second diagnostician.
- The report should be stated clearly for clinicians. It should include the method of PC identification, the probes used, the number of scored cells, and the percentage of cells with alterations. The European Myeloma Network [13] does not recommend International System for Human Cytogenomic Nomenclature (ISCN), but according to the guidelines of the European Cytogeneticists Association [14], a full ISCN should be stated on the report.
- The frequency of FISH testing is not well defined. It is accepted that primary abnormalities will not change over time. However, disease progression can be accompanied by genetic evolution. In the case of disease relapse, it is now recommended to test del(17p) and gain(1q) [6].

The extension of probes panel at the time of diagnosis may be necessary as it can yield more information regarding disease biology, clinical features and outcome. The extended, more comprehensive, panel may include testing for chromosome 1 abnormalities, t(11;14), t(14;20), chromosome 13 deletion, and ploidy status (to establish aneuploidy for any two chromosomes out of 5, 9, 11 and 15) [6, 7, 13, 14].

FISH diagnostics in myeloma — laboratory experience

FISH analysis of identified plasma cells in PCM became part of our laboratory practice in 2009. Between September 2009 and March 2021, bone marrow samples (or other extramedullary tissues) of 603 patients with suspected plasma cell myeloma or extramedullary plasmacytoma were investigated. Over that period, we performed nearly 2,050 FISH tests in myeloma patients (Table III).

For PC identification, we applied immunostaining of cytoplasmic immunoglobulin chains. In this method, AMCA anti-human kappa, anti-human lambda and anti-human IGG chains antibodies are used for staining of PC cytoplasm (Vector Laboratories, Burlingame, CA, USA). For this purpose, we use cultured cells fixed in 3:1 methanol:acetic acid. Excitation and emission parameters of our fluorescent microscopes (triple Filter set 25HE, Carl Zeiss Jena, Germany) enables us to see AMCA stained cytoplasm of plasma cells as brown/yellow (Figure 1).

In March 2018, we introduced target FISH as the second method of PC identification. In this method, bone marrow cells are separated by density gradient centrifugation to prepare cytospins. Cytospin slides are stained with MGG and scanned by multiparametric BioView system (Abbott Molecular, Abbott Park, IL, USA). The system automatically selects plasma cells, but review by a diagnostician is necessary to classify PCs for further FISH analysis. According to our experience, the optimal number of classified PCs is 200–250. This is many more than should be analyzed, but it is necessary because of hybridization failure or detaching of cells. The next steps are destaining of the slides, FISH procedure, and repeated scanning. The system automatically finds previously selected PCs and enables simultaneous observation of FISH results and MGG morphology of cells (Figure 2).

Currently we use both methods of PC identification (Figure 3) and we apply the FISH algorithm presented in Figure 4. If the amount of bone morrow (BM) is adequate, every sample is *in vitro* cultured for c-IG FISH (and karyotyping, if necessary) and prepared for target FISH. In the cases of extramedullary plasmacytoma, biopsies of other tissues are *in vitro* cultured for c-IG FISH and karyotyping. Employing two methods is more time-consuming, but provides advantages. The identification of PCs is crucial for proper FISH analysis. c-IG FISH is the established method of PC identification, but in some cases the labeling of cytoplasm is weak and the selection of plasma cells is very difficult. MGG morphology as the first step of PC identification minimizes the troubles with PC selection. On the other hand, the procedure of cytospin preparation can lead sometimes to destruction of PCs. Plasma cells are sensitive for centrifugations, because of the abundance of cytoplasm. In this case, c-IG of cultured cells facilitates PC selection.

Table III. Data regarding myeloma fluorescence *in situ* hybridization (FISH) tests performed in Cytogenetic Laboratory, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland

Year	Number of PCM patients	Number of EMP patients	Number of patients	Number of tests
2009	15	2	17	111
2010	44	5	49	310
2011	34	4	38	170
2012	42	2	44	179
2013	28	1	29	150
2014	48	0	48	214
2015	52	3	55	190
2016	44	3	47	145
2017*	45	2	47	97
2018#	82	6	88	185
2019	69	2	71	141
2020	53	2	55	120
2021 (January–-March)	15	0	15	39
Total: September 2009 to March 2021			603	2051

*Introduction of basic panel of probes: IGH breakapart, *TP53*/centromere 17; #introduction of target FISH; PCM — plasma cell myeloma; EMP — extramedullary plasmacytoma

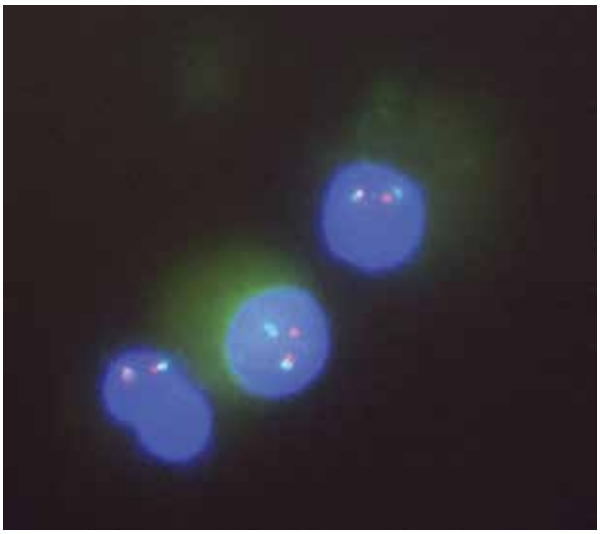

Figure 1. Plasma cell identified by labeling of cytoplasmic immunoglobulin light chains (c-IG). Fluorescence *in situ* hybridization (FISH) with immunoglobulin heavy chain (IGH) breakapart probe (Zytovison, Bremerhaven, Germany): separate green and red signals indicate rearrangement of *IGH*. Non-plasmatic cell has two not rearranged IGH (yellow) signals

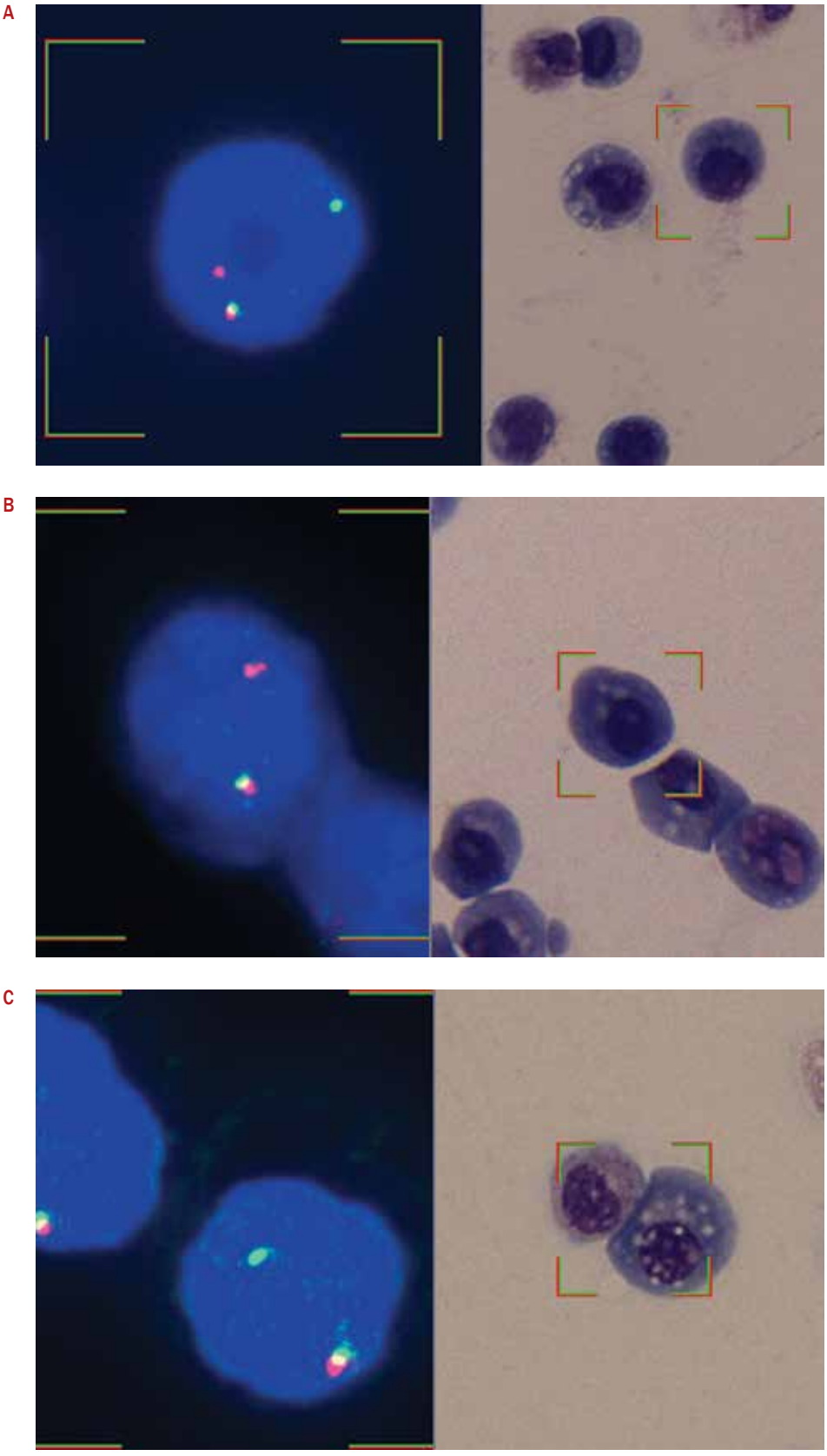

Figure 2. Plasma cells identified by target fluorescence *in situ* hybridization (FISH). FISH result of immunoglobulin heavy chain (IGH) break-apart (BAP) probe (Zytovison, Bremerhaven, Germany) on left, same cell stained with May-Grünwald-Giemsa (MGG) on right: **A.** Typical rearrangement of *IGH*: one 3'IGH (red) signal, one 5'IGH (green) signal and one IGH (yellow) signal (1Y1R1G); **B.** Deletion of 5'*IGH* region: one 3'IGH (red) signal and one IGH (yellow) signal (1Y1R); **C.** Deletion of 3'*IGH* region: one 5'IGH (green) signal and one IGH (yellow) signal (1Y1G)

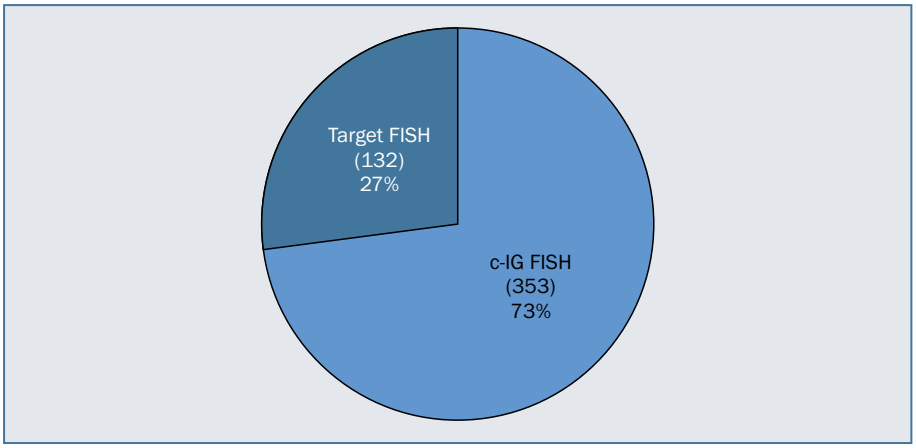

Figure 3. Fluorescence *in situ* hybridization (FISH) myeloma tests performed in Cytogenetic Laboratory, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland between 2018 and 2021. In this period, two methods of plasma cells identification for FISH were used: labeling of cytoplasmic immunoglobulin light chains [immunostaining of cytoplasmic immunoglobulin chains (c-IG) FISH] and target FISH. Total number of tests was 485, c-IG FISH represented 73% of all tests, target FISH represented 27% of all tests

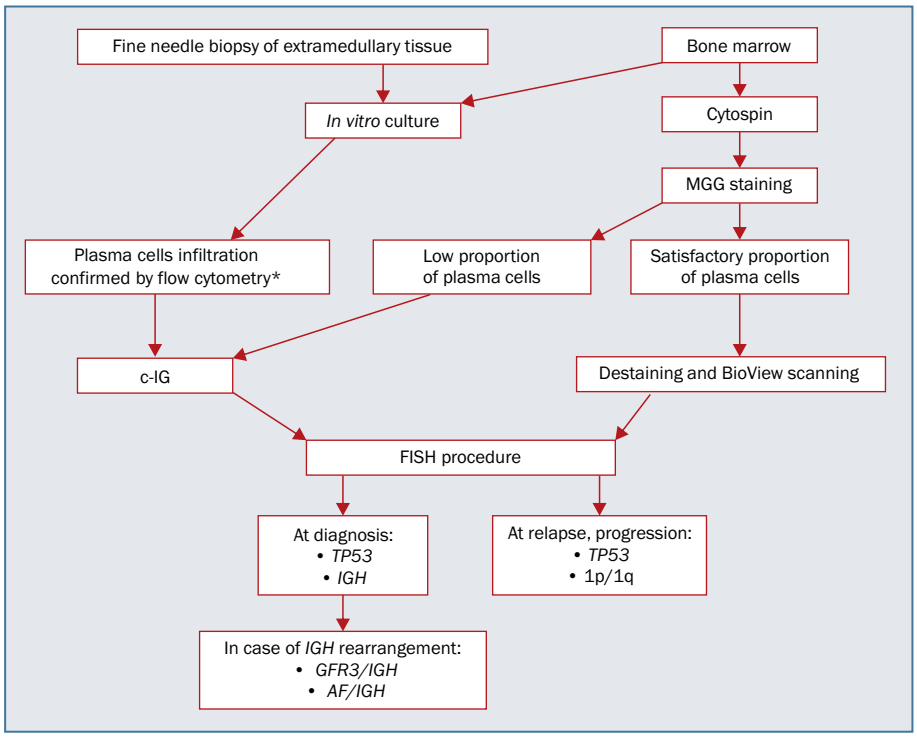

Figure 4. Scheme of myeloma fluorescence *in situ* hybridization (FISH) diagnostic algorithm used in Cytogenetic Laboratory, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw; *flow cytometry confirmation is needed only in biopsy specimens; MGG — May-Grünwald-Giemsa staining; c-IG — immunostaining of cytoplasmic immunoglobulin chains

In newly diagnosed patients, or in patients without any cytogenetic data, our basic panel consists of IGH BAP and TP53/centromere 17 probes. If IGH signal pattern suggests rearrangement, IGH/FGFR3 and IGH/MAF dual fusion probes are used to detect high risk fusions. It should be emphasized that not only typical split signal pattern (1Y1R1G — one yellow, one red, one green signal) point at the rearrangement of the *IGH* (Figure 2A). In 10–17% of PCM patients, partial deletions of the *IGH* locus are observed. These deletions are heterogeneous, most often including monoallelic deletion of 3'*IGH* (constant region) and monoallelic deletion of 5'*IGH* (variable region) [8, 15, 16]. Moreover, these deletions may be accompanied by duplications of the *IGH* regions. Various *IGH* deletion signal patterns can be observed, including 1Y1R, 1Y1G, and 2Y with diminished R or G signal (Figure 2B, 2C). As approximately 20% of these deletions coexist

with translocations, it is important to use dual fusion probes when deletion of the *IGH* is identified [16].

The aforementioned panel of FISH probes has been used in our laboratory since 2017. Prior to that, our panel was more extended. This panel followed valid myeloma FISH recommendations and included testing for *IGH/FGFR3*, *IGH/MAF*, *IGH/CCND1*, 13q14 deletion and *TP53* deletion.

In progression or at relapse of PCM we use CKS1B/CDKN2C and TP53/centromere 17 probes for testing of 1p/1q aberrations and *TP53* deletion.

In addition to the imperative of identifying PCs, intrapatient/intratumoral heterogeneity creates further difficulties in FISH diagnostics [2]. It often happens that there are discrepancies between the proportion of PCs assessed by examination of bone marrow aspirate smears or trephine sections, and the proportion of PCs in samples dedicated to FISH. In some cases, we have observed that despite a high proportion of PCs in morphological smears, FISH samples had too few PCs to allow an analysis. On the other hand however, regardless of a very low proportion of PCs in morphological smears, we have occasionally found an adequate number of PCs on c-IG slides or MGG cytospins.

In conclusion, accurate FISH analysis in PCM is more complicated and time-consuming than in other hematological FISH tests.

The proper FISH diagnostics in plasma cell myeloma should be carried out according to the recommendations of the European Myeloma Network and the European Cytogeneticists Association. Every laboratory which performs myeloma FISH tests should follow the latest advice regarding risk stratification in myeloma.

Author's contributions

RW — sole author.

Conflict of interest

The author declares no conflict of interest.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Anemia in cancer patients

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Abstract

Anemia is a common feature in c.40% of patients at the time of cancer diagnosis and in more than half of patients undergoing anticancer therapy. Cancer-related anemia does have an unfavourable impact on the functional capacity of the relevant tissue and organs. Its pathogenesis is complex and often difficult to identify. Symptoms related to cancer and chemotherapy-induced anemia may have a negative impact on the quality of life and may influence treatment efficacy, disease progression and even survival. Moreover, anemia causing tumor hypoxia leads to tumor progression through the increase of local tumor expansion and spreading of metastases. Tumor hypoxia directly or indirectly confers resistance to irradiation, some chemotherapeutic drugs, and photodynamic therapy. Therapeutic alternatives in cancer patients with anemia include the substitution of the lacking agents, red blood cell (RBC) transfusions, iron supplementation, and erythropoiesis-stimulating agents (ESAs). Using ESAs reduces the need for red blood cell transfusions, decreases the risk of post-transfusion adverse reactions, and improves the quality of life for cancer patients with chemotherapy-induced anemia. The immediate administration of RBC transfusions is justified in patients with hemoglobin (Hb) under 7–8 g/dL and/or severe anemia-related symptoms (even at higher Hb levels) and who require immediate Hb and symptom improvement.

Therefore, clinical evidence supports the need to closely monitor Hb level in cancer patients. Anemia should be corrected to improve chemo- and radiosensitivity and the quality of life.

Key words: anemia, cancer, cancer-related anemia, chemotherapy-induced anemia

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Introduction

Anemia is commonly encountered in cancer patients at various stages of disease progression, especially among those who receive active chemotherapy with or without radiotherapy [1]. In a group of patients with cancer, anemia can cause a wide range of signs and symptoms involving organs of the body. Their severity depends on the level of anemia, the speed of its onset, and existing co-morbidities and, above all, the type of cancer. The impact of anemia on survival is connected with a delay in onset of the therapy or failure to complete chemotherapy regimens on time. Furthermore, the cytotoxicity induced by chemotherapy drugs or/and radiotherapy requires adequate oxygen levels in tissue. Since tumor hypoxia boosts tumor resistance to radiation and chemotherapy, it can lead to the lack of tumor response [2, 3].

Multiple studies have suggested that, aside from its important role in QOL issues, anemia constitutes an independent factor of survival in patients with cancers, especially those who received chemotherapy and radiotherapy at the same time [4, 5].

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Epidemiology of anemia and its definition

In a group of cancer patients, anemia occurs frequently, according to the data, even more than in 40% of cases [1]. In patients who received chemotherapy or/and radiotherapy the incidence of anemia may rise even to 90% of cases [2]. A higher percentage of anemia is observed in some myelo- and lymphoproliferative cancers, e.g. acute leukemias (100%), myelodysplastic syndrome (95%), multiple myeloma (85.3%), Hodgkin lymphoma (66%), and other non-Hodgkin lymphomas (77.9%) [6].

The severity of anemia is defined by the level of hemoglobin (Hb) in the blood <14 g/dL for male population and <12 g/dL for women. It is additionally subdivided into a few types: mild - when the level of Hb is below lower normal range but more than 10 g/dL, moderate - when the level of Hb is between 8–10 g/dL, severe — with Hb level 6.5–8 g/dL and life-threatening — when Hb is below 6.5 g/dL types.

Pathophysiology

Despite the well-established knowledge about the pathophysiology of cancer-induced anemia (CIA), its multifactorial background often makes it difficult to clearly identify the cause of the decrease in hemoglobin concentration in the population of cancer patients [7]. Attempts are being made to systematize the causes of anemia, emphasizing the role of chronic blood loss and the associated iron deficiency, as well as the chronic inflammatory process that implies a reduction in hematopoiesis [8]. In the development of anemia, importance is also attached to excessive destruction of red blood cells, which is caused by the appearance of auto-reactive antibodies [8].

Cancer patients very frequently develop iron deficiency, either absolute or functional. Absolute iron deficiency is caused mainly by bleeding, whereas other underlying factors such as insufficient intestinal iron resorption are usually of minor importance. Moreover, iron homeostasis seems to be associated with coexisting inflammation, and hepcidin, which is a cytokine-induced protein, is of particular importance [9]. Hepcidin, being a key regulator of iron uptake and release, reduces its absorption in the gastrointestinal tract and regulates its metabolism in the bone marrow microenvironment. As a consequence, iron is not used effectively during erythropoiesis, resulting in its impairment.

It should not be forgotten that the neoplastic process itself is often associated with bone marrow infiltration, thus exerting a suppressive effect on hematopoiesis [10]. Moreover, neoplastic cells, demonstrating the ability to secrete cytokines, stimulate macrophage-dependent iron sequestration [10].

Another important cause of anemia, especially in the field of hematooncology, is the development of autoimmune hemolytic anemia (AIHA), most often in the course of chronic lymphocytic leukemia, lymphomas or adenocarcinoma [11]. Moreover, in the course of neoplasms, there are cases of non-immune haemolysis caused by thrombotic microangiopathy (TMA) [11]. It manifests as microangiopathic haemolytic anemia (MAHA), characterized by the absence of increased reticulocytosis (normal reticulocytes <2%) [11].

Finally, selected chemotherapeutic agents, depending on the dose and mechanism of action, induce anemia by impairing myeloid hematopoiesis [12]. No less important in the context of anemia development is the nephrotoxic potential of selected substances, such as platinum salts, which is associated with reduced erythropoietin (EPO) production by renal Epo-producing cells (REPs) [13]. Moreover, commonly used chemotherapy regimens involving cytostatics from various groups are associated with a synergistic effect. Considering that an advanced stage of cancer usually requires more and more intensive chemotherapy, the incidence of anemia increases with each new cycle.

Treatment options

It is necessary that efforts be made to identify the etiology of anemia and that its treatment be directed at the underlying cause. The main purpose of its treatment should aim at improving or resolving the symptoms of anemia, such as fatigue and dyspnea, enabling anticancer therapy and increasing quality of life, especially taking into account a cancer patient's life expectancy. It must be borne in mind that this goal should be achieved with the possible safest methods and least intensive one. What should be treated first are the diagnosed deficiencies (like iron, folic acid or vitamin B_{12}). If their correction does not lead to an increase of hemoglobin, the options of treatment of anemia in cancer patients include iron treatment, a transfusion of packed red blood cells (RBC), and an application of the erythropoiesis-stimulating agents (ESAs). The treatment of cancer anemia or chemotherapy-induced anemia depends on the level of hemoglobin and the severity of its symptoms. Transfusion of red blood cells is the main option for patients who due to the symptoms, which lead to deterioration of comorbidities, need immediate correction of their anemia. In cancer patients who do not need a quick improvement of Hb level, the alternatives include a transfusion, an ESA therapy, and sometimes an iron therapy.

Red blood cell concentrations transfusion

Guidance on the use of red blood cell concentrations in patients with cancers has been recently published as an expert group recommendation [14]. However, RBC transfusions ought not to be used as a universal method to correct anemia in patients with diagnosed cancer They should be restricted to those conditions, in which they constitute the

only effective way to increase the Hb concentration or in which there are indications for quick removal or relief from symptoms related to anemia.

The RBC transfusions, according to the latest recommendation should be given for patients whose hemoglobin level falls under 7–8 g/dL, or in situations when a quick correction of serious, symptomatic anemia is needed [European Society for Medical Oncology (ESMO)]. The choice to use a blood transfusion should never be based solely on the Hb concentration. In patients with symptoms of severe anemia or existing co-morbidities (e.g. ischemic heart disease) and an ongoing or planned chemotherapy or radiotherapy, a red blood cell transfusion should be given even at a higher level of Hb than 8 g/dL. Moreover, available data showed that restrictive transfusion policies for patients with cancer who present anemia, appear to decrease blood utilisation without increasing side effects including morbidity or mortality [15].

Although transfusions offer obvious advantages, they are not risk-free, These risks include some transfusion-related reactions, congestive heart failure, an increased incidence of thromboembolism and bacterial and viral infections, and iron overload [16, 17]. Indeed, iron overload is one of the most common side effects in patients with myelodysplastic syndrome (MDS) who need transfusions over a long period. On the other hand, these problems are rarely seen in a group of patients with solid tumors for whom the transfusion period lasts less than a year [18].

The immune-modulatory effect of blood transfusions in patients with diagnosed cancer is also well described. Large population-based studies and available data from a meta-analysis implied a presence of a link between RBC transfusions and an increased risk of recurrence of malignancy [19, 20].

Because several post-transfusion adverse reactions can occur, including some fatal ones and in most of them the reason is the presence of leukocytes in the blood components, and to limit an adverse reaction, one ought to choose the appropriate red cell concentrate (RCC) for each individual: leukocyte-depleted, irradiated, irradiated leukocyte-depleted, or washed RCC.

Iron treatment

Iron supplementation

The criteria for starting iron supplementation include:

- concentration of hemoglobin between (8 <Hb <10 g/dL);
- absolute iron deficiency (ferritin <100 ng/mL and transferrin saturation <20%);
- relative iron deficiency (ferritin >100 ng/mL and transferrin saturation <20%).

Iron administration should be started before or at the same time as the ESA is started [1]. Iron supplementation is available in both oral and intravenous (i.v.) forms. The clinical studies have shown a significantly faster and higher increase in hemoglobin concentrations in the group of patients receiving ESA who received intravenous iron supplementation than patients receiving iron orally or no iron supplementation at all [21, 22]. On the other hand, i.v. iron boasts the superiority of bypassing the intestinal hepcidin-ferroportin pathway that regulates iron absorption. Additionally, i.v. iron leads to a faster rise of Hb concentration and ensures better and more effective replenishment of iron storage in the body. A randomized investigation demonstrated no negative influence of i.v. iron treatment when given to patients with diagnosed lymphoid malignancies or patients after autologous hematopoietic stem cell transplantations [23]. However, intravenous iron is not recommended to be given to patients who present an active infection. It is recommended that injection of iron is not administered simultaneously with cardiotoxic chemotherapy (anthracyclines, alkylating drugs and vinca alkaloids) [14].

Erythropoietin-stimulating agents treatment

Erythropoietin-stimulating agents (ESA) are biological analogues of human erythropoietin (EPO). Currently on the market, erythopoietin with a short (alpha, beta, theta, zeta) and long (darbepoetin) duration of action is available. Epoetin has the same acid sequence as EPO. Darbepoetin has an additional oligosaccharide, which results in a longer half-life [24]. The use of ESA aims to reduce the number of blood units transfused and thus reduces the possible risk of side effect reaction, improves fatigue-related symptoms and QOL with chemotherapy-induced anemia. ESA therapy might be considered as an option in the case of asymptomatic patients who can deteriorate to more severe anemia [25, 26]. Several clinical data and meta-analyses have reported that ESAs treatment results in a meaningfully significant betterment of the quality of life (QoL) and fatigue-related symptoms [27]. It is worth mentioning that ESA, differently from RBC concentrates, has a beneficial impact on the immune system. It was also found that ESA reduces the expression of numerous pro-inflammatory cytokine genes [interleukin (IL)-1B, IL-6, IL-10, tumor necrosis factor-alpha], contributes to lowering the concentration of IL-1α and IL-6, and by influencing the immune system, it causes a decrease in the number of suppressive cells like (CD8+CD152+) [28–30].

ESA is recommended to use for a patient with non-myeloid malignancies including lymphomas and multiple myelomas with chemotherapy-induced anemia (CIA). Moreover, in compliance with the ESMO, the use of ESAs are recommended in the case of patients with the diagnosed myelodysplastic syndrome but only those with the lower-risk myelodysplastic syndromes, whose serum erythropoietin

level is below 500 U/L and who have a normal level of blastic cells [31]. In patients who progress to acute myeloid leukiemia (AML), ESAs should not be used. Iron replacement (i.v.) can be applied with a view to improving Hb response and reducing RBC transfusions for patients receiving ESA with or without iron deficiency. The inclusion of ESA in treatment is considered in patients with anemia during or after chemotherapy when the Hb level is <10 g/dL, and the target value is 12 g/dL. The effectiveness of ESA is demonstrated by the increase in Hb concentration by 1–2 g/dL after 4 weeks of using the drug. Treatment with ESA should not be extended beyond 6–8 weeks when there is no desired Hb increase.

It is necessary that clinicians weigh the possible complications and advantages of ESA treatment and always inform about the possible side effects of applied therapy to the patient [25, 26]. ESA in patients with a history of hypersensitivity to the drug and hypertension that is not under control, is not approved. In recent years, however, there have been many concerns about the use of ESA and its likely impact on mortality, thrombotic complications and possible impact on tumor progression. Indeed, despite the significant benefits of ESAs for CIA, few randomized clinical investigations and meta-analyses have shown the risk of thromboembolic complications to be comparatively lower in patients treated with ESAs compared to the placebo groups [27, 32]. Various meta-analyses that have assessed deadliness and thromboembolic complications may have been prejudiced because they included clinical reports where ESAs were used even when the level of hemoglobin was above 12 g/dL [24, 33, 34]. Furthermore, according to the available reports, no significant data is confirming, that the use of ESA, significantly further increase the risk of thromboembolic complications in a group of patients who are treated with thalidomide or lenalidomide [35, 36].

Conclusion

Over the past decade, understanding has expanded of many aspects of the pathophysiology of anemia in cancers. Nevertheless, a lot remains to be elucidated including the role of iron supplementation, some possible complications after the use of ESA, as well as, transfusion-related side effects.

Authors' contributions

JK wrote the first version of the manuscript, LB corrected the manuscript, linguistic correction

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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REVIEW ARTICLE

Anemia in children: a pediatrician's view

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Abstract

Anemia is defined as a hemoglobin level that is two standard deviations below the mean for age. After children reach the age of 12, the hemoglobin norm can be further divided into gender-specific ranges. When a patient presents with anemia, it is important to establish whether the abnormality is isolated to a single cell line [red blood cells (RBC) only] or whether it is part of a multiple cell line abnormality. In children, anemia is usually caused by decreased RBC production or increased RBC turnover. Anemia is usually classified based on the size of RBC (microcytosis, normocytosis, or macrocytosis) as measured by the mean corpuscular volume. Although iron deficiency anemia is usually microcytic, some patients may have normocytic blood cells. From a practical point of view, it is better to use in children the etiologic classification of anemia which includes impaired red cell formation, blood loss and hemolytic anemia. Most children with anemia are asymptomatic, and the condition is detected on screening laboratory evaluation. Iron deficiency can be treated with oral iron, intravenous iron, and/or blood transfusion, depending on the patient`s hemoglobin levels, tolerance and co-morbidity. Oral iron salts are usually the first line of treatment for uncomplicated iron deficiency, but are poorly absorbed and lead to gastrointestinal side effects. In some cases, iron refractory iron deficiency anemia (IRIDA), a hereditary recessive anemia refractory to oral iron, occurs. IRIDA shows a slow response to intravenous iron and partial correction of anemia.

Key words: anemia, iron deficiency, iron refractory iron deficiency anemia (IRIDA)

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Anemia is a public health problem that affects both rich and poor countries [1]. Worldwide, anemia affects up to 50% of children under 5, especially children from low-income families [2]. Anemia with iron deficiency is the most common form of anemia.

Infants and children needs iron for the proper neurological development, differentiation of brain cell, myelinization of neurons, and as a cofactor for enzymes that synthesize neurotransmitters.

This is why iron deficiency/anemia negatively impacts on the fundamental aspects of growth and intellectual developments with potential long-term consequences [2, 3]. We diagnose anemia when a hemoglobin level is two standard deviations below the mean for age. We should remember that the hemoglobin level in children above 12 of age is gender-specific [4]. In some patients anemia is limited only to the red blood cell line [RDC] and in others it coexists with damage to other cell lines in bone marrow [4] When anemia coexists with damage to other cell lines we should primary diagnose bone marrow diseases (aplastic anemia, leukemia) or an immunological disorders [5]. Iron deficiency is one of the main causes of anemia in patients with chronic kidney disease. We also diagnose anemia in patients with, celiac disease, non-celiac gluten sensitivity, an autoimmune atrophic gastritis and in patients with bowel disease in which it is more frequent than in Crohn's disease. Decreased production or destruction of RDC are the main cause of anemia in children [6].

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One of the classification of anemia is based on the size of RBC, which specifies the mean corpuscular volume (MCV). With MCV we distinguish microcytic anemia (MCV <80 fL), normocytic anemia (80–100 fL), or macrocytic anemia (>100 fL).

RBC distribution width is a measure of the size variance of RBCs. A low RBC distribution width suggests uniform cell size, whereas an elevated width (>14%) indicates RBCs of multiple size.

From a practical point of view, it is better to use in children the etiologic classification of anemia which includes impaired red cell formation (deficiency, bone marrow failure, infiltration) blood loss and hemolytic anemia (corpuscular, extracorpuscular). Due to the consequences of anemia, the American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend testing for anemia in children at one year of age [2]. In Polish children, we recommend testing for anemia at 3, 6 and 12 months, and also in children with feeding problem, poor growth, inadequate dietary iron intake, and during adolescence, especially in girls [7].

In most children anemia is asymptomatic, and we diagnose it based on a medical history, physical examination or additional tests. Symptoms indicative of anemia are irritability or pica, jaundice, shortness of breath, or palpitations.

In medical history important are questions about prematurity, low birth weight, diet, chronic disease, and a family history of anemia. In the physical examination, we pay attention to jaundice, tachypnoe, tachycardia, and heart failure, especially in children with severe or acute anemia. The basic research of anemia is a complete blood count, reticulocyte count, serum ferritin level reflecting iron stores and transferrin.

Though serum ferritin is a good indicator of stored iron, as an acute phase protein may be increased with inflammation or chronic diseases. Therefore, it should not be tested in these states for assessing iron stocks.

A blood iron test assesses its current concentration in blood, but does not specify the amount of iron available in the body, hence this result is interpreted along with others iron tests.

Total iron-binding capacity (TIBC) measures all the proteins in the blood that are available to bind with iron, including transferrin. unsaturated iron-binding capacity (UIBC) measures the portion of transferrin that has not yet been saturated. UIBC also reflects transferrin levels.

Transferrin saturation is a calculation that reflects the percentage of transferrin that is saturated with iron.

Soluble transferrin receptors (sTfRs) are proteins found in the blood which can be elevated with iron deficiency. The sTfR test is not available at all centers, but because it is not an acute phase reactant, it is useful for assessing iron stores in patients with chronic diseases.

If the anemia is microcytic, we should look for iron deficiency, thalassemia and anemia of chronic disease. Iron deficiency, chronic disease, hemolysis, immune-mediated destruction, and bone marrow disorders are the most common causes of normocytic anemia. Macrocytic anemia is uncommon in children. It is caused by deficiency of vitamin B_{12}, and folic acid, hypothyroidism and hepatic disease.

The main causes of anemia in newborns are hemorrhage, hemolysis or failure of red cell production. Anemia in infants and toddlers is caused by: failure of red cell production, hemorrage, hemolysis. Anemia in older children and adolescents is caused by failure of red cell production, hemorrage, hemolysis.

As previously stated, iron deficiency anemia is the most common type in children. Although iron deficiency anemia is usually microcytic, some patients may have normocytic blood cells [2, 8]. Anemia is most common in children during late infancy/early childhood because of rapid growth, exhaustion of gestational iron and low levels of dietary iron. The second period of increased occurrence of anemia is adolescence, due to rapid growth, suboptimal iron intake and menstrual blood loss in females [5].

Children at the aged 1–3 years should receive 7 mg elementary iron daily in food. We must remember that consumption of large quantities of non–iron-fortified cow's milk favors the occurrence of iron deficiency anemia.

For older children in areas of high anemia prevalence, the WHO recommends intermittent iron supplementation (potentially once or twice a week) for pre-school and school-age children and adolescents [9].

When we delayed umbilical cord clamping for 60––120–180 sec after delivery, iron status in infants aged 2–6 month may be improved, however, it does not last longer than 12 months [2, 9–11].

Exclusively breastfed preterm infants, except for those who have had multiple blood transfusions. should receive prophylactically 2 mg elementary iron per kg per day from age 1–12 month [2, 12].

Full-term infants do not require prophylaxis with iron, for their pregnant iron supplies are sufficient for the first 4–6 months of life [2, 13].

As recommended the AAP full-term exclusively breastfed infants should receive 1 mg per kg per day of elementary iron supplementation at age 4 months until introduced into the diet foods that contain the right amount of iron [2, 12, 13]. Formula-fed infants often receive adequate amounts of iron, and thus rarely require further supplementation [12]. Full-term infants (4–6 month to 1 year) require 11 mg iron per day and children aged 1–3 years require 7 mg iron per day [2, 14, 15].

Patient's hemoglobin levels, tolerance of anemia and co-morbidity decide on the form of iron administration, oral

iron, intravenous iron, and/or blood transfusion. Oral forms of iron as ferrous or ferric salts are are most often used for the sake of their availability, ease of administration, and relatively low cost. We currently have:

1) iron (II) compounds (ferrous sulphate, ferrous glycine sulphate, ferrous fumarate, ferrous gluconate);
2) iron (III) complexes [iron (III) hydroxide polymaltose complex, iron (III) succinyl protein complex];
3) elemental iron (carbonyl iron);
4) sucrosomal iron.

During the therapy with oral iron salts, in some patients they are observed the gastrointestinal side effects, caused by poor drug absorption [16]. Some new iron preparations increase their tolerability.

One of these is sucrosomal iron, absorbed as a vesicle-like structure, bypassing the conventional iron absorption pathway. Therefore sucrosomal iron is well tolerated and more bioavailable than other iron salts salts [17, 18].

The properties of sucrosomal iron make it recommended for patients at which iron salts are inefficacious and also in iron deficiency prophylaxis. This drug can be used for initial or maintenance treatment [17].

Intravenous iron is administered to the patients with intolerance to oral iron salt, or when the treatment is inefficacious [19]. Intravenous iron preparations include ferric gluconate, iron sucrose, low molecular weight iron dextran, ferric carboxymaltose, ferumoxytol and iron isomaltose. Due to the occasional anaphylactic reactions after the intravenous iron administration, treatment must lead only by staff trained to manage anaphylactic reactions, and where resuscitation facilities are immediately available [20].

An indication to the red cell transfusion are very severe iron deficiency anemia and hemodynamic instability. After the red cell transfusion we observe transient rise of haemoglobin, as a result, it increases oxygen-carrying capacity. In patients who achieved hemodynamic stability the iron supplementation should be considered [17, 21].

The first description of the patients with iron unresponsive anemia, malabsorption of medical iron and a partial but incomplete hematological response to parenteral dextran occured in 1981 [22, 23].

After 16 years, in 1997, there was a report about the 18-month old African child with iron resistant iron deficiency anemia and severe microcytosis [24]. His anemia was unresponsive to oral iron supplementation and persisted after iron stores were replete. Most of the reported cases have been children, who despite anemia, had normal growth, development and intellectual performance [22, 24, 25].

Iron refractory iron deficiency anemia (IRIDA), presented above, is a hereditary recessive anemia due to a defect in the *TMPRSS6* gene encoding matriptase 2. This protein plays a role in down-regulating hepcidin, the key regulator of iron homeostasis [22, 26].

The IRIDA patients are characterized by hypochromic, microcytic anemia, very low serum iron, and transferrin saturation levels. However, serum ferritin levels are mostly within the normal range or even slightly elevated following intravenous iron treatment. The degree of anemia varies, being mostly mild and more pronounced in childhood. Anemia is not detectable at birth. The phenotype develops only after the neonatal period [22]. In most patients, oral iron is ineffective in correcting anemia, and patients must receive intravenous iron. The response to parenteral administration of iron is variable but generally leads to a progressive increase in hemoglobin levels. Correction of anemia is much slower than in patients with acquired iron deficiency. Hemoglobin levels rarely normalize, microcytosis persists and transferrin saturation remains below normal value. Serum ferritin increases following iron injections, somehow in a dose-dependent manner [22].

Anemia classification and diagnosis in children is a very complex challenge, although it must be remembered that the main cause of anemia is iron deficiency. Oral iron is the first-line treatment for iron deficiency in pediatric populations [12].

Authors' contributions

MM — sole author.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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Jakie jest ryzyko zwiększonej transmisji HEV i jakie są rozwiązania tego problemu?

Zakażenie wirusem zapalenia wątroby typu E (HEV) nabyte w wyniku przeniesienia lub transfuzji może być przyczyną poważnej choroby lub nawet zgonu u pacjentów z immunosupresją. Zakażenia takie obserwowano sporadycznie, bezobjawowe zakażenia wśród dawców krwi uważa się za powszechne.[1] Europejska Agencja Leków oceniła bezpieczeństwo wirusologiczne produktów leczniczych pochodzących z osocza i odnotowała, że zdarzenia związane z przeniesieniem zakażenia HEV obserwowano w przypadku wszystkich produktów krwiopochodnych,[2] przy czym w kilku krajach europejskich udokumentowano coraz częstsze przypadki oddawania krwi zawierającej HEV o genotypie 3.[3 4]

Ryzyko przeniesienia wirusa HEV poprzez transfuzję krwi można zmniejszyć poprzez poddanie składników krwi procesowi redukowania patogenów (PRT) Mirasol®. System Mirasol redukuje wirusy bezotoczkowe w składnikach krwi,[5 6 7] takie jak HEV, wirus zapalenia wątroby typu A i modelowy parwowirus B19 – jest to kategoria wirusów, które wykazywały dotychczas oporność na inne dostępne technologie redukowania patogenów (takie jak INTERCEPT®9).

Więcej informacji na temat systemu PRT Mirasol

ZDOBĄDŹ WIEDZĘ NA TEMAT REDUKOWANIA RYZYKA TRANSMISJI HEV

Film: **Mechanizm działania systemu Mirasol**. Zobacz, jak system Mirasol wykorzystuje połączenie ryboflawiny (witaminy B2), nietoksycznego, niemutagennego związku, oraz swoistego spektrum światła ultrafioletowego (UV) do inaktywowania wirusów, bakterii, pasożytów i krwinek białych, które mogą być obecne w uzyskanych preparatach krwi. *Więcej informacji*

Poster: Ojea A i wsp. **W Asturii (Hiszpania) bezobjawowi dawcy przenoszą HEV poprzez transfuzję koncentratu krwinek czerwonych, ale nie koncentratu płytek krwi, dzięki zastosowaniu procesu PRT z użyciem ryboflawiny/UV.** Vox Sang. 2020;115(suppl. s1):224-225. P-433. *Więcej informacji*

Artykuł: Owada T i wsp. **Fotochemiczna inaktywacja wirusa zapalenia wątroby typu E (HEV) w próbkach koncentratu płytek krwi (PC) przy użyciu systemu technologii redukowania patogenów (PRT) Mirasol.** Transfusion. 2014;53(suppl. 2):215A. *Więcej informacji*

Prezentacja: **Tiberghien P. Strategie badań przesiewowych dawców pod kątem HEV: brak przesiewowych/uniwersalnych/selektywnych metod. Praca przedstawiona podczas konferencji EFS-Francja, 24. Międzynarodowe Warsztaty IPFA/PEI na temat Nadzoru i Badań Przesiewowych w kierunku Patogenów Krwiopochodnych;** St Denis, Francja; maj 2017. *Więcej informacji*

Kliknij tutaj, aby uzyskać dostęp do dodatkowych zasobów

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REVIEW ARTICLE

Methods of pathogen inactivation in whole blood and red blood cells: current state of knowledge

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Abstract

Although pathogen reduction technology was implemented for platelet concentrates and plasma, the risk of pathogen transmission has not been completely eliminated as no inactivation procedures were implemented for red blood cells and whole blood. Research was therefore focused on developing methods for effective pathogen inactivation in red blood cell components. Attempts were made to apply either chemical compounds (porphyrins and Sylsense compounds) or photosensitizers such as methylene blue (Theraflex MB Plasma System) and amotosalen hydrochloride (Intercept System) already in use for pathogen inactivation in plasma. None proved effective for pathogen inactivation in red blood cells.

Approval was recently given to pathogen inactivation methods based on S-303 compound (for red blood cells) and with riboflavin (for whole blood). Clinical trials are ongoing. Pilot studies have shown that packed red blood cells subjected to pathogen inactivation with S-303 demonstrated slight loss of red blood cells, decrease in hemoglobin concentration, significantly lower lactate concentration, and lower pH. Pathogen inactivated whole blood stored at room temperature for up to seven days showed slight hemolysis (within the normal range).

This paper presents several pilot clinical trials with pathogen inactivated red blood cells or whole blood. It focuses primarily on the recovery of red blood cells in the recipient's organism and on hemoglobin concentration.

Key words: pathogen inactivation, red blood cells, clinical trials

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Introduction

Since the end of the 20th century, many blood establishments (BEs) worldwide have begun the implementation of pathogen inactivation methods for blood components intended for clinical use. These methods were based on the experience gained from the pathogen inactivation methods developed for plasma fractionation. In the 1980s, the solvent/detergent (SD) method appeared which reduced the risk of enveloped virus transmission with blood products. In the 1990s, the method was modified and applied also to plasma intended for clinical use [1].

In 2000, Macopharma developed the Theraflex MB-Plasma system based on methylene blue and visible light for pathogen inactivation in plasma. Amotosalen hydrochloride and ultraviolet A (UVA) were used in the Intercept system, initially developed for pathogen inactivation in platelet concentrates (2002) and in plasma (2006). In 2007, the Mirasol®PRT system was developed, which was based on riboflavin and UV and intended for pathogen inactivation in platelet concentrates (PC), and a year later in plasma (Table I) [2–4].

Although pathogen reduction technology (PRT) was implemented for PC and plasma, the risk of pathogen

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Table I. Systems of pathogen inactivation in blood components

System	OctaplasLG®	Theraflex MB Plasma	Intercept	Mirasol PRT
Company	Octapharma	Macopharma	Cerus	Terumo BCT
Type of blood component	Plasma from apheresis or WB	Plasma from apheresis or WB	Plasma from WB (pooled) or from apheresis, PC in plasma or PAS (InterSol, SSP$^+$)	Plasma from apheresis or from WB PC in plasma or PAS (SSP$^+$)
Chemical compounds/ photosensitizer	Tri (n-butylo) phosphate (TNBP) and 1% Octoxinol 9	Methylene blue	Amotosalen hydrochloride (S-59)	Riboflavin (vit. B_2)
Chemical compounds/ photoproducts	Residual amounts of chemical compounds	Azur A, B, C; tionona	Dimers S-59	Lumichrome, lumiflavin 2-ketoflavin, 4-ketoflavin, mononuceotide flavins, formylomethyloflavin
Inactivation conditions	4 h incubation at 30°C	Visible light (590 or 630 nm) 180 J/cm^2	UVA (320–400 nm) 3 J/cm^2	UV (280–400 nm) 6.24 J/cm^2
Additional steps	Removal of chemical compounds (extraction in vegetable oil, ion exchange chromatography or gel filtration and sterile filtration (0.22 mm)	Leukoreduction (Plasmaflex) Removal of photosensitizer and photoproducts (Bluflex)	Removal of photosensitizer and photoproducts (CAD)	Not applicable

PRT — pathogen reduction technology; WB — whole blood; PC — platelet concentrates; PAS — platelet additive solution; UVA — ultraviolet A; CAD — compound adsorption device

transmission was not completely eliminated as there were no inactivation procedures for red blood cells (RBC) and whole blood (WB). Research studies were therefore focused on developing methods that would effectively inactivate pathogens in red blood cell components. but also maintain an acceptable quality level during storage for longer than the 5–7 day limit for platelets.

First attempts to develop pathogen inactivation methods for red blood cells and whole blood

The first trials to develop methods of pathogen inactivation in RBC and WB were based on reactions involving light and photosensitizers. One of the first groups of compounds used as photosensitizers were porphyrins. During trials, it turned out that most porphyrins are amphiphilic which leads to their aggregation in cell membranes. They are therefore effective only for inactivation of enveloped viruses. Hematoporphyrin and dihematoporphyrin derivatives are examples of such compounds effective for inactivation of various enveloped viruses, but inactive against non-enveloped viruses. Benzoporphyrin, on the other hand, is a photosensitizer with a high affinity for lipoproteins. The compound inactivates vesicular stomatitis virus (VSV) and human immunodeficiency virus (HIV) — both free and bound — with limited damage to red blood cells [5, 6]. Research on these compounds has been halted due to the emergence of new photosensitizers of more promising characteristics. The Dutch developed cationic compounds called "Sylsense compounds", and their effectiveness for the inactivation of red blood cell components has been confirmed in pilot studies. These photosensitizers are activated by visible light (>600 nm) and effectively inactivate pathogens such as enveloped viruses [HIV, VSV and bovine viral diarrhea virus (BVDV), hepatitis C virus (HCV) and West Nile virus] and Gram positive and Gram negative bacteria. Qualitative studies of RBCs subjected to pathogen inactivation with Sylsense compounds demonstrated that the mean degree of haemolysis after five weeks of storage slightly exceeded 1%. All other parameters, adenosine triphosphate (ATP) concentration included, were comparable to those for control RBCs [7]. Methylene blue (phenothiazine dye), effective for pathogen inactivation in plasma with the Theraflex MB Plasma system could not be applied to PCs or RBCs due to high protein and lipoprotein binding affinity; envelope membranes of viruses and nucleic acids contain protein and lipoproteins. Moreover, the hydrophilic nature of methylene blue impeded penetration into cells so the compound could not be used for inactivation of intracellular pathogens. Another promising compound for pathogen inactivation in RBC was hydrophobic silicone found effective *in vitro*

for inactivation of the so-called model enveloped viruses. No *in vitro* detrimental effect on the quality of RBCs was confirmed but their survival time was significantly reduced. Research studies were therefore discontinued.

Under consideration was also the inactivation method based on a photochemical reaction with amotosalen hydrochloride and UVA radiation (320–400 nm). It could not however be applied to RBCs because hemoglobin absorbs UVA radiation. During pilot studies on development of PRT for RBCs several classic photosensitizers were tested. Despite the promising efficacy of these methods, studies have been halted due to increased haemolysis, significant oxidative damage, significant ATP reduction during storage, or unacceptable toxicity profiles [8].

Promising results were obtained *in vitro* with the light-independent alkylating compound PEN110 (Inactine). Positive outcome after transfusing healthy individuals with autologous RBCs pathogen inactivated with PEN110 led to initiation of clinical trials with sickle cell anemia patients and patients after cardiac surgery. The studies were stopped when anti-PEN100 antibodies were detected in some patients transfused with pathogen inactivated RBCs [9, 10].

Modern methods of pathogen inactivation in RBCs and whole blood

Method of pathogen inactivation in RBCs

The method is based on the reaction in which the S-303 compound cross-links with nucleic acids through a di-alkylating group. When added to RBCs the S-303 compound rapidly penetrates virus envelopes and integrates into host DNA. S-303 also reacts with other nucleophilic compounds of RBCs such as phosphates and proteins. To minimize these non-specific reactions, particularly the reactions with proteins, glutathione is added which is a natural antioxidant present in most cells. Following the first clinical reports on the formation of antibodies directed against inactivated RBC in patients with chronic anemia, the S-303 method was modified to include neutral pH glutathione in the reaction mixture, instead of unbuffered glutathione [11].

RBCs subjected to pathogen inactivation with S-303 demonstrate a slight loss of red blood cells, a decrease in hemoglobin concentration, significantly lower lactate concentration and a lower pH during storage. No differences in blood count and CD47 antigen expression on the surface of red blood cells were determined in either pathogen inactivated or non-inactivated RBCs [12, 13].

Pathogen inactivation in whole blood

The Terumo BCT company developed an option for pathogen inactivation in whole blood (with riboflavin and UV) which could replace the systems used for inactivation of individual blood components. This would significantly reduce the workload and implementation cost since one unit of whole blood provides 2 or 3 blood components. The procedure still requires multi center studies to document the minimum acceptable effect of PRT on WB quality but also on the quality of blood components obtained from fractionation of inactivated WB [14].

Reddy and Marschner [15] evaluated the quality of pathogen inactivated WB stored at room temperature for up to 7 days to find slight haemolysis (but within normal). No significant differences in adhesion and aggregation were observed when the functions of platelets obtained from inactivated WB stored for 7 days and blood cells from the control group were compared. For fresh frozen plasma (FFP) obtained from inactivated WB, stored for up to 28 days, protein recovery was not significantly reduced while mean concentrations of fibrinogen, factors V, VIIIc and XI were even higher than for plasma inactivated with the Mirasol®PRT system. In RBC obtained from inactivated WB, haemolysis increased (on average <1% on the 35th storage day) while sodium and potassium levels decreased as compared to standard RBC. The system is currently under validation in several transfusion centers (in Ghana among others), where transfusion of Mirasol inactivated WB prevents the spread of malaria (28% of donations was infected with malaria parasites) [15–19].

Clinical trials

Clinical trials on pathogen inactivated RBC and PCs have been conducted for over 10 years. Table II presents data from 3 evaluation studies on transfusion of autologous RBCs and 4 clinical studies on RBCs inactivated with the Intercept system (2nd generation) evaluating the outcome of transfusions of autologous RBC obtained from WB inactivated with the Mirasol system.

In the studies of Cancelas et al. RBCs pathogen inactivated with the Intercept system were stored 35 days prior to transfusion and RBCs obtained from WB were subjected to pathogen inactivation with Mirasol and stored for 21 days before transfusion. In general, the PRT-RBC recovery 24 hours after transfusion was lower that for reference RBCs although the mean values were in line with United States Food and Drug Administration (US FDA) guidelines (minimum 75%). No safety issues for autologous RBC recipients were reported [20, 21].

Allain et al. [22] presented the protocol from the AIMS study, which stressed the possibility of preventing malaria spread through transfusion of Mirasol-inactivated WB to acute anemia patients in the endemic region of Sub-Saharan Africa. The AIMS study reported statistically significant reduction in malaria transmission in recipients of WB units inactivated with Mirasol system and stored for 3–4-days [22]. Brixner et al. reported no significant differences between the use of conventional RBCs and RBC inactivated

Table II. Autologous transfusion studies in healthy subjects and clinical trials in adults and children using second generation Intercept RBC–PRT and Mirasol WB–PRT

Reference	PRT	Type of study (location)	Subjects (n) and condition	Outcomes (mean ± SD)
Cancelas et al. [19]	I	Autologous RBC crossover study (USA)	27 healthy subjects	24-h RBC recovery: • 88.0 ±8.5% (T) • 90.1 ±6.9% (C) Mean RBC survival: • 74.6 days (T) • 88.3 days (C)
Cancelas et al. [20]	I	Autologous RBC crossover study (EU and USA)	42 healthy subjects	24-h post-transfusion recovery: • 83.2 ±5.2% (T) • 84.9 ±5.9% (C) Mean RBC survival: • 62.8 days (T) • 75.1 days (C)
Cancelas et al. (IMPROVE II) [21]	M	Autologous RBC crossover study (USA)	24 healthy subjects	24-h post-transfusion recovery: • 82.5 ±3.9% (T) • 91.7 ±6.8% (C) Mean RBC survival: • 60.5 days (T) • 81.6 days (C)
Allain et al. (AIMS) [22]	M	Allogeneic WB transfusion, RCT (Ghana)	227 anemic patients	Incidence of TTM in 65 non-parasitemic patients exposed to parasitemic blood: • 1/28 =4% (T) • 8/37 =22% (C)
Brixner et al. (2018) (STARS)	I	Allogeneic RBC *in vitro* study and RCT (EU)	51 cardiovascular surgery recipients of 148 RBC units	Hb content in RBC (g): • 53.6 ±5.6 (T) • 56.3 ±6.0 (C) Clinical safety (T vs. C) — comparable renal and hepatic insufficiency
Trakhtman et al. (2019)	M	Allogeneic RBC *in vitro* study and RCT (Russia)	70 paediatric onco-hematology patients	Post-transfusion Hb (g/L): • 100.0 ±8.30 (T) • 101.6 ±7.57 (C)
Aydinok et al. (2019) (SPARC)	I	Allogeneic RBC crossover RCT (EU)	86 thalassemic patients	Transfused Hb (g/kg/day): • 0.113 ±0.04 (T) • 0.111 ±0.04 (C) No antibodies to S-303 RBC

RBC — red blood cells; PRT — pathogen reduction technology; WB — whole blood; SD — standard deviation; I — Intercep; USA — United States of America; T — treatment; C — control; EU — European Union; M — Mirasol; RCT — randomized controlled trial; TTM — transfusion transmitted malaria; Hb — haemoglobin concentration

with the Intercept system in patients subjected to cardiovascular procedures within the framework of the STARS RCT program. RBCs were transfused after an average storage period of 18.9 days. No antibodies to S-303 RBC were detected [23]. In a study involving pediatric onco-hematology patients transfused with RBCs from WB pathogen inactivated with the Mirasol system, the storage time was limited to 14 days. With longer storage time, increased hemolysis was reported. No antibody formation was observed [24]. Similar hemoglobin consumption was reported in the SPARC RCT study with multiple RBC recipients with thalassemia. During two separate periods, pediatric and adult patients were transfused a total of 1,024 Intercept and 1008 control RBCs stored for approximately 9 days prior to transfusion. No antibodies to S-303 RBC were detected [25].

It should be emphasized that the clinical trials with RBC-PRT and WB-PRT published so far were conducted in small groups of hematological patients with anemia who required rapid or repeated RBC support or patients subjected to cardiac surgery. Further research is required to assess the safety and efficacy in other clinical settings. Up-to-date information on new, larger clinical trials in different settings is available in international clinical trial registries. Additional studies are currently underway to expand knowledge on the safety and efficacy of RBC inactivated with the Intercept system and WB inactivated with the Mirasol system. "Study to evaluate the efficacy & safety of the INTERCEPT blood system for RBCs in complex cardiac surgery patients (ReCePI)" began in March 2018 and the program is expected to terminate on June 30, 2021. The aim of the study is to evaluate the efficacy and safety of transfusions of RBC subjected to pathogen inactivation with the Intercept system in patients undergoing complex cardiac surgery. The main outcome of the ReCePI project includes the assessment of renal failure, adverse events and emerging antibodies. "The efficacy and safety of WB-derived RBCs inactivated with Mirasol system and transfused to patients requiring multiple transfusions" are assessed within the framework of the PRAISE project (NCT03329404).

Author's contributions

EL — sole author.

Conflict of interest

None.

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None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

VIA MEDICA

Artificial tears to treat dry eye syndrome

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Abstract

Dry eye syndrome (DES) is caused by insufficient tear discharge, abnormal tear composition, or excessive evaporation of the tear film. It is a source of anxiety and discomfort. Treatment of DES is long-lasting and often unsatisfactory. Various treatment regimens are recommended based on the application of tear-imitating liquids produced by the pharmaceutical industry. The use of products approved for clinical use can however be limited by allergic reactions of some patients. In such cases it is recommended to use artificial autologous serum tears.

In some patients, blood collection is impeded and autologous tears cannot be processed. Other ways of obtaining artificial tears from material of human origin are therefore sought. Allogeneic preparations from blood donors are becoming more common. There is ongoing research into obtaining artificial tears with different techniques and from source material other than serum, namely platelet lysate and umbilical cord blood.

In Poland, there are no legal regulations regarding the preparation of artificial tears. The Institute of Hematology and Transfusion Medicine in Warsaw has been preparing autologous artificial tears from serum for over 40 years. Since 2019, research has explored the possibility of using allogeneic preparations.

Finding optimal treatment options for patients unable to use medicinal products, or for whom such products are ineffective, is a huge challenge worldwide. Efforts should be directed at developing the most uniform preparation methods and quality standards for each type of preparation. The growing number of publications on the subject shows the necessity of satisfying this need.

Key words: dry eye syndrome, autologous eye drops, allogenic eye drops

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Introduction

Dry eye syndrome (DES) is a condition caused by insufficient secretion, abnormal composition or excessive evaporation of the tear film which leads to injury and peeling of the eyeball epithelium. It may be attributed to endogenous cause and/or occur as result of physical, chemical or biological external factors. The condition is increasingly common [1].

One cause of DES is atrophy of the lacrimal gland as a result of abnormal immune responses or surgical intervention. The condition may also be attributed to damage to numerous lacrimal glands and reduced tear production. Among other causes are: Sjögren's syndrome, graft-versus-host disease (GvHD), sarcoidosis, infectious mononucleosis, acquired immunodeficiency syndrome (AIDS), and neurological conditions. DES may also be caused by dry, well-ventilated or air-conditioned rooms as well as dust, chemicals or smoke which irritate and dry out the eye.

Other causes may include lacrimal duct air (eyelid) regurgitation when the eyeball is not sufficiently moistened although tear production is normal. Another cause is incorrect composition of the mucus layer as a side effect of oral drug intake or topical drug application into the conjunctival

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sac. Bacterial infections inside the edge of the eyelid and conjunctiva may also be responsible for disorders in the fatty layer of the tear film.

DES troubles patients, causing physical as well as emotional discomfort. The most common symptoms include redness, itching, a feeling of burning and "sand under the eyelids" as well as the presence of a foreign body, increased mucus, watery eyes, photophobia and eye pain. Visual disturbances are reported, and images are blurry and out of focus. Some patients experience photosensitivity or ocular pressure.

Dry eye syndrome treatment

Unfortunately, the treatment is long-lasting and often unsatisfactory. Various treatment regimens are recommended based on the application of liquids imitating tears, i.e. artificial tears produced by the pharmaceutical industry [1].

Products approved for clinical use are safe, but their use however may be limited by allergy in some patients to substances in the drops, most often to preservatives. These may be responsible for local anaphylactic reactions. In such cases, it is recommended to use artificial serum tears.

Serum obtained from human blood consists mainly of water, and to a small extent of proteins, electrolytes, growth factors, vitamins, and other components secreted from platelets in the clotting process. Viscosity and composition ensure adequate moisturising of the eye epithelium, and the effect is maintained for a certain period of time. Artificial serum tears are a safe product. They contain no preservatives. Moreover, they help induce regeneration of damaged epithelium [2].

Worldwide, there is growing demand for artificial tears. In Australia for example, demand increased by 30% just between 2014 and 2015. Also, the number of publications on autologous preparations used to treat DES is steadily growing [3–5].

There is however, the problem of eligibility of patients for the procedures of collecting blood for artificial tears preparation. Indication for the use of artificial tears should be determined by an ophthalmologist and the patient referred to a special center where artificial tears are prepared according to procedures which guarantee the safety of both patient and product.

Autologous serum eye drops

In Poland, there are no legal regulations regarding the preparation of artificial tears. Other European countries follow recommendations of the Guide with regard to the quality and safety of tissues and cells for human application [6].

The first center in Poland to prepare artificial tears was the Institute of Hematology and Transfusion Medicine (IHTM) which started this activity in the late 1980s. Since then, IHTM staff have trained representatives from other entities, and currently several blood transfusion centers (CKiK) also provide this service. Of crucial importance is the fact that CKiK are well able to prepare sterile and safe artificial eye drops. Moreover, the activities of CKiK are subject to constant supervision by IHTM. Regrettably, there are increasing reports of offers of artificial tears from entities not subjected to any supervision; little is known about the conditions in which that product is prepared.

Selection of patients and their background

It is extremely important for the patient to be well prepared in order to obtain a high-quality product. In the case of autologous artificial tears, the patient should inform the physician responsible for the procedure of any medications that he or she takes. Of particular significance is information on any type of anticoagulant. At least three days prior to procedure, the patient should discontinue the anticoagulant medication including aspirin and aspirin derivatives. The patient should prepare himself for the procedure just like for blood donation i.e. on the previous day no fatty products are allowed to avoid lipemia. The patient should be in good general condition. On procedure day, he or she should have a light breakfast and be well hydrated.

In some patients however, blood collection is impeded by difficult vein access, intake of certain type of medications, or by an underlying disease. Autologous tears cannot be processed. Therefore, other ways of obtaining artificial tears from material of human origin are being sought.

Allogenic serum eye drops

Allogeneic preparations from blood donors are becoming more common [7, 8]. Their use is considered for both patients for whom autologous tears can be prepared as well as those for whom autologous tears are out of reach, e.g. children, people with difficult vein access, abnormal test results, or who are on drugs that might affect autologous preparations, *etc.*

Such patients can rely on artificial tears obtained from allogeneic serum of healthy male donors of the AB blood type. In such cases blood should be collected from donors tested for human leukocyte antigen (HLA) and/or human neutrophil antigen (HNA) antibodies or with no history of transfusions. To date, the risk related to the application of eye drops with antibodies has not been determined, but it is likely that antibodies may adversely affect therapy safety and outcome. Such donors must meet all eligibility criteria for infection markers as donors of blood dedicated for transfusion. They must test negative for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), syphilis and other infectious agents routinely tested in the country.

The advantages of autologous preparations are considerable: easier donor-patient access, quick collection procedure, no need for infectious marker testing, no risk of immunological reactions, and less anxiety related to application. Moreover, the procedure can be performed on an outpatient basis.

On the other hand, allogeneic preparations are produced in volumes that serve more patients. They can be mass produced and the quality standards more precisely defined and subject to strict legal regulations. Indeed, for safety reasons, legal regulations should be defined with regard to both allogeneic and autologous preparations.

Alternative sources of artificial tears

There is ongoing research into obtaining artificial tears with different techniques and from source material other than serum, namely platelet lysate and umbilical cord blood. Platelets are rich in growth factors that support wound healing. Approximately 70% of growth factors are secreted during clot formation. Current investigations are focused on finding an optimal preparation method to obtain artificial tears containing more growth factors than serum. A simple method, which has been known for years, is that of preparing platelet lysate through repeat freezing-thawing. There is no one standardized method of obtaining artificial eye drops from platelets. However, platelets seem an important source material for production of eye drops richer in some growth factors than autologous or allogeneic serum. This should directly translate to better therapeutic efficacy [9].

The scope of research into the possible use of umbilical cord blood (CB) is very broad. Well-recognized is the use of CB as a source of stem cells for managing malignant and non-malignant hematological disorders and immunological diseases. Research indicates that CB can also be used for the regeneration of other tissues. Numerous studies have demonstrated the efficacy of eye drops obtained from cord blood serum. Studies have been performed on preparates obtained from pooled CB [10]. The content of epidermal growth factor (EGF) was found to depend on the mother's age and type of delivery (vaginal vs. Cesarean section). Such a method of obtaining eye drops (EDs) allows for better choice of the starting material, performance of testing before eye drops are issued to the patient, and the selection of units with a higher content of healing factors [11].

Regardless of the preparation method, a universal challenge surrounds the storage of eye drops. EDs from both serum and platelet lysate require storage at temperatures below minus 18°C. An alternative could involve the preparation of lyophilisate plasma rich in growth factors, but this requires special techniques and equipment [12]. Preliminary research indicates that such preparates have good healing properties.

Our own experiences

IHTM has been preparing autologous artificial tears from serum for over 40 years. Currently, about 150 preparates are obtained annually. Each preparation gives c.600–1,500 single doses of artificial tears. Preparates are issued to patients immediately after the procedure together with instructions for freezing within 18 hours of collection and storage below –18°C. For artificial tears stored in such conditions, the expiry date is 12 months. No serious adverse reactions have been reported. Patients are instructed to discontinue applying artificial tears if dry eye symptoms exacerbate. Immediately after applying artificial tears, some patients experience sticking eyelids and blurred vision due to serum viscosity. Observations of patients with chronic GvHD and co-existing DES demonstrate their effectiveness as supportive therapy [13].

In cooperation with children's treatment centers (Children's Health Center, Clinic of Pediatrics, Hematology and Oncology the Medical University of Warsaw) the IHTM has participated several times in the preparation of autologous artificial tears for children with diseases other than DES. The outcome of such therapy was also found to be effective [14]. In the case of children, such preparates are difficult to obtain and other options must be considered, e.g. allogeneic tears or tears from the serum of an adult relative.

In 2019, following the approval of the IHTM Bioethics Committee, research was launched into the possible use of allogeneic preparations. Preliminary results are very promising [15]. During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, allogeneic preparations have gained a special significance. Patients are discharged with pre-prepared frozen allogeneic preparations and therefore their stay on the IHTM premises is minimized, as is the risk of infection transmission. Patients who consent to this study are required to evaluate the effectiveness of the artificial tears by completing a questionnaire developed by IHTM. The questionnaire is based on a standard survey used in ophthalmology to assess the severity of the DES. In 2020, such preparates were issued to 14 patients.

Summary

DES is a growing problem which affects not only elderly people and patients subjected to stem cell transplants. It contributes to deterioration in the quality of life. Finding optimal treatment options for patients who cannot use medicinal products, or for whom such products are inefficient, is a huge challenge worldwide. The growing number of publications on the subject shows the necessity of satisfying the needs of such patients [3]. In order to evaluate the best therapeutic option, specialists in various fields should cooperate. Development of an optimal method of obtaining EDs cannot be achieved without collaboration

between transfusion medicine and other specialties including ophthalmology. Our study also indicates that therapy should be adjusted to the individual characteristics of each patient.

In many countries, ophthalmology societies issue guidelines for the use of preparations of human origin in patients with DES. Serious challenges to the development of uniform standards are the variety of preparation methods, the origin of the starting material, and the use of quality control or the applicable legal regulations.

Procedures for EDs preparation from material other than autologous serum are being gradually implemented in different centers, which proves that patient needs are diverse and many cannot benefit from autologous EDs. It has been pointed out that allogeneic artificial tears may have advantages over autologous tears. The latter may not be sufficiently effective and can sometimes even aggravate symptoms because they come from people with autoimmune diseases.

In view of the above, further research is called for. Efforts should be directed at the development of the most uniform preparation methods and quality standards for each type of preparation.

Author's contributions

JA-P — sole author.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Anticoagulant treatment of venous thromboembolism in pregnant women

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Abstract

Venous thromboembolism (VTE), in particular pulmonary embolism (PE), remains the leading cause of death among pregnant women. Low-molecular-weight heparin (LMWH), with preference for therapeutic doses given twice daily according to European guidelines, is the drug of choice for the treatment of VTE in pregnancy and the puerperium. The recommended therapeutic dose is calculated on early pregnancy body weight. Evidence to support anti-Xa monitoring in pregnancy is weak. Unfractionated heparin (UFH) with multiple activated partial thromboplastin time measurements is still used in the acute treatment of high-risk PE. American experts have suggested considering initial outpatient therapy over hospital admission also in pregnant women with low-risk acute VTE, but European experts suggest adopting such a strategy selectively, for example in isolated distal leg thrombosis. Scheduled delivery with prior discontinuation of anticoagulant therapy in pregnant women who received a therapeutic dose of LMWH is suggested with the restart of therapy 4–6 h after a vaginal birth and 6–12 h after a cesarean delivery. It is recommended that UFH, LMWH, warfarin, acenocoumarol, or fondaparinux, but not direct-acting oral anticoagulants, should be used in breastfeeding women.

This review summarizes the key messages from current guidelines mainly based on low-quality evidence and expert consensus.

Key words: venous thromboembolism, pregnancy, anticoagulation

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Introduction

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), occurs four or five times more frequently in pregnant women compared to nonpregnant women of a similar age. It is estimated that VTE occurs in 0.05–0.20% of all pregnancies [1–4], with predominance of DVT over PE [5, 6]. However, PE remains the leading cause of death among pregnant women, with mortality of about 4% [7]. The risk of VTE rises with each month of pregnancy, and peaks within the first two weeks after birth [5, 8], but increased risk is still seen during the first six post-partum weeks [1, 5, 8]. The incidence rate of VTE antepartum is estimated to be 118 [95% confidence interval [CI]: 101–137) per 100,000 person-years, and 424 (95% CI: 238–755) per 100,000 person-years postpartum [1–4]. The multiple mechanisms behind the elevated risk of VTE in pregnant women involve pelvic venous compression by the gravid uterus, venous stasis, compression of the left iliac vein by the right iliac artery, and prothrombotic alterations to blood coagulation including increased factor VIII, fibrinogen, thrombin generation and reduced free protein S, accompanied by enhanced platelet activation and hypofibrinolysis largely driven by elevated plasminogen activator inhibitor-1 (PAI-1) [1, 5, 8].

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Risk factors and VTE prevention in pregnancy

It is recommended that women who plan pregnancy, or those in early pregnancy, should be assessed in terms of risk factors for VTE [9]. Women are classified to be at low, intermediate, or high risk of VTE, and prevention should be administered accordingly [9]. There is no established VTE risk assessment scoring system during pregnancy [10]. Recently, it has been demonstrated that infection, varicose veins, preeclampsia/eclampsia, emergency cesarean delivery, stillbirth, and medical comorbidities predict VTE after childbirth [11]. There is consensus that unprovoked VTE, hormone-related VTE, antiphospholipid syndrome, severe thrombophilia, and concomitant cancer represent high risk factors [12]. Approximately 6–12% of women who have experienced unprovoked or hormone-associated VTE in the past will suffer from VTE during pregnancy if thromboprophylaxis is not initiated, but the risk of recurrent episodes is still higher than in women without such previous events [13, 14]. It has been suggested that thromboprophylaxis during pregnancy should be initiated if the estimated VTE risk is approximately 2% [10]. Thrombophilia-associated VTE risk is highly heterogenous in pregnancy. In young women heterozygous for the factor V Leiden (FVL) polymorphism, the risk is about 0.5%, while in those with heterozygosity for both FVL and the prothrombin gene *G20210A* polymorphism, the risk is much higher, c.5.5%. In antithrombin (type I) deficient women, it is 11.6% during pregnancy without thromboprophylaxis [10].

A 2014 Cochrane systematic review of randomized trials led to the conclusion that "there is insufficient evidence on which to base recommendations for thromboprophylaxis during pregnancy (and that) large scale, high–quality randomized trials of currently used interventions are warranted" [15].

Prospective studies have however indicated that thromboprophylaxis can reduce VTE risk in pregnancy from 2.4–12.2% in its absence to 0.5–5.5% observed in women on heparin-based prevention [14, 16].

For many years, low-molecular-weight heparin (LMWH; in Poland enoxaparin, dalteparin, and nadroparin) has been the drug of choice for the prevention and treatment of VTE in pregnant women [17]. Preventive strategies are based on expert opinion and low–quality evidence, and therefore pharmacological thromboprophylaxis should be used wisely, taking into account commonly reported easy bruising, minor bleeding, skin allergic reactions (about 2%), pain, bone loss, heparin-induced thrombocytopenia (in <0.5%) and also high out–of–pocket costs for pregnant women. The initial dose of LMWH for thromboprophylaxis should be based on body weight in early pregnancy, i.e. 8–10 weeks [18]. Consequently, patients at high risk for VTE should receive LMWH i.e. enoxaparin at 0.5 IU/kg of body weight once daily [18] or at equivalent doses. In obese women (>100–120 kg), weight-based dosing (enoxaparin 40 mg bid) is commonly recommended based on the concept that the daily dose should be high enough to achieve adequate anti-Xa activity estimated at 0.2–0.6 IU/mL [19]. Despite controversy surrounding the optimal thromboprophylaxis in pregnancy, there is consensus that pregnant women with prior VTE who are not receiving anticoagulation should receive six weeks of postpartum prophylaxis. Importantly, experts underscore that all pregnant women at risk of VTE should be educated as to its signs and symptoms and the need to consult a physician if they develop [20].

Diagnosis of acute VTE in pregnancy

Dyspnea, poor exercise tolerance, pleuritic chest pain, cough, tachycardia, tachypnea, and hemoptysis represent the common symptoms and signs of PE during pregnancy that are identical to those observed in other PE patients. In the case of suspected DVT, physicians should pay attention to unilateral leg edema and increased swelling of one leg, in particular the left. In >85% of pregnant women with DVT, the veins of the left lower extremity are affected at least in part due to compression of the left iliac vein by both the left iliac artery and the gravid uterus. Persistent pain in the buttock, groin, flank, or even abdomen, can herald iliac vein thrombosis which is relatively common in pregnancy and associated with a 50% risk of subsequent acute PE.

Compression ultrasound is the diagnostic imaging procedure of choice for suspected DVT in pregnancy, with a high sensitivity and specificity for proximal DVT [12]. It has been proposed that the absence of the three following features: left leg presentation, >2 cm calf circumference difference, and first trimester, has a nearly 100% negative predictive value in the diagnosis of iliac vein thrombosis if ultrasonography of the leg veins does not detect thrombosis [21]. Its value is much lower in the detection of either distal DVT or pelvic DVT compared to proximal DVT, which is of particular importance in pregnancy. Serial compression ultrasound imaging on days 0, 3, and 7 in pregnant women has been reported to have almost 100% negative predictive value, which allows the exclusion of DVT [22]. If the initial compression ultrasound is negative, then MRI venography may be considered to exclude a pelvic DVT, but not DVT at other locations [22]. If the clinical suspicion is high, the use of heparin should be initiated and compression ultrasonography should be repeated on days 3 and 7. If the initial clinical suspicion is low, then anticoagulation can be stopped after a negative result of compression ultrasonography, but repeat imaging should be performed on days 3 and 7 [22]. If such a strategy is unfeasible in practice, heparin administration should be continued with clinical evaluation of symptoms and signs.

In pregnant women, clinical prediction scores for assigning pre-test probabilities of VTE and diagnostic algorithms used in patients suspected of PE have not been validated [23]. Given the risk of death, all pregnant women in whom acute PE is suspected should be assessed and therapeutic anticoagulation should be initiated until the diagnosis is made.

Interpretation of D-dimer concentrations in pregnant women is challenging. It is well known that D-dimer levels rise in each pregnancy and each trimester. It has been estimated that there is a 39% relative increase in D-dimer concentration for each trimester [24]. A positive D-dimer test, defined as a D-dimer concentration of above 500 ng/mL, in pregnant women is not necessarily a marker of developing acute VTE, while normal D-dimer concentrations have been observed despite objective confirmation of acute VTE by imaging [25]. Imaging is needed to confirm or refute the suspicion of VTE in this clinical setting [26].

There is no consensus on the best diagnostic strategy for pregnant women suspected of acute PE [27]. A modified Wells score has been suggested to be used in combination with D-dimer measurement to identify pregnant women who require imaging [28, 29].

If the index of suspicion of DVT remains high, then compression USG should be performed. If this is abnormal, then anticoagulation is indicated. If compression USG is negative, then further testing is required and MRI should be performed. Where PE is suspected and all other investigations are being normal, low-dose CT should be undertaken [12].

Treatment of acute VTE in pregnancy

LMWH is the drug of choice for the treatment of VTE in pregnancy and the puerperium. In acute VTE, treatment with therapeutic doses of weight-adjusted LMWH should be given twice daily according to the European guidelines on the management of PE [12].

The American Society of Hematology (ASH) guidelines panel strongly recommends therapy with LMWH over unfractionated heparin (UFH) in pregnant women in whom acute VTE has been diagnosed, with no clear preference for either once-per-day or twice-per-day dosing regimens given the limited evidence to support one of these two options in practice [30].

In a systematic review and meta-analysis, treatment of pregnancy-associated VTE with LMWH or UFH led to an estimated antepartum mean VTE recurrence incidence of 1.97% (95% CI: 0.88–3.49), accompanied by a risk of major bleeding of 1.41% (95% CI: 0.62–2.41%) prior to delivery and of 1.20% (95% CI: 0.3–2.50%) during the 24 h after delivery [31]. The results of two meta-analyses of studies performed on a nonpregnant population showed that the risks of bleeding occurring during the initial therapy of acute VTE with LMWH and UFH did not differ [32, 33]. Pregnant women on heparin therapy are most likely exposed to the same risks while on LMWH or UFH.

As in the non-pregnant population, it is strongly recommended that in all subjects with suspected DVT or PE, therapeutic LMWH should be given until the diagnosis has been excluded by objective testing [32–34]. Anticoagulation is very effective in decreasing the risk of PE-related death. Therefore, pregnant women especially should not be sent to other specialists for further tests or to hospital if the appropriate therapy has not been initiated.

The recommended therapeutic dose is calculated on early pregnancy body weight (i.e. enoxaparin 1 mg/kg body weight twice daily or dalteparin 100 IU/kg body weight twice daily) [34]. The target peak anti-Xa values, typically determined 4–6 h after injection, range from 0.6 to 1.2 IU/mL [34]. However, evidence to support anti-Xa monitoring is weak. Some, but not all, observational studies have reported a need for dose adjustments when anti-Xa levels have been used to guide therapy [35–41]. However, none demonstrated a clear clinical benefit from the LMWH dose adjustments e.g. reduced blood loss at the time of delivery in women with FXa monitoring [42]. Given the available evidence, the risk and benefits related to anti-FXa monitoring in pregnant women are probably small. With regard to the risk of thrombocytopenia in heparin-treated women, experts in Canada have suggested assessing platelet count seven days after the start of therapy. However, the risk of clinically relevant thrombocytopenia while on LMWH in pregnancy is 0.1–0.2%, and therefore this approach is rarely used in practice if pregnant women are treated exclusively with LMWH [20].

UFH intravenous (i.v.) with multiple activated partial thromboplastin time (APTT) measurements is used in the acute treatment of high-risk PE.

Thrombolytic therapy, in most cases with alteplase i.v., should only be used in acute PE patients with severe hypotension or shock [43]. Following thrombolysis, UFH should be started at a rate of 18 U/kg/h without administration of the loading dose and initiation of therapeutic-dose LMWH as soon as stabilization has been achieved [12]. Thrombolysis is rarely used in limb-threatening DVT in pregnancy [20].

Fondaparinux (7.5 mg once a day in normal weight or 10 mg if weight exceeds 100 kg) can be considered if LMWH is not well tolerated or causes adverse events e.g. skin allergy or if heparin-induced thrombocytopenia develops, or also if this life-threatening adverse event is even only suspected based on a drop in platelet count by 50% or more, usually after 5–15 days of therapy.

The insertion of vena cava filters is not recommended in most cases of massive proximal DVT with PE, since the procedure is associated with several risks, in particular if the presence of a filter is prolonged [43, 44]. In some centers, a temporary vena cava filter is inserted prior to planned

delivery in women at highest risk of fatal PE, in particular those who developed proximal DVT (i.e. iliac vein thrombosis) or massive PE within the 2–4 preceding weeks, in particular in the presence of contraindications to anticoagulation (e.g. intracranial bleeding). The filter should be removed a few weeks postpartum [20].

Importantly, the ASH panel advises against the addition of catheter-directed thrombolysis therapy to anticoagulation in pregnant women who develop both massive proximal DVT and/or acute PE with right ventricular dysfunction in the absence of hemodynamic instability [30]. In the case of hemodynamic instability, the panel suggests administering systemic thrombolytic therapy in addition to anticoagulant therapy [30]. To date, there have been two analyses of observational studies in which the efficacy and safety of systemic thrombolysis in a total of 31 pregnant women were evaluated; they demonstrated five neonatal deaths not related to bleeding or thrombolytic therapy, with no cases of maternal death [45, 46].

American experts suggest considering initial outpatient therapy over hospital admission also in pregnant women with low-risk acute VTE [30]. European experts however suggest adopting such a strategy only in certain circumstances, for example in isolated distal DVT or popliteal vein DVT in young patients free of other conditions increasing morbidity e.g. diabetes.

Anticoagulation and delivery

In women on therapeutic LMWH, delivery should be planned at a maximum of 39 weeks to minimize the possibility of unexpected labor following the administration of full-dose heparin, as protamine sulfate can reverse 50% of anticoagulant effects of LMWH, which might lead to major bleeding. Whether to stop anticoagulation before delivery depends on the VTE risk. In high-risk women on therapeutic LMWH, LMWH should be withdrawn and replaced by i.v. UFH at least 36 h prior to delivery, and the infusion of UFH should be stopped 4–6 h prior to anticipated delivery. Normal APTT, determined after 4–6 h, is needed to decide on the use of regional anesthesia.

In contrast, if the VTE risk is low in women on therapeutic LMWH or those on thromboprophylaxis with a higher-than-standard dose administered twice daily, the evening dose of LMWH should be omitted and induction of delivery or cesarean section performed the next morning, with regional anesthesia started more than 24 h after the last dose of LMWH and if no antithrombotic agents e.g. aspirin are used [47]. In the case of therapeutic anticoagulation prior to delivery, and if neuroaxial anesthesia was used, monitoring for the development of spinal hematoma should be carried out.

In women who received therapeutic-dose heparin before delivery, European experts recommend (to decrease the risk of postpartum major bleeding) that in the third stage of labor a modified dose of oxytocin should be administered, namely 2 IU oxytocin over 5 min added to a standard infusion for 4 h [10 U of oxytocin in 500 mL of normal saline given i.v. at 36 mL/h for 4 h (12 mU/min)], as such a protocol has been demonstrated to reduce blood loss [47].

In women with VTE who received heparin therapy prior to childbirth, the treatment should be restarted 4–6 h after a vaginal birth and 6–12 h after a cesarean delivery unless major bleeding has occurred. Some experts from the United Kingdom suggest initiating VKA at least five days after delivery, which is common practice. The overlap of LMWH with VKAs for at least five days should be recommended, then LMWH withdrawn and VKA continued for at least three months, or six months if PE was diagnosed in the third trimester. The target INR is 2–3 and its determination should be performed every 1–2 weeks. In women who preferred LMWH over the entire period of postpartum anticoagulant treatment, parenteral therapy could be continued ideally once a day without any anti-Xa measurements [12].

The ASH guideline panel suggests scheduled delivery with prior discontinuation of anticoagulant therapy in pregnant women who received therapeutic dose LMWH and "against scheduled delivery with discontinuation of prophylactic anticoagulation compared to allowing spontaneous labor" if a prophylactic dose of LMWH was administered [30].

Anticoagulant use in breastfeeding women

The ASH panel recommends in favor of using UFH, LMWH, warfarin, acenocoumarol, or fondaparinux in breastfeeding women, and recommends against using direct oral anticoagulatns (DOACs) [30].

UFH is not excreted to breast milk due to its large size and negative charge [48], while LMWH can be found in breast milk at negligible levels based on the measurement of anti-FXa activity (below 0.04 IU/mL) in treated women [49], with no risk of clinically relevant bleeding in the infant. Vitamin K antagonists are nonlipophilic and highly protein bound and are not excreted into breast milk [50]. There is no published data on the excretion of fondaparinux into breast milk, but orally taken heparins have low availability [49]. Although it has been reported that rivaroxaban is detectable in breast milk at very low levels [51], DOACs are strongly contraindicated in breastfeeding women, as in pregnancy.

Conclusions

Anticoagulation in pregnant women with VTE is challenging and based mainly on low-quality evidence. The prompt initiation of LMWH therapy with its continuation up to six weeks after delivery is the cornerstone of anticoagulant strategy, which is effective in reducing the risk of life-threatening PE. The decision as to how long anticoagulation should be administered after pregnancy-related VTE should be individualized.

Authors' contributions
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Ethics
The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

VIA MEDICA

Emicizumab in severe hemophilia A

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Abstract

Emicizumab is a recombinant, humanized, bispecific, asymmetric monoclonal antibody that bridges activated factor IX and factor X (FX) and leads to activation of FX, thus mimicking the hemostatic function of activated factor VIII (FVIIIa).

The clinical trial program showed that emicizumab prophylaxis maintains low bleed rates and is well tolerated by patients with hemophilia A of all ages with and without factor VIII (FVIII) inhibitors. Emicizumab prophylaxis in severe hemophilia A patients with high titer inhibitor against FVIII was launched in Poland in 2020. As of April 2021, 42 patients were receiving emicizumab in Poland, not including clinical trials. The aim of this paper was to review the most recent data on the role of emicizumab in the management of patients with severe hemophilia A.

Key words: hemophilia A, inhibitor, factor VIII, emicizumab, rFVIIa, aPCC

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Long-term prophylaxis in severe hemophilia A

Severe hemophilia A, defined as a complete deficiency of factor VIII (FVIII) (plasma activity <1 IU/dL) manifests with devastating, inherited bleeding tendency in which repeated, spontaneous and trauma-related hemorrhages into joints, muscles, and various critical organs inevitably lead to disability, reduced health-related quality of life, and premature death. The natural course of the disease however can be modified, or completely averted, by intravenous infusions of deficient clotting factor. Such treatment is referred to as replacement (or substitution) therapy [1].

There are two basic approaches to replacement therapy. One is on-demand therapy consisting in administration of the missing factor at the time of clinically evident bleeding, the other is prophylaxis based on administration of the deficient clotting factor before bleeding occurs with the aim of avoiding bleeding episodes. Prophylaxis can be short-term or long-term, or even lifelong to avoid many, or ideally all, spontaneous and traumatic bleeds [1, 2].

Nowadays, long-term prophylaxis is generally accepted as the best form of treatment for patients with severe hemophilia A. However, FVIII replacement therapy is invasive, expensive, and not widely available. Due to the short half-life of standard FVIII concentrates [standard half-life (SHL)], of about 10 h, no less than three intravenous infusions per week may be required to maintain FVIII levels at >1 IU/dL, which is effective at reducing incidence of breakthrough bleeds [3]. The use of novel recombinant FVIII concentrates with extended half-life (EHL) has slightly increased the interval between treatments but there is still a requirement for lifelong intravenous infusions, which considerably alter patients' quality of life. On top of that, in about 30% of previously untreated patients (PUPs) with severe hemophilia A, treatment with FVIII concentrates is further complicated by the development of FVIII inhibitors, which render FVIII replacement therapies ineffective [4].

Because prophylactic treatment with SHL and EHL FVIII concentrates does not completely eliminate bleeding episodes in patients with severe hemophilia A, is associated

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with the burden of lifelong intravenous injections, and is ineffective in patients with FVIII inhibitors, more effective therapies are urgently needed. One such is emicizumab.

Emicizumab

Emicizumab (Hemlibra®, F. Hoffmann, La Roche, Basel, Switzerland) is a recombinant, humanized, bispecific, asymmetric monoclonal antibody that bridges activated factor IX (FIXa) and factor X (FX) to restore the function of FVIII [5, 6]. In the coagulation process, emicizumab functions like activated FVIII (FVIIIa), although the two molecules show no structural similarity [7]. In fact, the completely different structure of the two molecules has a significant therapeutic benefit: the antibodies that neutralize factor VIII (FVIII inhibitors) are not capable of neutralizing emicizumab. Therefore, emicizumab is able to restore thrombin generation in the plasma of patients with hemophilia A and inhibitors against FVIII.

Unlike clotting factor concentrates for intravenous use, emicizumab can be injected subcutaneously. This is another advantage much appreciated by patients who thus avoid frequent intravenous injections. Moreover, SHL FVIII concentrates for long-term prophylaxis are usually administered every 2–3 days, while emicizumab can be injected every one, two or even every four weeks, depending on the selected dosing schedule [8].

Emicizumab is injected subcutaneously once weekly, at a dose of 3 mg/kg during the first four weeks (loading dose) which results in the steady-state of plasma concentration of the drug. This is the so-called saturation phase. In the 5th week, the mean trough plasma concentration of emicizumab in hemophilia A patients was around 50 µg/mL [8]. This concentration corresponds to c.15% of factor VIII activity; in other words, the hemostatic status of a severe hemophilia A patient on regular emicizumab dosage can be compared to that of a patient with mild hemophilia A with FVIII activity approximately 15% of normal [9]. Detailed descriptions of the pharmacokinetic and pharmacodynamic properties of emicizumab can be found elsewhere [10–12].

Clinical development program for emicizumab

The program of clinical trials to evaluate prophylactic emicizumab involves multi-center, open-label phase III clinical trials, including HAVEN (1 to 4), STASEY, and HOHOEMI [13–18]. Emicizumab was approved in the USA in 2017, and in the EU, Japan and other countries in 2018 on the basis of positive results from the HAVEN 1 trial [13]. The study demonstrated the superiority of emicizumab for long-term bleeding prophylaxis compared to that of by-passing agents (BPAs) administered either prophylactically or on demand. In the HAVEN 1 trial, 63% of patients on emicizumab prophylaxis reported no bleeding episodes which required hemostatic treatment. In HAVEN 2, the percentage of such patients was even higher, at 87%. To date, no such good results have been obtained in evaluation trials of various forms of prophylaxis in severe hemophilia, regardless of the inhibitor status. Table I presents the major outcomes of four HAVEN clinical trials on emicizumab in the management of hemophilia A patients with and without inhibitors against FVIII [13–16].

Recently, Callaghan et al. [19] presented long-term data on the efficacy, safety, and pharmacokinetics of emicizumab used in the HAVEN 1–4 studies. A total of 401 pediatric and adult patients enrolled in the phase III Haven 1–4 studies were followed for a median of 120.4 weeks. The model-based treated annualized bleed rate (ABR) was 1.4 [95% confidence interval (CI): 1.1–1.7]. ABRs declined and then stabilized at <1 in an analysis of 24-week treatment intervals; at weeks 121 to 144 (n =170), the mean treated ABR was 0.7 (95% CI: 0–5.0). During weeks 121 to 144, 82.4% of participants had 0 treated bleeds, 97.6% had ≤3 treated bleeds, and 94.1% reported no treated target joint bleeds [19]. Unsurprisingly, long-term prophylaxis with emicizumab led over time to decreased FVIII consumption in patients without inhibitors, and by-passing agents in those with inhibitors.

Safety profile of emicizumab

The most common adverse events associated with the use of emicizumab are injection site reactions (ISR) [13, 19]; normally they are mild to moderate in intensity. In the HAVEN 1–4 studies, no participants discontinued emicizumab because of ISR [19]. However, the most important adverse events reported in the HAVEN 1 trial were thromembolic events (TE) and thrombotic microangiopathy (TMA) episodes in five patients [13]. All episodes were preceded by the administration of activated prothrombin complex concentrate (aPCC) at a dose of >100 U/kg/day for >24 h. In 4/5 patients, TE and TMA symptoms resolved completely within 1–4 weeks of aPCC discontinuation. One TMA patient died, although the symptoms were reported to have resolved before death. Another TMA patient required several days of intensive therapeutic plasma exchange and renal replacement therapy. In all patients, both aPCC and emicizumab were discontinued. None of the TE and TMA patients received anticoagulant medication. Two of the four patients resumed emicizumab therapy, and no further events were reported.

Based on these results, guidelines have been changed to recommend recombinant factor VIIa (rFVIIa) use and avoid aPCC or, if impossible, use the lowest aPCC doses for the management of bleeding episodes in patients on emicizumab [12, 20–23].

Table I. Data for HAVEN 1–4 trials (based on [13–16])

Study population	No. of bleeds requiring treatment per 12 months* during emicizumab prophylaxis (95% CI)	% of reduced bleeding episodes during emicizumab prophylaxis vs. on demand	% of patients with no treated bleeds reported during study	% of reduced bleeding episodes during emicizumab prophylaxis vs. prior prophylaxis in NIS study
HAVEN 1 Hemophilia A +inhibitor (≥5 UB/mL) Age: ≥12 years Body weight: >40 kg In 24-week period before the trial: ≥6 bleeding episodes (BPAs 'on demand') or ≥2 bleeding episodes (BPA prophylaxis) N =109	2.9 (1.7–5.0)	87	Emicizumab: 63 BPAs ('on demand'): 6	79
HAVEN 2 Hemophilia A +inhibitor (≥5 UB/mL) Age: <12 years Body weight: 3–40 kg In 24-week period before trial: ≥6 bleeding episodes (BPAs 'on demand') or ≥2 bleeding (BPA prophylaxis) N =88	0.2 (0.06–0.62)	Not asssed	Emicizumab: 87 BPAs ('on demand'): not assessed	99
HAVEN 3 Hemophilia A without inhibitors Age: ≥12 years Before trial: n =89 On episodic therapy n =63 and on prophylaxis with FVIII n =152	1.5 (0.9–2.5) (QW) 1.3 (0.8–2.3) (Q2W)	96 (QW) 97 (Q2W)	56 (QW) 60 (Q2W) 0 (no prophylaxis)	68 reduction with emicizumab QW vs. prior FVIII prophylaxis (ABR on emicizumab 1.5 vs. 4.8 on FVIII prophylaxis in NIS)
HAVEN 4 Hemophilia A with and without inhibitors Age: ≥12 years N =48	2.4 (1.4–4.3) (Q4W)	Not assessed	56 (Q4W)	Not applicable (no comparator)

*This value is estimated as follow-up period was <12 months; CI — confidence interval; NIS — a non-intervention study conducted before main clinical trial; BPA(s) — by-passing agent(s); QW — once weekly; Q2W — every two weeks; FVIII — factor VIII; ABR — annualized bleed rate; Q4W — every four weeks

In the study by Callaghan et al. [19], two additional TE episodes not associated with aPCC use were reported; one was device occlusion of a peripherally inserted central catheter (HAVEN 1) and the second was acute myocardial infarction (MI) (HAVEN 3). Both were assessed as unrelated to emicizumab by the investigators, both were resolved, and each individual continued emicizumab. The patient with MI was aged over 65, had previously undiagnosed coronary artery disease, was treated for the event, and recovered [19].

Emicizumab is an immunogenic protein that can stimulate the recipient's immune system to produce the so-called anti-drug antibodies (ADA). As of 2020, in the whole HAVEN trial, 14 out of 398 (3.5%) patients developed ADA, and three (<1%) developed neutralizing anti-drug antibodies to emicizumab [24]. One pediatric hemophilia A patient with

Table II. Laboratory assays to use in presence of emicizumab (acc. to [12, 26], modified)

Assay	Guidance
FVIII activity	Recommended use of chromogenic method with bovine reagents or with human factor IXa and bovine factor X
FVIII inhibitor titer	Recommended to use chromogenic method with bovine factors IXa and X (or with human factor IXa and bovine factor X). Same method should be used for inhibitor titration in blood sample collected prior to inclusion of emicizumab, in order to facilitate interpretation of results in long-term monitoring
ADA	No available commercial test for ADA. If neutralizing ADA suspected, recommended to control emicizumab concentration (*see below*)
	Prolongation to APTT may indicate presence of drug neutralizing antibodies
Emicizumab concentration	Recommended to use a test based on measurement of FVIII activity with one-stage clotting assay with specific emicizumab calibrators to which results are referred

FVIII — factor VIII; ADA — anti-drug antibodies; APTT — activated partial thromboplastin time

inhibitor developed anti-drug antibodies that completely eliminated the pharmacokinetic effect of emicizumab. It was therefore necessary to go back to BPAs [14]. Callaghan et al. [19] have announced a separate paper on long-term immunogenicity of emicizumab, to be published soon.

As of May 2020, 31 fatalities in patients with hemophilia A taking emicizumab had been reported [25]. Median age at death was 58 years; 51% had FVIII inhibitors. The most frequent cause of death was hemorrhage (11/31). No death related to thrombosis or TMA was reported. The authors of this paper concluded that no unique risk of death was associated with emicizumab prophylaxis.

Emicizumab effect on coagulation tests

Emicizumab reduces the activated partial thromboplastin time (APTT) and may therefore be responsible for false APTT-dependent coagulation assay results, FVIII activity included [12, 26]. In severe hemophilia A patients, normalization of the APTT will occur, even at minimum (>5 µg/mL) concentration of emicizumab in plasma. Emicizumab has little effect on prothrombin time (PT) and practically no impact on thrombin time (TT) and fibrinogen concentration in plasma measured with the Clauss method. The effect of emicizumab on PT is minimal, and therefore the results of PT-dependent coagulation tests are considered reliable. Table II sets out selected coagulation assays to use in the presence of emicizumab [12, 26].

Perioperative management of patients with hemophilia receiving emicizumab

Across the HAVEN studies, 215 minor and 18 major surgical procedures were performed [27]. Most of the minor interventions were dental and central venous access device procedures, which went uneventfully without any additional hemostatic therapy in the perioperative period. Nevertheless, in some patients bleeding complications were observed, mostly following dental procedures and in these cases coagulation factor therapy might have been required.

Most major surgeries were successfully managed with prophylactic coagulation factor infusions; in patients without inhibitors, FVIII concentrates were given at usual doses, while in patients with hemophilia A complicated by FVIII inhibitor, rFVIIa was used in all but one patient who underwent laparoscopic appendectomy after a single injection of aPCC at a dose of c.50 U/kg [28, 29]. Suggested management of minor and major surgeries in patients with hemophilia A on emicizumab is set out in Table III.

Real-world experience

Recently, the first results of single-center studies have been published which report physicians' experience with emicizumab in various clinical circumstances [30–32]. Berg et al. [30] evaluated the safety, efficacy, and laboratory monitoring of emicizumab prophylaxis in a cohort of 40 children with severe HA, including 22 non-inhibitor patients and nine babies aged under 12 months. During a median of 45 weeks of follow-up, 20 patients experienced zero bleeds; all breakthrough bleeds were trauma-related. Sixteen surgical interventions were performed in 12 patients, with no thrombotic complications or thrombotic microangiopathy. Prolonged aPTT values normalized after emicizumab initiation, correlating with an increase in emicizumab plasma levels. Emicizumab prophylaxis was safe and well tolerated.

In another observational study, McCary et al. [31] evaluated the efficacy and safety of emicizumab in 93 patients with a median age of 8.6 years, including 49 under 12 years without inhibitors. ABR dropped from 4.4 (inhibitors) and 1.6 (non-inhibitors) to 0.4 (both groups) on emicizumab (p =0.0012 and 0.0025, respectively). There were 28 minor (21 port removals) and two major procedures. Three patients received 1–2 doses of unplanned factor postoperatively to treat minor bleeding events. No patient discontinued therapy, and there were no thrombotic events or deaths.

Table III. Management of minor and major surgical procedures in patients with hemophilia A on emicizumab prophylaxis

Type of surgery	1st-line option	2-nd line option	3-rd line option
Minor surgery in patient without inhibitor	No additional treatment	Tranexamic acid	FVIII concentrates (FVIII plasma activity monitoring) ±tranexamic acid
Major surgery in patient without inhibitor	FVIII concentrates (FVIII plasma activity monitoring) ±tranexamic acid	N/A	N/A
Minor surgery in patient with inhibitor	No additional treatment	Tranexamic acid	FVIII concentrates (FVIII plasma activity monitoring) or rFVIIa ±tranexamic acid* aPCC if FVIII and rFVIIa ineffective**
Major surgery in patient with inhibitor	FVIII concentrates (FVIII plasma activity monitoring) or rFVIIa ±tranexamic acid* aPCC if FVIII and rFVIIa ineffective**	N/A	N/A

*Depending on current inhibitor titer; **activated prothrombin complex concentrate (aPCC) should be given in low doses i.e. <50 U/kg/dose and <100 U/kg/24 h; FVIII — factor VIII; N/A — not applicable; rFVIIa — recombinant factor VIIa

Lewandowska et al. [32] reported on 20 minor and five major surgeries performed in 17 and five patients on emicizumab prophylaxis respectively. Overall, 9/20 minor surgeries were planned to occur with emicizumab as the sole hemostatic agent; of these, four required additional coagulation factor. Three of the 11 minor surgeries with planned additional coagulation factor resulted in non-major bleeds; all were safely managed with additional coagulation factor. All five major surgeries were planned with additional hemostatic agents; there was one bleed, likely triggered by physical/occupational therapy in a patient after elbow surgery. Four patients with hemophilia A complicated by inhibitors underwent three minor and one major surgeries. Three of them received additional therapy with rFVIIa; no thrombotic complications occurred. However, two patients developed minor bleeding complications following dental extraction and port removal respectively. Overall, there were no major bleeds, thrombotic events or deaths.

Emicizumab in Poland

Emicizumab prophylaxis was launched in Poland in 2020 in a group of 30 severe hemophilia A patients with high titer inhibitor against factor VIII. As of April 2021, a total of 42 pediatric and adult patients with hemophilia A complicated by FVIII inhibitor were receiving emicizumab in Poland (not including clinical trials). Eligibility criteria for long-term bleeding prophylaxis with emicizumab are set out in the National Program for 2019–2023. For Polish hemophilia A patients with no inhibitor, Hemlibra® is not as yet available. Results of the interim analysis of the efficacy and safety of emicizumab in adult Polish patients with hemophilia A complicated by FVIII inhibitor will be presented at the Polish Society of Haematology and Transfusion Medicine, (PTHiT, *Polskie Towarzystwo Hematologów i Transfuzjologów*) Congress 2021. It is worth noting that in 2020, the Group for Hemostasis of the Polish Society of Hematology and Transfusion Medicine published guidelines on emicizumab use in hemophilia A patients with inhibitors against factor VIII [12].

Author's contributions

JW — sole author.

Conflict of interest

None.

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None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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Prevention and treatment of venous thromboembolism in patients with hematological neoplasms

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Abstract

Venous thromboembolism (VTE) risk-assessment models are not always useful in predicting VTE risk in patients with hematological neoplasms. Newly updated guidelines recommend primary prevention of VTE in selected patients with cancer using Khorana Risk Score points. The decision to use anticoagulants for primary prophylaxis should be individualized, taking into account the risk of VTE as well as the risk of bleeding. Randomized trials with direct oral anticoagulants (DOACs) have confirmed their safety, good treatment tolerance, and efficacy in both cancer-associated thrombosis (CAT) primary prevention and CAT treatment in cancer patients. In all clinical trials, patients with hematological malignancies have been underrepresented. Individualized use of DOACs for primary thromboprophylaxis should be based on a patient risk/benefit assessment including thrombocytopenia and drug interactions. Although rivaroxaban or apixaban are safe and efficacious for VTE treatment compared to low-molecular-weight heparin, the choice of optimal anticoagulation in patients with hematological malignancies should be individualized and based on the type of malignancy, the bleeding risks, the concomitant medications, and patient preferences. Further research on primary prophylaxis is required, especially in patients with hematological malignancies.

Key words: venous thromboembolism (VTE), cancer-associated thrombosis, hematological neoplasms, DOACs, VTE prophylaxis, VTE treatment

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Introduction

Patients with cancer including hematological neoplasms have a significant risk of developing a venous thromboembolism (VTE) [1, 2]. Overall, patients with cancer have a four-fold to seven-fold higher risk of cancer-associated thrombosis (CAT) than do patients without cancer [1]. Symptomatic VTE occurs in approximately 10–15% of *de novo* diagnosed patients with hematological malignancies [3]. VTE and arterial thrombosis account for 9% of deaths, aggravate the clinical course of the disease, and worsen the survival prognosis; they constitute the leading causes of death [4]. It has been estimated that patients with CAT have a tripled morbidity [5]. CAT also prolongs hospitalization by up to three times [6]. Various factors can have an influence on the risk of VTE in patients with cancer, and these can be categorized into four main groups: patient-related risk factors (e.g. comorbidities or hereditary risk factors); cancer-related risk factors (e.g. site of cancer); cancer treatment-related risk factors (e.g. selected anticancer or supportive therapy such as thalidomide or erythropoiesis-stimulating agents); and biomarkers (e.g. D-dimer levels). See Table I [6, 7]. The risk of VTE development is also higher in the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [8, 9].

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Table I. Risk factors for cancer-associated thrombosis (CAT) in patients undergoing treatment for hematological malignancies

Patient-related risk factor	Treatment-related risk factor
Age	Hospitalization and immobility
Comorbidities	Surgery
Prior VTE	Systemic therapy/anti-angiogenic agents/platinum-based regimen/anthracycline-containing therapy
Hereditary risk factors (e.g. factor V Leiden)	Central venous catheters
Presence of varicose veins	ESA/blood transfusion
	Hormonal therapy
Cancer-related risk factor	**Biomarker**
Site of cancer — lymphoma high risk	Hematologic biomarkers (e.g. platelets, hemoglobin, leukocyte counts)
Primary site — CNS	D-dimers
Histology	P-selectin
Grade	Tissue factor-positive microvesicles
Stage	Elevation in plasminogen activator inhibitor 1
Initial period	Others

VTE — venous thromboembolism; ESA — erythropoiesis-stimulating agents; CNS — central nervous system

Several hematological neoplasms and/or location of neoplasms are considered to be high risks of VTE occurrence [10–13]. It has been documented that the risk of thrombosis in patients with lymphoma is similar to patients with solid tumors. Although lymphomas belong to one of the most heterogeneous group of neoplasms, in patients treated for lymphoma the incidence of VTE is 7–15%. But in patients with central nervous system (CNS) lymphoma, the incidence of VTE can be up to 60% [14]. Moreover, novel therapies such as chimeric antigen receptor (CAR) T-cell can lead to coagulopathy and increase the risk of thrombosis [15, 16]. Patients with cancer are not only at increased risk of VTE, but also have an increased risk of major bleeding [17–19]. That is why any consideration of thromboprophylaxis or treatment for patients with cancer should be based on an assessment of the patient's individual risk for thrombosis and major bleeding, after full exploration of the potential benefits and risks.

Prediction models

Several laboratory biomarkers for VTE prediction have been identified [20, 21], including in patients with hematological malignancies [17, 22, 23]. Based on selected biomarkers, several VTE-risk-assessment models in ambulatory patients with cancer have been proposed [24]. The most common for cancer patients is the Khorana Risk Score (KRS; *see* Table II) [25]. A meta-analysis of 34,555 patients with cancer showed that CAT occurs in 10% of patients with cancer within six months, with high risk defined as a score of more than 2 points [26]. In recent clinical guidelines [27–31], thromboprophylaxis should be considered in selected ambulatory patients with cancer and with a high Khorana score (2 or more points) but with a low risk of major bleeding and without drug–drug interactions. In patients with cancer who are starting chemotherapy, primary prophylaxis with apixaban (2.5 mg twice daily) or rivaroxaban (10 mg once daily) or low-molecular-weight heparin (LMWH; in cases of a high risk of bleeding) is recommended. The KRS has been evaluated in patients with lymphoid malignancies, but did not adequately stratify or predict VTE events in patients at a higher risk of VTE [32]. This finding suggests the need for the development of a disease-specific VTE assessment model. For patients with lymphoma, Antic et al. developed and validated a multivariable model for thromboembolic events in lymphoma patients known as the Thrombosis Lymphoma (ThroLy) Score, *see* Table III [33]. The association of ThroLy with VTE in patients treated for diffuse large B-cell lymphoma (DLBCL) or Hodgkin lymphoma (HL) undergoing ambulatory first-line chemotherapy did not show improved prediction of VTE events because 48% of VTE events occurred in the low-risk ThroLy Score group [34].

Assessment of bleeding risk

Overall, in patients with neoplasms, the rate of bleeding complications is higher than in non-cancer patients, ranging from 7% to 33% [18, 35].

Table II. Khorana Risk Score

Characteristic	Score
Site of cancer Lymphoma — high risk	1
Platelet count ≥350 ×10^9/L	1
Hb <10 g/dL or use of ESA	1
Leukocyte count >11 ×10^9/L	1
BMI ≥35 kg/m^2	1
Risk category	
High-risk group: ≥3 points	
Intermediate-risk group: 1–2 points	

Hb — hemoglobin; ESA — erythropoiesis-stimulating agents; BMI — body mass index

Table III. ThroLy risk score

Patient characteristic	Score
Previous VTE/acute myocardial infarction/stroke	2
Reduced mobility (ECOG 2–4)	1
Obesity (BMI >30 kg/m^2)	2
Extranodal localization	1
Mediastinal involvement	2
Neutrophils <1 ×10^9/L	1
Hb level <10 g/dL	1
ThroLy score points	
0–1 — low risk	
2–3 — intermediate risk	
>3 — high risk	

VTE — venous thromboembolism; ECOG — Eastern Cooperative Oncology Group; BMI — body mass index; Hb — hemoglobin

There are several risks for major bleeding in patients with malignancies: recent major bleeding, abnormal renal function, gastrointestinal manifestation, genitourinary or gynecological localization, thrombocytopenia (<100 ×10^9/L), uncompensated coagulopathy, and metastatic disease in solid tumors [35]. The risk of thrombocytopenia during therapy is relatively high among patients with hematological malignancies [36–38]. Neoplasm localization and concomitant gastrointestinal disease should be evaluated before selecting the appropriate drug. Due to a 36% higher risk of major bleeding on direct oral anticoagulants (DOACs) compared to LMWH, special caution is needed in patients at high risk of bleeding [39–41]. Among cancer patients with an acute diagnosis of VTE and a high risk of bleeding, for patients with luminal gastrointestinal cancers, patients with genitourinary tract cancers, bladder, or nephrostomy tubes, or patients with active gastrointestinal mucosal abnormalities such as duodenal ulcers, gastritis, esophagitis, or colitis, LMWH should be offered. Several anticancer therapies are associated with gastrointestinal toxicities including alkylating agents in high doses (e.g. antimetabolite (e.g. cytarabine, methotrexate), checkpoint inhibitors (e.g. nivolumab) and antimitotic agents (e.g. vinblastin, vincritin) [42].

There are several clinical situations in which anticoagulation may be contraindicated including thrombocytopenia (particularly thrombocytopenia resistant to transfusion), active major bleeding, uncompensated coagulopathy and recent or planned surgery, or invasive procedures (lumbar puncture). If there is active bleeding on therapeutic anticoagulation with contraindication to oral or parental anticoagulation, the placement of an inferior vena cava (IVC) filter may be considered [43].

Drug interactions

In general, LMWH or DOACs can be used as long as there are no contradictions to the selected agent. The choice of anticoagulation should be based both on specific risk factors for patients with malignancy, such as specific risk of bleeding, thrombocytopenia and drug interactions, as well as on factors applying to the general population such as renal/hepatic insufficiency, comorbidities with gastrointestinal disorders or hereditary bleeding diathesis, obesity, *etc.* In general, VKA is not recommended for VTE treatment in cancer patients. LMWH is preferred over VKA because of its superior efficacy and comparable safety based on a meta-analysis [41, 44, 45]. It must be underscored that all hematological malignancies have been underrepresented in clinical trials with DOACs and have constituted 2.5–10.6% of the whole cohort (Table IV) [46].

In patients with renal insufficiency and creatine clearance below 30 mL/min, and where both LMWH is contraindicated and DOACs have not been studied or included in clinical trials, intravenous unfractionated heparin (UFH) may be administered, or small doses of LMWH with monitoring of anti-factor Xa levels. Compared to LMWH, oral anticoagulants, both vitamin K antagonist (VKA) and DOACs, have potential drug interactions with concurrent use of potent P-glipoprotein (minor or none for VKA but major for DOACs) or cytochrome P3A4 inhibitors (major interactions for VKA, apixaban and edoxaban but no metabolic interaction with rivaroxaban).

According to American Society of Clinical Oncology (ASCO) guidelines, both inhibitors or inducers of P-glipoprotein can affect the concentration of all DOACs, while inhibitors or inducers of cytochrome P3A4 may influence rivaroxaban and apixaban to some extent, but without any effects on dabigatran and edoxaban [27]. There is only limited data on specific drug-drug interactions from clinical trials, such as the Hokusai VTE cancer study [47], and therefore the potential benefits and risks between DOACs and

Table IV. Percentage of patients with hematological malignancies included in clinical trials with direct oral anticoagulants (DOACs) for treatment of venous thromboembolism (VTE) in patients with cancer

Study	Hematological malignancies [%]	N	DOAC studied
Hokusai VTE	10.6	1,046	Edoxaban
SELECTED-D	2.5	406	Rivaroxaban
ADAM VTE	9.3	287	Apixaban
CARAVAGGIO	7.4	1,155	Apixaban

cancer therapies should be assessed individually. Doxorubicin, dexamethasone and vinblastine must be mentioned among anti-cancer agents or supportive drugs that may reduce the level of DOACs, including rivaroxaban, apixaban and dabigatran [48]. Combined imatinib with dabigatran, rivaroxaban, and apixaban decreases the level of DOACs [42]. On the other hand, many drugs can increase the level of DOACs and increase the risk of bleeding with nolitinib concomitant use with dabigatran (P-glipoprotein) or rivaroxaban/apixaban by metabolic activity via P-glipoprotein or cytochrome P3A4. Moreover, many anti-mycotic agents, and also cyclosporine, increase plasma factor Xa through P-glycoprotein or CYP3A4 induction and it is suggested to avoid these combinations [42, 48]. Both ibrutinib and venetoclax are P-glycoprotein inhibitors and may increase the level of DOACs.

Recommendations for CAT prevention and treatment

The 2019/2020/2021 updated guidelines from the International Initiative on Thrombosis and Cancer (ITAC), the ASCO, the National Comprehensive Cancer Network (NCCN; version of March 2021), and the American Society of Hematology (ASH) recommend DOACs in the prevention and treatment of CAT [27–31]. Most of the recommendations and suggestions concerning hospitalization, surgery, ambulatory thromboprophylaxis, and CAT treatment are in line with the ASH 2021 guidelines.

VTE prevention in hospitalized patients with cancer

According to the ASH 2021 guidelines, in primary prophylaxis for hospitalized patients with cancer without VTE, the use of thromboprophylaxisis is recommended instead of no thromboprophylaxis. Furthermore, pharmacological thromboprophylaxis is preferred over mechanical thromboprophylaxis. Additionally, in place of a combination of both pharmacological and mechanical thromboprophylaxis, only pharmacological thromboprophylaxis is advised. When using pharmacological thromboprophylaxis for this group of patients, according to the ASH guideline recommendations, LMWH is preferred over unfractionated heparin (UFH), with discontinuation at hospital discharge [28].

Primary prophylaxis for patients with cancer undergoing surgery

The current updated ASH guidelines for patients with cancer without VTE undergoing a surgical procedure with a lower bleeding risk recommend using pharmacological rather than mechanical thromboprophylaxis, except for patients at high bleeding risk, where only mechanical thromboprophylaxis is advised.

A combination of mechanical and pharmacological thromboprophylaxis, rather than mechanical prophylaxis alone, is recommended for patients with cancer without VTE undergoing a surgical procedure and at a high risk of thrombosis, except in patients at high risk of bleeding. Among the available drugs, LMWH or fondaparinux for thromboprophylaxis rather than UFH are recommended in this group of patients. There have been no studies into the use of VKA or DOACs for thromboprophylaxis in patients with cancer undergoing a surgical procedure. Postoperative thromboprophylaxis over preoperative thromboprophylaxis is suggested. In the case of patients with cancer who have undergone a major abdominal/pelvic surgical procedure, pharmacological thromboprophylaxis post-discharge should be continued [28].

Primary prophylaxis in ambulatory patients with cancer receiving systemic therapy

According to the ASH guidelines, no thromboprophylaxis is advised rather than parenteral thromboprophylaxis for ambulatory patients with cancer and a low or intermediate risk of CAT who are receiving systemic therapy. Neither VKA nor DOACs should be offered in the low-risk group, although DOACs (apixaban or rivaroxaban) are advised for the intermediate and high-risk groups. Meanwhile, for ambulatory patients with cancer at a high risk of CAT undergoing systemic therapy, the ASH guidelines recommend parenteral thromboprophylaxis (LMWH) over no thromboprophylaxis.

Patients with multiple myeloma undergoing treatment with thalidomide, lenalidomide or pomalidomide-based regimens can be offered thromboprophylaxis with

low-dose acetylsalicylic acid (ASA) or fixed low-dose VKA or LMWH [28].

Treatment of CAT

Initial treatment (first week) for patients with active cancer and VTE

According to the ASH guidelines, initial treatment of CAT (first week) should comprise DOAC (apixaban or rivaroxaban) or LMWH (over UFH) for patients with active cancer and VTE.

Short-term treatment for patients with active cancer (initial 3–6 months)

According to the ASH guidelines, DOACs (apixaban, edoxaban, or rivaroxaban) over LMWH are suggested for the short-term treatment of VTE (3–6 months) for patients with active cancer. In the current ASH guidelines, incidental (unsuspected) pulmonary embolism (PE) and/or subsegmental PE in patients with cancer should be treated with short-term anticoagulation, rather than just being observed. For patients with cancer and visceral/splanchnic vein thrombosis, there is a choice between treatment with short-term anticoagulation or observation.

Long-term treatment (>6 months) for patients with active cancer and VTE

The ASH guidelines recommend the implementation of long-term anticoagulation for secondary prophylaxis (>6 months) rather than only short-term treatment (3–6 months) in patients with CAT. Meanwhile, the guidelines recommend the continuation of indefinite anticoagulation rather than complete cessation in patients with active cancer and CAT after completion of a definitive period of anticoagulation. Among anticoagulation agents, DOACs are preferred over LMWH in this group of patients, continuing anticoagulation >6 months [28].

Patients with cancer with central venous catheter

The updated ASH guidelines for 2021 do not recommend the administration of parenteral or oral thromboprophylaxis for patients with cancer and a central venous catheter (CVC). They recommend not removing the CVC in patients with cancer presenting CVC-related VTE receiving anticoagulants, and instead leaving the CVC in place [28].

Conclusion

The available VTE risk-assessment models are not useful in predicting VTE risk in patients with hematological neoplasms. Further research on primary prophylaxis is required, especially in patients with hematological malignancies. The ASH guidelines suggest that mortality, pulmonary embolism, deep venous thrombosis, and major bleeding including risks of thrombocytopenia, are major factors when considering thromboprophylaxis and CAT treatment in cancer patients including hematological malignancies.

Author's contributions

JR-M — sole author.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

VIA MEDICA

Risk assessment of recurrent venous thromboembolism

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Abstract

Venous thromboembolism (VTE) recurrence risk is determined by risk factors that were present at the time of the initial VTE episode. The most significant determinant of risk for recurrent VTE is whether the VTE occurred in the setting of provoked or unprovoked condition. As anticoagulation reduces the risk of recurrent VTE, initial anticoagulant treatment at the time of VTE diagnosis is indicated with consideration given to an associated risk of bleeding. After three months of initial anticoagulation, recurrence risk and bleeding risk should be assessed again to decide if anticoagulation should be stopped or continued indefinitely. If indefinite anticoagulation is recommended, annual assessment of both risks should guide decisions about further treatment. Knowledge about the various risk factors for VTE recurrence and the risk factors for bleeding associated with anticoagulation should guide anticoagulant duration.

Key words: venous thromboembolism recurrence, venous thromboembolism risk factors, anticoagulant treatment, anti-vitamin K drugs, direct oral anticoagulant drugs, bleeding risk assessment

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Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), affects 1–2 in 1,000 individuals annually and is the third most common cause of vascular death worldwide [1]. VTE is also a cause of considerable long-term disability connected with post-thrombotic syndrome, chronic exertional dyspnea, and chronic thromboembolic pulmonary hypertension. Anticoagulant therapy is the mainstay of VTE treatment, and can substantially reduce morbidity, mortality and health costs [2]. However, such therapy, especially using vitamin K antagonists (VKA), confers an increased risk of potentially devastating bleeding complications. For this reason, recent guidelines recommend after a VTE episode an obligatory 3-month primary anticoagulant treatment, after which a decision should be made to either stop anticoagulation or continue it as a long-term secondary prevention [3]. This decision is based on the balance between the risk of recurrence if treatment is stopped, and the risk of bleeding when treatment is continued [3]. Recent guidelines recommend indefinite duration of anticoagulation in patients with high recurrence risk and a low risk of bleeding, with consideration given to patient preference [3, 4]. Risks of VTE recurrence and bleeding should be reassessed at least annually [4].

Risk factors for VTE recurrence

Traditionally, according to the circumstances in which a VTE event occurred, the risk of VTE recurrence was dichotomized. Patients with a high risk of recurrence, namely those with unprovoked VTE (c.10% at 1 year, 30% at 5 years), and those with VTE provoked by a major persistent risk factor, such as cancer (15% during first year), required extended (indefinite) anticoagulant therapy. On the other hand, patients with low recurrence rate with VTE provoked by a major transient risk factor (e.g. major surgery or

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Table I. One- and 5-year recurrence rates following discontinuation of anticoagulant therapy according to risk factor category [5]

Risk factor category	VTE recurrence rate	
	1-year [%]	5-year [%]
Major transient	1	3
Major persistent	15	NC
Minor transient	4–6	15
Minor persistent	11	~30
Unprovoked	8–10	30

NC — not calculable (due to high cancer mortality)

trauma) required anticoagulant therapy for three months only [4]. More recently, this simplistic distinction has been challenged. Minor risk factors, persistent and transient, have been identified with risk of recurrence often similar to that of unprovoked VTE (see Table I) [5]. According to the International Society on Thrombosis and Hemostasis (ISTH), risk factors were defined as minor if they were associated with half the risk of recurrent VTE after stopping anticoagulants compared to patients with no risk factors, or were associated with a 3–10-fold increased risk of having a first VTE [6]. Those minor risk factors broadened the group of VTE patients with the recommendation for long-term secondary VTE prevention [7].

A systematic review published just over 10 years ago showed yet another example of these differences between various transient risk factors, demonstrating that while after 24 months patients with a surgical risk factor have a VTE recurrence rate of 0.7%, those with a nonsurgical transient risk factor showed a recurrence rate of 4.3% [8].

Recently, based on the index VTE event, two major guidelines have provided a framework for categorizing VTE risk factors. Although they differ a little in terminology, their categorization is broadly similar (Table II) [3, 9].

Anticoagulant treatment was dominated for decades by heparin and VKA. The advent of direct oral anticoagulants (DOACs) changed this picture dramatically. With their fixed-dose oral administration, and markedly reduced rate of devastating central nervous system bleeding, DOACs are now replacing VKA in initial and extended anticoagulant VTE treatment [3, 9]. Several trials have been performed to assess their use showing that dabigatran [10], apixaban [11], and rivaroxaban [12] are both safe and effective in this setting. To further individualize patients' VTE recurrence risk, several other persistent and transient minor risk factors have been included in these trials. Their conferred risk of recurrence has been described by a hazard ratio (HR) and its 95% confidence interval (CI) compared to patients not carrying this risk. Those additional risk factors not mentioned in Table II are listed in Table III.

Table II. Categorization of venous thromboembolism (VTE) risk factors (modified after [3, 9])

VTE PROVOKED BY TRANSIENT RISK FACTOR (Resolves after it has provoked VTE)
Major transient risk factors (during three months before VTE episode; recurrence risk <3%/year)
Recent major surgery (with general anesthesia for ≥30 min.) or major trauma with fractures
Confinement to bed in hospital (at least three days; with an acute illness)
Cesarean section
Minor transient risk factors (during two months before VTE episode; recurrence risk 3–8%/year)
Surgery (with general anesthesia for <30 min.)
Admission to hospital for <3 days with an acute illness
Estrogen therapy (e.g. oral contraceptives, hormone replacement therapy)
Pregnancy and puerperium
Confined to bed out of hospital for ≥3 days
Leg injury associated with reduced mobility for ≥3 days
Long haul flight
VTE PROVOKED BY PERSISTENT RISK FACTOR (Persists after it has provoked VTE)
Major persistent risk factors (recurrence risk >8%/year)
Active cancer (e.g. ongoing chemotherapy, recurrent or progressive disease)
Antiphospholipid syndrome (triple positive)
Minor persistent risk factors
Inflammatory bowel disease
Autoimmune disorders (systemic)
Chronic immobility (e.g. spinal cord injury)
UNPROVOKED VTE (No identified provoking risk factor)

Hormone-provoked VTE includes a VTE episode related to pregnancy and puerperium, and hormone use (mainly of estrogen-containing oral contraceptives and hormone replacement therapy). Optimal duration of pregnancy-related VTE treatment has yet to be established, but it is recommended to use low molecular heparin during pregnancy and at least six weeks postpartum and then use VKA for a total of three months, as DOACs are contraindicated during lactation [7, 13].

Oral hormone therapy is considered a minor transient risk factor with low risk of VTE recurrence providing that estrogen therapy is stopped. Of note, the risk is highest in the 6–12 months after initiating therapy. Additional risk factors may modulate the recurrence risk in women with hormone-provoked VTE (see infra; HERDOO2 rule).

Table III. Risk for venous thromboembolism (VTE) recurrence: minor persistent and transient risk factors (modified after [7])

Persistent factor	Reported risk HR (95% CI)	Remarks
Renal impairment	5.32 (1.49–18.95)	eGFR <60 mL/min/1.73 m^2
Thrombophilia	1.4–1.9 (1.0–2.2)	Depending on inherited defect
Chronic heart failure	1.43 (1.04–1.97)	
Family history of VTE	1.2–1.92 (1.10–2.58)	Depending on whether both parents, a sibling, or just one parent suffered an episode
Obesity	1.6 (1.0–2.4)	BMI ≥30 kg/m^2
Transient factor		
Oral estrogen therapy	6.4 (1.5–27.3)	Estrogen based replacement therapy
Immobilization	2.9 (1.2–7.5)	Due to chronic medical disease

HR — hazard ratio; CI — confidence interval; eGFR — estimated glomerular filtration rate; BMI — body mass index

Among non-environmental risk factors, the influence of inherited thrombophilia on recurrent VTE is less clear. Most studies show a VTE recurrence rate not different from those without such genetic polymorphism/mutation (for review see [12]). Male sex carries a doubled risk of VTE recurrence compared to women, but only in those with unprovoked VTE [14]. The available evidence suggests that many patients with minor persistent risk factors may benefit from extended anticoagulant therapy, as do patients with major risk factors and those with truly unprovoked VTE.

A few other factors have been implicated as markers of personal risk of VTE recurrence with a potential to guide the decision as to the optimal duration of anticoagulation. They include: D-dimer levels (during anticoagulant treatment, or one month after stopping anticoagulation), and residual vein thrombosis (assessed by ultrasonography) or occlusion [13].

Predicting risk of recurrent thromboembolism and bleeding

Attempts have been made in the past to create more or less complex tools to predict personal risk of VTE recurrence in subjects after their first VTE episode. Risk prediction models may also help physicians to inform patients about risks and benefits of proposed treatment and then take into account patient preferences.

Several risk prediction models have been developed, including: the HERDOO2 rule [15, 16], the Vienna prediction model [17], and the DASH score [18]. Most of these risk models include only patients with unprovoked VTE. They use various combinations of factors: sex, age, body mass index (BMI), D-dimer levels, location and type of VTE event, and hormonal therapy to predict the risk. Only one of these models has been prospectively validated. This study (REVERSE II) used the HERDOO2 rule [hyperpigmentation, edema, and redness (HER) in either leg; D-dimer ≥250 µg/L; BMI ≥30 kg/m^2; age ≥65 years]) to determine if low risk patients with unprovoked VTE could safely stop anticoagulant therapy after 5–7 months of treatment [16]. It was shown that low-risk women (score ≤1) who stopped treatment had a recurrence rate of 3% (CI 1.8–4.8%)/patient-year, while high-risk women and men who discontinued treatment showed a recurrence rate of 8.1% (CI 5.2–11.9%). However, if they both continued treatment, the recurrence rate was only 1.6% (CI 1.1–2.3%) [16]. Recently, the REVERSE study investigators showed that in low-risk women (according to the HERDOO2 rule) with combined oral contraceptive (COC)-associated VTE, the risk of recurrent VTE was clearly lower (0.4% a year, 95% CI: 0.0–2.1%), compared to high-risk women (3.5% a year; 95% CI: 0.4–12.5%) [19].

Anticoagulation with VKA is associated with a 1–2% annual risk of major bleeding but it may vary substantially depending on additional risk factors. The two most used prognostic models for bleeding in VTE are the ACCP model [4] and VTE-BLEED [20, 21]. The first includes the following bleeding predictors: age >65 years, previous bleeding, cancer, metastatic cancer, renal failure, liver failure, thrombocytopenia, previous stroke, diabetes, anemia, antiplatelet therapy, non-steroidal anti-inflammatory drugs, poor anticoagulant control, comorbidity and reduced functional capacity, recent surgery, frequent falls, and alcohol abuse. One point is ascribed to each factor. Low risk of bleeding =0 points (0.8%); intermediate risk =1 point (1.6%); high risk ≥2 points (≥6.5%) [4].

VTE-BLEED bleeding risk factors, assigned points and associated risk of bleeding are shown in Table IV.

However, due to methodological limitations and insufficient predictive accuracy, recent guidelines [3] and a systematic review of available data [22] do not support, and in fact suggest against, routine use of prediction models in patients with venous thromboembolism. However, American Society of Hematology (ASH) guidelines [3] consider the use of scores for recurrence and bleeding in certain individual situations where their use may aid final

Table IV. VTE-BLEED bleeding prognostic model in venous thromboembolism

Predictor	Assigned points
Active cancer	2
Male sex	1
Uncontrolled hypertension (men)	1
Anemia	1.5
History of bleeding	1.5
Renal dysfunction (eGFR 30–60 mL/min/1,73 m^2)	1.5
Age ≥60 years	1

Bleeding risk 0–1 point: 2.8%; ≥2 points: 12.6%
eGFR — estimated glomerular filtration rate

decision-making regarding whether to continue or stop anticoagulation.

Long-term secondary VTE prevention in era of DOAC

The decision to extend anticoagulant treatment beyond three months as a secondary prevention of VTE recurrence depends on the associated benefits versus risks. These risks may change over time. For this reason, patients receiving extended anticoagulation should be reassessed at least annually [4]. Recent guidelines define low risk of recurrence at <3% a year if anticoagulation is stopped after 3-month primary treatment [3, 9]. It has to be remembered that while anticoagulation reduces the risk of recurrent VTE, this benefit does not persist after discontinuation of anticoagulation [23].

The introduction of DOAC completely changed the picture of VTE treatment. They are easy-to-use fixed-dose oral drugs with no requirement for laboratory monitoring. Moreover, DOACs are associated with a significantly (40%) lower risk of major bleeding and an impressively (60%+) lower risk of intracranial bleeding compared to VKA [24].

Successful DOAC trials in the primary treatment of VTE were followed by trials with DOAC use in secondary VTE prevention. Drugs were administered usually for 12 months after completing primary treatment (6–12 months). Dabigatran in a dose of 150 mg bid was used in the RE-MEDY trial and compared to warfarin [10]. Dabigatran was shown to be noninferior to warfarin in reducing the rate of recurrent or fatal VTE with a significantly lower (c.45%) rate of major or clinically relevant non-major bleeding (CRNM) compared to warfarin (5.6% vs. 10.2%, HR 0.54, 95% CI: 0.41–0.71, p <0.001). Interestingly, the EINSTEIN CHOICE and AMPLIFY-EXT trials used not only full anticoagulant doses but also reduced doses of rivaroxaban (20 mg and 10 mg od) and apixaban (5 mg and 2.5 mg bid), respectively [11, 12]. It was shown that both doses were effective and comparable in reducing the risk of recurrent VTE and all-cause mortality (by about 65%, to 3.8–4.2%; apixaban) as compared to placebo and of recurrent VTE as compared to aspirin (by about 70%, to 1.2–1.5% a year; rivaroxaban). While apixaban did not significantly increase the rate of major and CRNM bleeding as compared to placebo, rivaroxaban in both doses showed similar rates of major bleeding as aspirin (c.0.3–0.5%).

Pooled analysis of rivaroxaban used for secondary VTE prevention in patients with minor persistent risk factors showed that, even if numerically different, overall rate of recurrence in patients with minor persistent risk factors was statistically similar to that observed with unprovoked VTE (HR 0.68, 95% CI: 0.32–1.30%) [6]. This opens up an intriguing possibility of safely prolonging anticoagulation with a low-dose DOAC regimen in a large group of patients with various minor persistent or transient VTE risk factors deemed at higher risk of VTE recurrence. At this point, it is suggested that before switching to a low-dose regimen, patients should complete six months of full-dose primary treatment with rivaroxaban or apixaban, as for now all secondary prevention clinical trials start after six months of primary anticoagulation [6]. Probably, patients who should remain on high-dose regimens include those at high risk of recurrence (cancer, triple positive antiphospholipid syndrome), or those who had a recurrence on a reduced-dose regimen.

Patient preference would also be important as in those with a life-threatening pulmonary embolism or family history of fatal PE. On the other hand, a low-dose regimen could be preferred by patients participating in contact sports or those with a history of bleeding [25].

The benefit-risk profile of extending anticoagulation in the era of low-dose DOACs is clearly different to what it was in the VKA era. More trials are needed to determine the optimal duration of secondary VTE prevention in patients with initial VTE provoked by minor transient or persistent risk factors (*see also* Figure 1). Studies are also needed to identify the highest risk minor transient or persistent risk factors.

Author's contributions

JM — sole author.

Conflict of interest

No conflict of interest declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments

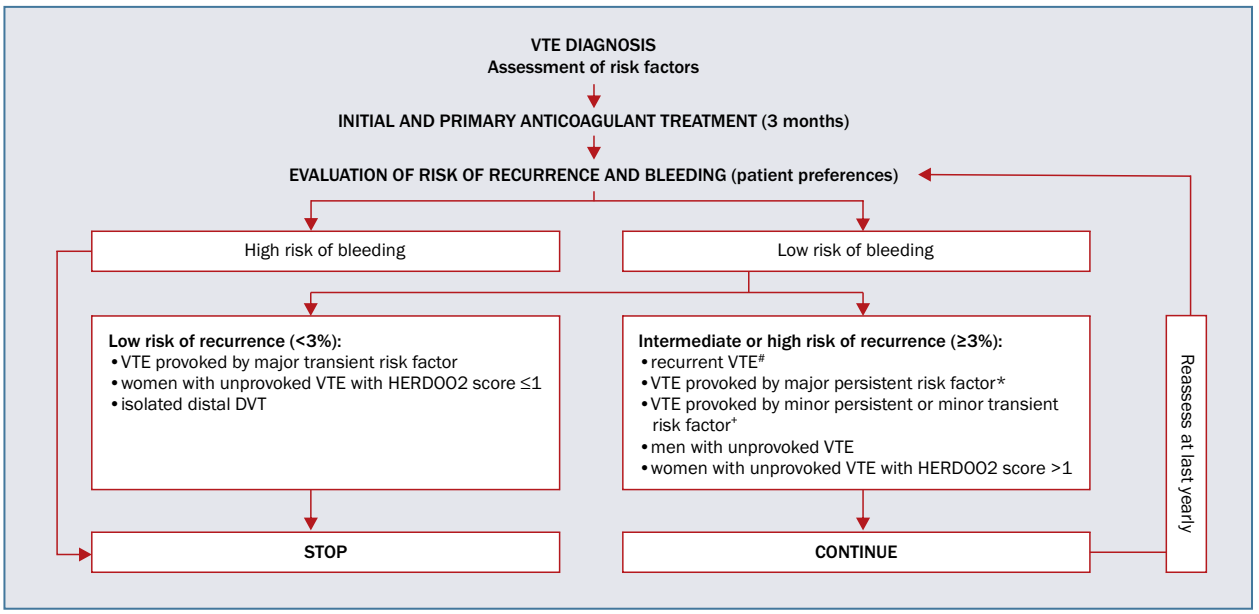

Figure 1. Approach to duration of anticoagulation in patients with venous thromboembolism (VTE) (modified after [7]); #previous VTE episode not related to a major transient or reversible risk factor; *see Table II; +see Table II and III; HERDOO2 — hyperpigmentation, edema, and redness in either leg; D-dimer ≥250 µg/L; body mass index ≥30 kg/m^2; age ≥65 years; DVT — deep vein thrombosis

involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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REVIEW ARTICLE

Viscoelastic methods in clinical laboratory hematology: a narrative review

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Abstract

Viscoelastic methods (VEMs) such as thromboelastography (TEG) and thromboelastometry (TEM) offer a comprehensive assessment of hemostasis, starting with early stages of coagulation, through fibrin clot formation, and ending with fibrinolysis.

They offer several advantages compared to the standard coagulation tests i.e. they allow a detailed assessment in real time of the coagulation process with the contribution of platelets and fibrinogen levels in the whole blood. TEG or TEM point-of-care devices are widely used in managing intraoperative bleeding, especially in the context of cardiac surgery. The latest study results indicate a growing interest in VEMs in various fields of hematology. TEG and TEM have been used in congenital bleeding disorders such as hemophilia and von Willebrand disease. Both assays offer more objective and complete laboratory evaluation of an individual patient's phenotype, effective personalized prophylaxis, the management of bypassing agent therapy, and the management of spontaneous bleeding episodes or surgery.

We have therefore carried out a narrative review to summarize evidence on the usefulness of VEMs in the assessment of blood coagulation and fibrinolysis in these bleeding disorders.

Key words: viscoelastic methods (VEMs), thromboelastography (TEG), thromboelastometry (TEM), hemophilia, von Willebrand disease

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Introduction

Viscoelastic methods (VEMs) have been widely developed in recent decades, although the first mention of thromboelastography was made much earlier, in 1948 [1]. Since then, many investigations have been made to optimize and improve this technique, which has become a useful tool for extensive, bedside evaluation of whole blood clotting in real time. VEMs are based on the physicochemical and rheological properties of blood, in that it becomes less viscous and more elastic as it begins to clot [2].

Principles of viscoelastic methods

There are two major and equally important viscoelastic assays: thromboelastography (TEG) and thromboelastometry [TEM and rotational thromboelastometry (ROTEM)]. The principle of the methods is the same for both TEG and ROTEM. A blood sample is transferred to the cup together with appropriate reagents and a pin is placed in the cup. The major difference between these viscoelastic devices concerns the element that rotates. TEG instruments use a fixed pin and oscillating cup (4°45' every 5 s), while ROTEM uses a rotating pin (4°75' every

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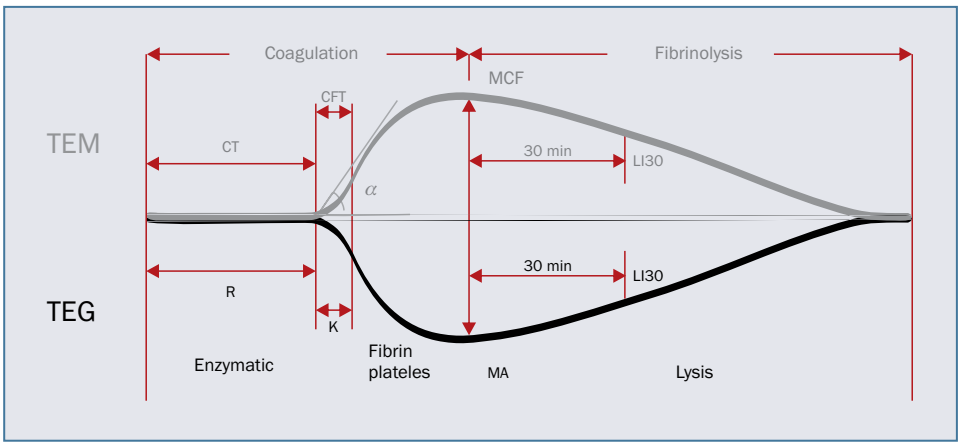

Figure 1. Comparative analysis of thromboelastography (TEG) and thromboelastometry [TEM and rotational thromboelastometry (ROTEM)] tracing and accompanying parameters; CT (clotting time) — period from start of analysis until start of clot formation (until a 2 mm amplitude occurs); CFT (clot formation time) — period until an amplitude of 20 mm is reached (represents clot formation dynamics); MCF (maximum clot firmness) — maximum amplitude of TEMogram; R-time (reaction time) — time to initial fibrin formation; K-time (kinetics time) — time to clot formation; a (alpha angle) — rate of clot formation; MA (maximum amplitude) — absolute clot strength; LI30 (LY30) — fibrinolytic activity 30 minutes after maximum amplitude

6 s) and a stationary cup [3]. There are several activators of blood coagulation that are used in each device to get a detailed evaluation of clot formation, its strength as well as the rate and efficiency of fibrinolysis, such as phospholipid and ellagic acid, tissue factor (TF), TF with cytochalasin D (platelet-independent measurement), and TF with aprotinin or tranexamic acid. Both instruments offer a series of variables as test results, such as clotting time (CT), maximal clot firmness (MCF), clot formation time (CFT), the alpha angle (α) or maximum lysis (ML), although the most characteristic feature of both methods is the graphical presentation of hemostatic processes (Figure 1) [4]. Although TEG and ROTEM traces look similar, the variables are not directly interchangeable and should be interpreted with caution.

TEG and TEM have several advantages compared to standard coagulation tests. They allow for a real-time assessment of the coagulation process with the contribution of platelets number and fibrinogen levels. The absence of one piece of a 'hemostatic jigsaw' is almost immediately visible on the chart. For this reason, both assays enable differential diagnosis of coagulopathy due to thrombocytopenia and coagulopathy due to a- or hypofibrinogenemia. Furthermore, TEG and TEM tests, if carried out for long enough, provide detailed information on the fibrinolytic activity that falls outside the scope of routine tests [4]. Due to its many advantages, viscoelastic methods are used to diagnose coagulopathies, monitor hemostatic treatment, and guide transfusions, primarily in the field of cardiac surgery [5–7].

Role of TEG and TEM in laboratory diagnostics of congenital bleeding disorders

Hemophilia A

Hemophilia A is the most common X-linked recessive congenital bleeding disorder. An absence or severe deficiency of coagulation factor VIII, which is critical for sufficient activation of the coagulation cascade, manifests as spontaneous hemorrhage and abnormal bleeding. Routine coagulation test i.e. activated partial thromboplastin time (aPTT) and factor VIII activity assay are useful in making the diagnosis and severity grading of hemophilia patients. However, standard laboratory tests have only limited use in the monitoring of routine FVIII prophylaxis, as well as the clinical response to different forms of therapy, especially bypassing agents such as recombinant activated factor VII (rFVIIa) and activated prothrombin complex concentrate (aPCC). At this point, viscoelastic methods have proved to be of great utility. Tran et al. have shown a great potential of ROTEM to assess the effectiveness of bypassing agents in hemophilia patients with inhibitors [8]. These results are consistent with the research by Chen et al. who also found that TEG is more sensitive to FVIII inhibitors than other global hemostatic tests [9]. Pivalizza and Escobar [10] used TEG and ROTEM devices to monitor hemostasis during emergency surgery. Both devices have guided large doses of rFVIIa, which were used pre- and intraoperatively. Furukawa et al. [11] also demonstrated that ROTEM tests are helpful in the estimation of appropriate doses of bypassing agents. A comprehensive review by Ramiz et al.

demonstrated that viscoelastic methods could be useful in the management of patients with inhibitors and perioperative management [12]. However, the authors underscore the need for better reproducibility and sensitivity, which are currently operator-dependent.

Furthermore, TEG and ROTEM have been used to monitor the effect of novel therapeutics such as a bispecific antibody to factor IXa (during phase I of clinical studies), or emicizumab that requires more than just standard coagulation tests, to measure response efficacy [12–14]. Yada et al. used ROTEM to monitor patients with hemophilia A taking emicizumab for three consecutive years. The data suggested that ROTEM facilitates assessment of global coagulation in these patients [15].

Von Willebrand disease

Von Willebrand disease (vWD) is a hereditary hemorrhagic disorder which can lead to severe complications due to prolonged mucosal bleeding, bleeding from the gums, epistaxis, easy bruising and heavy menstrual bleeding. Laboratory diagnostics of vWD disease is difficult and time-consuming. Regling et al. [16] have shown that TEG parameters are sensitive in detecting patients with VWF:RCo below 30 IU/dL. Contrary to other laboratory tests, e.g. VWF:RCo, TEG results are performed within an hour and significantly improve the diagnostic process. Moreover, the recent study by Topf et al. [17] showed that new modifications of the standard ROTEM test make it possible to diagnose critical vWD cases. Furthermore, the authors recommend including a ROTEM assay in the current management algorithm of acute microvascular bleeding, especially in severe von Willebrand disease patients [17].

TEG and TEM in laboratory hematology — application perspectives

There are many fields of application of VEMs, especially relating to their use in cardiac surgery or in sepsis [5–8, 18, 19]. These methods could be of great importance in the clinical management of patients with bleeding disorders. In addition, during coronavirus disease 2019 (COVID-19), many studies have demonstrated the indiscriminate utility of TEG and TEM in assessing hemostatic imbalance in the course of this new disease [20–22], showing that both these laboratory techniques indicate hypercoagulability and fibrinolysis shutdown in severe forms of COVID-19, despite the use of thromboprophylaxis [20–22]. Further research should be done on the use of VEMs in both laboratory hematology and COVID-19.

Author's contributions

JB and EKD performed literature search and wrote the manuscript. AS designed Figure 1. AS and EŻ developed the concept of the manuscript, supervised, and critically read the manuscript. All the authors contributed to the review of the manuscript, and all authors read and approved the final manuscript.

Conflict of interest

None.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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ORIGINAL RESEARCH ARTICLE

Therapeutic monitoring of direct oral anticoagulants — an 8-year observational study

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Abstract

Introduction: For years, anticoagulants have been the basic group of drugs that slow down, inhibit or prevent blood clotting by inhibiting thrombin formation or reducing its activity. Treatment with direct oral anticoagulants (DOACs) does not require routine anticoagulation monitoring due to their wide therapeutic index. However, there are circumstances where it may be necessary to know the drug levels to manage the risk of side effects or to confirm laboratory efficacy. In such a situation, an indication for DOAC laboratory testing could be the suspicion of excessively high or low DOAC levels. The aim of this article was to determine trends in concentrations of DOACs in a real life population of patients.

Material and methods: Consultations in clinical pharmacology extended with DOAC level assessments were performed in the Department of Cardiology and Clinical Pharmacology. The trial was designed as a retrospective analysis of laboratory analyses and included 480 laboratory tests performed in 236 patients.

Results: Mean CHA_2DS_2-VASc scoring 3.91 ±1.92 and HAS-BLED scoring 3.57 ±1.75 indicated high risks of both thrombosis and bleeding. Geometric mean of trough concentrations for dabigatran, rivaroxaban and apixaban were 91.53 ng/mL, 62.74 ng/mL and 124.81 ng/mL, whereas peak concentrations for all DOACs were significantly higher, at 220.80 ng/mL (p <0.0001), 116.59 ng/mL (p <0.0001) and 186.18 ng/mL (p =0.0354), respectively. Values for males and females did not differ significantly. Dose adjustment, performed according to rules described for every drug in registered drug characteristics, did not change significantly concentrations. Trough concentrations higher than 20 ng/mL were found at the 10th percentile for all DOACs, but higher at 40 ng/mL at the 5th percentile was found only for apixaban. Peak concentration lower than 400 ng/mL were for the 95th percentile for apixaban and for the 90th percentile for dabigatran and rivaroxaban.

Conclusion: Monitoring-based pharmacotherapy with DOACs should be restricted only to specific clinical settings; in the general population, it is not necessary. Recently, in many experienced centers, therapeutic drug monitoring of DOACs has gained great importance in selected clinical settings and it very likely will soon become commonplace in clinical practice.

Key words: therapeutic drug monitoring, TDM, dabigatran, rivaroxaban, apixaban, direct oral anticoagulant, DOAC

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Introduction

For years, anticoagulants have been the basic group of drugs that slow down, inhibit or prevent blood clotting by inhibiting thrombin formation or reducing its activity directly or indirectly, or by inhibiting the post-translational modification of clotting factors II, VII, IX, X and proteins C and S. The general indications for anticoagulant therapy are atrial fibrillation and flutter, acute coronary syndromes, venous thromboembolism, condition after prosthetic valve replacement, stenosis of the mitral valve, pulmonary hypertension, intracardiac thrombosis, percutaneous coronary interventions, and extracorporeal circulation [1]. For more than 60 years, pharmacological intervention was limited to only one option: treatment with the vitamin K antagonists (VKA) warfarin or acenocumarol.

In 2009, a new class of drugs was introduced into pharmacotherapy, which for the first time provided a viable alternative to VKA in the indications for the prevention of embolic complications in non-valvular atrial fibrillation and for the treatment of patients with deep vein thrombosis and pulmonary embolism. These new generation oral anticoagulants were originally referred to as novel oral anticoagulants (NOAC). As the group is no longer new, other abbreviations are now more appropriate. The best abbreviation is either direct oral anticoagulant (DOAC) or target specific oral anticoagulant (TSOAC) [1].

DOACs were approved for the treatment and prevention of thromboembolic events such as prevention of deep vein thrombosis, especially in its most dangerous form — pulmonary embolism. Next crucial indication is most important for atrial fibrillation adult patients. DOACs are now key drug used in stroke prevention as well as peripheral embolism. Rivaroxaban is the first DOAC approved for the prevention of atherosclerotic events in patients with a history of acute coronary syndrome, peripheral artery disease or stroke [2].

Nowadays, due to the favorable efficacy and safety profile of DOACs, the group has become the first line option for all patients without contraindications [1, 3].

DOACs fall into two main groups: Xa inhibitors (rivaroxaban, apixaban and edoxaban); and direct thrombin inhibitors (dabigatran) [1, 4]. These drugs are clinically similar, but their pharmacokinetics significantly differ.

Factor IIa (active thrombin) inhibition is possible with the reversible and competitive inhibitor, dabigatran. This kind of potent blockade prevents conversion from fibrinogen to fibrin. Dabigatran inhibits not only free thrombin but also fibrin-associated thrombin and fibrin-induced platelet activation [1].

The family of xabans include rivaroxaban, apixaban, edoxaban and betrixaban. These drugs act as direct inhibitors of factor Xa. They bind free and clot-bound forms and decrease thrombin concentration. In this condition, xabans block the enzyme involved in the production of thrombin. Generally, concentrations of dabigatran and xabans are higher in older people than in younger people [1, 5].

Dabigatran etexilate is effectively and completely converted to dabigatran. Its bioavailability is 6.5%, the T_{max} in healthy volunteers is 0.5–2 h, but in postoperative patients can be elongated especially on the day of surgery by up to 6 h. Bioavailability of dabigatran is not affected by food, but time necessary to obtain the maximal level can be extended by up to 2 hours. Plasma protein binding is 34–35%, half elimination time ($T_{0.5}$) is dose independent, in healthy volunteers it is 12–14 h. Volume of distribution (Vd) is 50–70 L. Dabigatran is excreted 85% in the urine, mainly unchanged, and 6% in the faeces. In patients with decreased creatinine clearance 50–30 mL/min, the effect of dabigatran is approximately 2.7 times greater than in patients without renal failure. For creatinine clearance <30 mL/min, the effect of dabigatran on the body is approximately six times greater, and $T_{0.5}$ twice as long as in patients with normal renal function, thus treatment with dabigatran is not recommended in that population. There are many drug/drug interactions, especially with verapamil, anti-cancer agents *etc.* [1, 6, 7].

Bioavailability of rivaroxaban depends on dose, for 10 mg is 80–100%; food has no effect on bioavailability or C_{max}. The drug at a dose of 15 mg and 20 mg has to be taken with a meal, as taking on an empty stomach significantly reduces its bioavailability. T_{max} is 2–4 h, Vd 50 L, $T_{0.5}$ 5–9 h and in older people 11–13 h. Plasma protein binding is 92–95%. Approximately 66% of the administered dose is metabolized and excreted equally by the kidneys and the liver. The remaining 33% is excreted unchanged by the kidneys, mainly by active renal secretion. The drug is metabolized by CYP3A4 and CYP2J2, and metabolism is independent of cytochrome P-450 [1, 6, 7].

Similarly to rivaroxaban, apixaban is rapidly absorbed and taking a 10 mg dose with a meal has no effect on AUC or C_{max}. Bioavailability for doses up to 10 mg is about 50%. T_{max} is 3–4 h, V_d 21 L. Apixaban binds to plasma proteins in 87%. 25% of the administered dose is excreted as metabolites, mainly in the faeces. The drug is also eliminated by the kidneys. $T_{0.5}$ is 12 h. Apixaban is metabolized mainly by CYP3A4/5 [1, 6, 7].

Edoxaban is rapidly absorbed, protein binding 55%, V_d 107 L, T_{max} 1–2 h, $T_{0.5}$ 10–14 h. Metabolism is minimal, elimination mainly by the kidneys. Betrixaban protein binding is 60%, T_{max} 3–4 h, $T_{0.5}$ 19–27 h, Elimination is mainly in the faeces (85%) and urine (11%). V_d 120 L [1, 6, 7].

Clinical trials have confirmed the efficacy and safety in typical populations of patients with atrial fibrillation, deep vein thrombosis, pulmonary embolism [7–10]. For all DOACs vs. VKA the significant reduction in the risk of major bleeding by 32% to 69% was revealed in randomized clinical trials [11]. The most important seems to be 50% reduction in the risk of intracranial bleeding in comparison to warfarin [12–14].

Pharmacotherapy monitoring

According to the current guidelines routine laboratory checks of coagulation parameters are not recommended because of very wide therapeutic index of DOACs [1]. However, there are clinical setting when therapeutic drug monitoring may help to manage the risk of side effects, especially bleeding, or confirm laboratory efficacy. Results of pre-registration trials suggests that one of key elements is body weight. Obesity or body weight over 110 kg may be risk factor of presence of low DOACs concentration, whereas body weight lower than 50 kg may lead to overdosage. DOACs laboratory testing may be useful tool to solve underdosage/overdosage problem especially in recurrent thrombotic episodes in adherent patients, or in pharmacokinetic disturbances such as malabsorption, elimination failure, as well as elderlies [15–17].

The presence of DOACs in plasma may be confirmed by routine clotting tests i.e. prothrombin time (PT) and activated partial thromboplastin time (APTT), but changes are not dependent on the type of drug and its concentration. Thrombin time (TT) and APTT are elongated in the presence of dabigatran, whereas PT elongation can be seen during treatment with xaban family agents. Coagulation reagents sensitivity vary for different DOACs e.g. lower sensitivity of PT chemicals is seen for apixaban in comparison to the rivaroxaban or edoxaban [18–20].

Laboratory monitoring of DOAC treatment

Laboratory monitoring can form the basis of classical laboratory tests or tests specific to DOACs. In the family of classical tests, there are TT, APTT and PT. TT is sensitive for dabigatran. Unfortunately, it is a kind of binary relationship, because at low concentrations of dabigatran, TT becomes maximal for method and therefore is not suitable for monitoring. APTT is recommended by registered drug characteristic for dabigartan as an indicatior of action. There are no linear relation between dabigatran concentration and APTT, moreover APTT elongation depends on reagents set type of and type of coagulometer, thus APTT cannot be used in therapy monitoring. APTT and PT, measured after high-dose xaban treatment, are prolonged, but the effect is variable. Moreover, high level of shaping of PT results depends on thromboplastin type used in a laboratory, especially for rivaroxaban. This is why these parameters are not recommended for monitoring of xaban treatment. To perform quantitative analysis for dabigatran, diluted thrombin time (dTT) or ecarin clotting time (ECT) should be used [21, 22].

According to the mechanism of xabans action measurement of anti-Xa activity is the best test to monitor its effectivity, but research to standardize and validate the assay are in progress [23].

The decision-making process based on laboratory testing is difficult because we have no detailed data and ranges for specific populations. In pre-registration pharmacokinetic trials, DOAC levels were distributed in a wide range of concentrations. For values below 20 ng/mL there is high probability of underdosage and risk of ineffective treatment, whereas results above 400 ng/mL suggest an increased risk of bleeding [23]. Personalized medicine in DOACs may improve decision taking in special populations where assessment of thrombosis or bleeding risk is crucial. Whereas for almost all patients therapeutic drug monitoring is not recommended, in some cases the changes in DOACs pharmacokinetics may make it necessary [15, 16, 24].

The main aim of this analysis was to determine trends in concentrations of DOACs in a real life population of patients.

Material and methods

Patients

Consultations in clinical pharmacology extended with DOAC level assessments were performed in the Department of Cardiology and Clinical Pharmacology between 2012 and 2021. The trial was designed as a retrospective analysis of laboratory analyses and included 480 laboratory tests performed in 236 patients. At that time, it was the only such center in the region that performed consultations in clinical pharmacology extended by therapeutic drug levels monitoring [15, 16, 24]. The study population took DOACs in the form of oral tablets or capsules (dabigatran).

Procedures

DOACs routine determination of the concentration in the serum was performed in all patients. To do this, we used Hemoclot assay and BIOPHEN DiXal assay (Hyphen Biomed, Neuville-sur-Oise, France), Direct Thrombin Inhibitor Assay by HemosIL cooperating with ACL TOP Family Analyzers (ACL TOP 700, ACL TOP 500 CTS, and ACL TOP 300 CTS), Liquid Anti-Xa's HemosIL cooperating with the ACL TOP Family Systems in conjunction with HemosIL, rivaroxaban and apixaban Calibrators. The range of detection values for dabigatran concentrations provided by the manufacturer is 20–2,000 ng/mL. The method's analytical sensitivity (lower limit of detection for the assay) is determined to be 2 ng/mL. A dabigatran concentration of 45 ng/mL is referred to as the 'low control' and 196 ng/mL as the 'high control'. The range of detection for rivaroxaban concentrations stated by the manufacturer is 20–1,000 ng/mL, and for apixaban 15–1,000 ng/mL. The analytical sensitivity of the method (lower limit of detection for the assay) is determined at 10 ng/mL for rivaroxaban and 6 ng/mL for apixaban. A rivaroxaban concentration of 79 ng/mL is defined as the low control and 299 ng/mL as the high control, whereas for apixaban it is 80 ng/mL and 322 ng/mL respectively.

Because in 2012 there were no therapeutic ranges, concentration ranges were divided into several. For trough values less than 30 ng/mL, treatment was considered ineffective. Therapeutic range was from 40 ng/mL to 400 ng/mL, but for values from 200 ng/mL to 400 ng/mL, additional analyses of risk factors of bleeding were performed. Values more than 400 ng were considered too high, thus clinical analysis of factors increasing the concentration was performed.

Statistics

Statistical software Statistica 12.0 in a Polish version (StatSoft, Tulsa, OK, USA) was used to calculate statistical parameters. The Shapiro-Wilk test assay revealed that the distribution of continuous variables did not comprise the criteria of normal distribution. According to that, quantitative variables were shown as medians and percentile ranges. To compare medians of independent variables, the Mann-Whitney test was performed. Values $p < 0.05$ were treated as statistically significant. Values $p \geq 0.05$ and < 0.10 were treated as a trend towards statistical significance. Values $p \geq 0.10$, being not significant, were replaced with the abbreviation ns (not significant).

Results

480 samples taken from 236 patients were analyzed. The clinical characteristics of the study population including DOAC drug therapy are set out in Table I. The average age of the study population was 68, weight 84 kg. The most common indication for treatment with DOAC was atrial fibrillation, in 98.96%. In five cases, the main indication was pulmonary embolism, for others comorbidity included atrial fibrillation and other diseases such as stroke, deep vein thrombosis, malabsorption syndrome or possible drug/drug interactions. Mean CHA_2DS_2-VASc scoring and HAS-BLED scoring indicated high risks of both thrombosis and bleeding. Creatinine clearance was calculated by the Cockcroft-Gault formula as 66.50 ±23.16. A plot of the DOAC concentration distribution is set out in Figure 1. Concentrations of DOACs are set out in Table II. Geometric mean of trough concentrations for dabigatran, rivaroxaban and apixaban were 91.53 ng/mL, 62.74 ng/mL and 124.81 ng/mL, whereas peak concentrations for all DOACs were significantly higher at 220.80 ng/mL ($p < 0.0001$), 116.59 ng/mL ($p < 0.0001$) and 186.18 ng/mL ($p = 0.0354$), respectively. Values for males and females did not differ significantly. Dose adjustment, performed according to rules described for every drug in registered drug characteristics, did not change significantly concentrations. Trough concentrations higher than 20 ng/mL were found at the 10th percentile for all DOACs, but higher at 40 ng/mL at the 5th percentile were found only for apixaban. Peak concentration lower than 400 ng/mL were for the 95th percentile for apixaban and for the 90th percentile for dabigatran and rivaroxaban.

Table I. Clinical characteristics of study group

Study feature	Property value
Number of consultations (patients)	236
Number of tests	480
Age (years)	68.44 (43–98)
Body weight [kg]	84.0 (48.0–118.0)
Sex	
Men	245 (51.0%)
Women	235 (49.0%)
Main indication for treatment/consultation	
Atrial fibrillation	475 (98.96%)
Stroke	88 (18.33%)
Drug/drug interactions	24 (5.00%)
Pulmonary embolism/deep vein thrombosis	10 (2.08%)
Malabsorption	3 (0.63%)
CrCl [mL/min]	66.50 ±23.16*
CHA_2DS_2-VASc	3.91 ±1.92
HAS-BLED	3.57 ±1.75

*Creatinine clearance (CrCl) calculated by Cockcroft-Gault formula

Discussion

According to the current guidelines regarding therapy with DOACs, therapeutic drug monitoring can be considered in expert centers to manage risk of bleeding. This is why in specific clinical setting assessment of drug levels can be necessary. The most important conditions are serious bleeding especially in patients with drug-drug or drug-disease interactions, in obese patients or with very low body weight, in patients on DOACs with deplessed kidney function, before emergent surgery to confirm necessarity of reversal agent usage [15, 16, 23, 24]. The role of therapeutic drug monitoring (TDM) increased recently in experienced centers and probably next years become more common [1]. Lin et al. [25] described a similar population of Asian patients treated with dabigatran. Similar effects of monitoring were found. Pharmacokinetic analysis of the RE-LY study (a pre-registration clinical trial) by Reilly et al. [5] described a huge population of 9,183 patients. They concluded that there is a correlation between side effects, especially bleeding and high concentrations of dabigatran, thus they found monitoring in a specific population to be effective. A real life population of 44 patients receiving dabigatran was analyzed by Šinigoj et al. [26]. Patients with bleeding had significantly higher average trough dabigatran concentrations than patients without bleeding, while peak

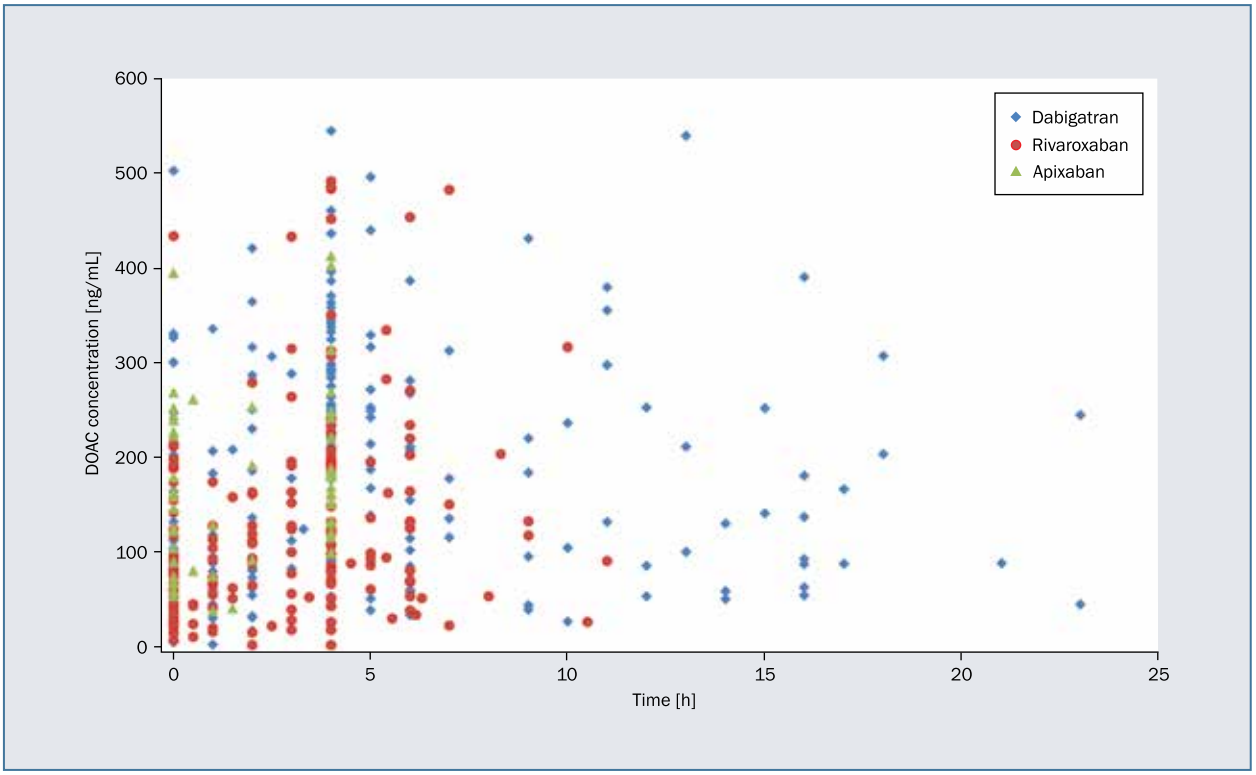

Figure 1. Concentrations of dabigatran, rivaroxaban and apixaban; DOAC — direct oral anticoagulant

dabigatran values had no predictive value. Dabigatran dose selection according to the guidelines resulted in appropriate trough concentrations with acceptable repeatability, whereas high trough concentrations predisposed to the risk of minor bleeding. These results supported the thesis that the most important factor is dosage that aligns with drug characteristics [26]. Monitoring of apixaban and rivaroxaban has been performed in many studies [27–30]. Generally, these have confirmed that DOACs are a safe group of anticoagulants, and that dose adjustment according to the drug characteristics will lead to effective and safe treatment, but it is extremely important to check all possible factors interfering with the drug pharmacokinetics during the first qualification to treatment visit. As DOACs are the first line option in many clinical settings due to safety and high efficacy, drug monitoring should be considered to enable the use of a better therapeutic option in a larger group of patients, including borderline patients.

Conclusion

Monitoring-based pharmacotherapy with DOACs should be restricted only to specific clinical settings. In the general population, it is not necessary. Recently, in many experienced centers therapeutic drug monitoring of DOACs has gained great importance in selected clinical settings, and it very likely will soon become more common in everyday clinical practice.

Authors' contributions

GG — sole author.

Conflict of interest

The author received honoraria from Bayer, Boehringer Ingelheim and Pfizer for lectures.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

Table II. Plasma concentrations of direct oral anticoagulants: dabigatran, rivaroxaban and apixaban

		C_{trough}	C_{peak}	F	M	Reg. dose	Adj. dose
DABIGATRAN [ng/mL]							
N	200	57	65	100	100	57	143
gMean	137.82	91.53	220.80**	146.21	129.90$^{\#}$	127.69	142.07$^{\#\#}$
gCV [%]	62.04	73.77	37.20	55.25	68.30	64.65	60.81
Median	166.81	95.09	249.00	175.50	159.20	135.25	173.00
P5	32.25	25.20	84.05	43.79	29.84	42.96	31.14
P10	51.93	39.00	99.00	52.26	51.88	51.71	52.54
P90	349.57	268.20	379.44	363.70	337.40	346.48	348.40
P95	396.64	337.20	438.49	396.64	387.99	380.39	429.02
Min	2.49	2.49	38.53	4.61	2.49	2.49	4.61
Max	544.00	502.00	544.00	538.80	544.00	544.00	538.08
RIVAROXABAN [ng/mL]							
N	170	64	57	77	93	125	45
gMean	87.80	62.74	116.60**	98.49	79.83$^{\#}$	89.21	83.98$^{\#\#}$
gCV [%]	69.15	57.29	69.96	62.30	73.73	64.68	70.69
Median	92.85	69.51	125.70	98.00	85.85	90.51	93.31
P5	17.85	15.19	24.34	20.37	16.73	17.85	20.27
P10	25.76	21.45	41.41	27.58	26.01	26.07	26.38
P90	264.44	184.03	328.41	292.11	231.78	237.59	274.97
P95	342.59	196.86	457.34	339.24	340.15	416.38	312.60
Min	2.01	7.10	2.01	7.10	2.01	2.01	7.10
Max	490.90	433.00	490.90	482.70	490.90	490.90	432.40
APIXABAN [ng/mL]							
N	56	30	22	31	25	42	14
gMean	145.28	124.81	186.18*	155.29	133.76$^{\#}$	151.05	129.26$^{\#\#}$
gCV [%]	38.60	40.82	24.70	32.01	45.04	34.86	39.40
Median	160.80	126.85	173.85	162.00	160.00	161.80	132.95
P5	53.50	53.50	116.20	71.05	43.34	53.76	62.15
P10	67.45	58.18	121.02	74.60	53.50	68.75	68.96
P90	264.60	252.81	308.58	253.10	265.32	268.74	243.28
P95	333.25	264.96	397.36	353.50	268.68	389.95	245.37
Min	38.30	38.30	99.10	58.70	38.30	38.30	53.50
Max	412.00	394.00	412.00	412.00	401.80	412.00	247.90

*p =0.0354, **p <0.0001 vs. C_{trough}; $^{\#}p$ ns vs. F group; $^{\#\#}p$ ns vs. reg. dose group; C_{trough} = concentration at steady state taken just before test dose, trough concentration; C_{peak} = concentration at steady state, after test dose, for time predicting maximal concentration, peek concentration; F — females; M — males; reg. dose = regular dose, dabigatran 150 mg bid, rivaroxaban 20 mg qd, apixaban 5 mg bid; adj. dose = adjusted dose, dabigatran 110 mg bid, rivaroxaban 15 mg qd, apixaban 2.5 mg bid; gCV = geometric coefficient of variation; gMean = geometric mean; P5 = 5th percentile; P10 = 10th percentile; P90 = 90th percentile; P95 = 95th percentile

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1. NAZWA PRODUKTU LECZNICZEGO: Yescarta, 0,4-2 $\times$ 10^8 komórek, dyspersja do infuzji. **2. SKŁAD JAKOŚCIOWY I ILOŚCIOWY: 2.1 Opis ogólny:** Yescarta (aksykabtagen cyloleucel) to genetycznie zmodyfikowane autologiczne limfocyty T skierowane przeciw CD19 stosowane w immunoterapii. Aby przygotować produkt Yescarta, od pacjenta pobiera się limfocyty T, które są następnie genetycznie modyfikowane w warunkach *ex vivo* metodą transdukcji retrowirusowej w celu uzyskania ekspresji chimerowego receptora antygenowego (ang. *chimeric antigen receptor*, CAR) zawierającego mysi jednołańcuchowy fragment Fv anty-CD19 połączony z domeną kostymulującą CD-28 i domeną sygnalizacyjną CD3-zeta. Żywotne limfocyty CAR-T anty-CD19 są namnażane i z powrotem wprowadzane za pomocą infuzji do organizmu pacjenta, gdzie mogą rozpoznawać i eliminować komórki docelowe prezentujące CD19. **2.2 Skład jakościowy i ilościowy:** Każdy worek do jednorazowej infuzji ze swoistym dla danego pacjenta produktem Yescarta zawiera około 68 ml dyspersji limfocytów CAR-T anty-CD19, co umożliwia otrzymanie docelowej dawki wynoszącej 2×10^6 żywotnych limfocytów CAR-T anty-CD19/kg masy ciała (zakres od 1×10^6 do 2×10^6 limfocytów/kg) z maksymalną liczbą limfocytów CAR-T anty-CD19 wynoszącą 2×10^8. Substancje pomocnicze o znanym działaniu: Każdy worek z produktem Yescarta zawiera 300 mg sodu. Pełny wykaz substancji pomocniczych, patrz punkt 6.1 ChPL. **3. POSTAĆ FARMACEUTYCZNA:** Dyspersja do infuzji. Klarowna do opalizującej dyspersja w kolorze od białego do czerwonego. **4. SZCZEGÓŁOWE DANE KLINICZNE: 4.1 Wskazania do stosowania:** Produkt Yescarta jest wskazany w leczeniu nawrotowego lub opornego na leczenie chłoniaka rozlanego z dużych komórek B (ang. *diffuse large B-cell lymphoma*, DLBCL) i pierwotnego chłoniaka śródpiersia z dużych komórek B (ang. *primary mediastinal large B-cell lymphoma*, PMBCL) u dorosłych pacjentów, którzy uprzednio otrzymali co najmniej dwie linie leczenia systemowego. **4.2 Dawkowanie i sposób podawania:** Produkt Yescarta musi być podawany w kwalifikowanym ośrodku leczniczym przez lekarza mającego doświadczenie w leczeniu złośliwych nowotworów krwi oraz przeszkolonego w zakresie podawania i leczenia pacjentów produktem Yescarta. Na wypadek wystąpienia zespołu uwalniania cytokin (ang. *cytokine release syndrome*, CRS) przed infuzją musi być dostępna przynajmniej 1 dawka tocilizumabu i sprzęt ratunkowy. Ośrodek, w którym odbywa się leczenie musi mieć zapewniony dostęp do dodatkowej dawki tocilizumabu w ciągu 8 godzin od uprzedniego podania każdej dawki. Dawkowanie: Yescarta to produkt przeznaczony wyłącznie do stosowania autologicznego (patrz punkt 4.4). Pojedyncza dawka produktu Yescarta zawiera 2×10^6 żywotnych limfocytów CAR-T na kilogram masy ciała (lub maksymalnie 2×10^8 żywotnych limfocytów CAR-T dla pacjentów o masie ciała równej 100 kg i większej) w około 68 ml dyspersji w worku do infuzji. Należy potwierdzić dostępność produktu Yescarta przed rozpoczęciem limfodeplecji. *Leczenie wstępne (chemioterapia limfodeplecyjna):* W 5., 4. i 3. dniu przed infuzją produktu Yescarta należy zastosować chemioterapię limfodeplecyjną, składającą się z cyklofosfamidu w dawce 500 mg/m² podawanego dożylnie i fludarabiny w dawce 30 mg/m² podawanej dożylnie. *Premedykacja:* Zaleca się podanie paracetamolu w dawce 500-1000 mg doustnie i difenhydraminy w dawce 12,5 do 25 mg dożylnie lub doustnie (lub równoważnej) na około 1 godzinę przed infuzją produktu Yescarta. Nie zaleca się podawania ogólnoustrojowych kortykosteroidów w ramach profilaktyki, ponieważ mogą mieć wpływ na działanie produktu Yescarta. *Monitorowanie:* Po infuzji pacjentów należy monitorować codziennie przez pierwsze 10 dni po infuzji pod kątem objawów przedmiotowych i podmiotowych potencjalnego CRS, zdarzeń neurologicznych i innych toksyczności. Lekarze powinni rozważyć hospitalizację pacjenta przez pierwsze 10 dni po infuzji lub w momencie pojawienia się pierwszych objawów przedmiotowych lub podmiotowych CRS i (lub) zdarzeń neurologicznych. Po pierwszych 10 dniach od infuzji o monitorowaniu pacjenta zdecyduje lekarz. Pacjentów należy poinformować o konieczności pozostania w pobliżu kwalifikowanej placówki klinicznej przez co najmniej 4 tygodnie po infuzji. Specjalne grupy pacjentów: *Pacjenci z zakażeniem ludzkim wirusem niedoboru odporności (ang. human immunodeficiency virus, HIV), wirusowym zapaleniem wątroby typu B (ang. hepatitis B virus, HBV) oraz wirusowym zapaleniem wątroby typu C (ang. hepatitis C virus, HCV):* Brak doświadczenia klinicznego ze stosowaniem u pacjentów z aktywnym zakażeniem HIV, HBV lub HCV. *Dzieci i młodzież:* Nie określono jeszcze bezpieczeństwa stosowania ani skuteczności produktu leczniczego Yescarta u dzieci i młodzieży w wieku poniżej 18 lat. Dane nie są dostępne. *Osoby w podeszłym wieku:* Nie jest wymagane dostosowywanie dawki u pacjentów w wieku 65 lat i starszych. Skuteczność leczenia była zgodna ze skutecznością leczenia w całej leczonej populacji pacjentów. Sposób podawania: Produkt Yescarta jest podawany drogą infuzji dożylnej. Produktu Yescarta nie wolno naświetlać. NIE używać filtra do deplecji leukocytów. *Środki ostrożności, które należy podjąć przed użyciem lub podaniem produktu leczniczego:* Ten produkt leczniczy zawiera genetycznie zmodyfikowane ludzkie komórki krwi. Fachowy personel medyczny przygotowujący produkt Yescarta powinien stosować odpowiednie środki ostrożności (nosić rękawice i okulary), aby uniknąć potencjalnego przeniesienia chorób zakaźnych. *Przygotowanie do infuzji:* Zweryfikować zgodność tożsamości pacjenta (ang. *identity,* ID) z oznaczeniami na kasecie z produktem Yescarta. Nie wolno wyjmować worka z produktem Yescarta z metalowej kasety, gdy brak zgodności informacji na etykiecie dotyczącej danego pacjenta z danymi pacjenta, dla którego przeznaczony jest ten produkt. Po potwierdzeniu ID pacjenta wyjąć worek z produktem Yescarta z metalowej kasety. Sprawdzić, czy dane pacjenta na etykiecie metalowej kasety są zgodne z danymi na etykiecie worka. Przed rozmrożeniem sprawdzić, czy worek z produktem nie ma jakichkolwiek uszkodzeń. W razie uszkodzenia worka postępować zgodnie z lokalnymi wytycznymi dotyczącymi postępowania z odpadami materiałów pochodzenia ludzkiego (lub niezwłocznie przekazać informację do firmy Kite). Umieścić worek do infuzji w drugim worku. Rozmrozić produkt Yescarta w temperaturze wynoszącej około 37°C w kąpieli wodnej lub metodą suchego rozmrażania do momentu, gdy w worku infuzyjnym nie będzie widocznego lodu. Delikatnie wymieszać zawartość worka, aby rozprowadzić grudki materiału komórkowego. Jeśli nadal widoczne są grudki materiału komórkowego, kontynuować delikatne mieszanie zawartości worka. Powinno to doprowadzić do rozproszenia małych grudek materiału komórkowego. Produktu Yescarta nie należy myć, wirować ani powtórnie odtwarzać zawiesiny na nowych nośnikach przed infuzją. Rozmrażanie powinno trwać około 3 do 5 minut. Po rozmrożeniu produkt Yescarta zachowuje trwałość w temperaturze pokojowej (od 20°C do 25°C) do 3 godzin. Infuzję produktu Yescarta należy jednak rozpocząć w ciągu 30 minut od rozmrożenia. *Podawanie:* Tylko do stosowania autologicznego. Tocilizumab oraz sprzęt ratunkowy powinny być dostępne przed infuzją i podczas monitorowania. Nie wolno używać filtra do deplecji leukocytów. Zaleca się założenie centralnego dostępu żylnego w celu podania produktu Yescarta. Ponownie zweryfikować ID pacjenta w celu potwierdzenia zgodności z oznaczeniami pacjenta na worku z produktem Yescarta. Przed infuzją wypełnić zestaw do infuzji roztworem chlorku sodu (0,154 mmol sodu/ml). Cała zawartość worka z produktem Yescarta powinna zostać podana w infuzji w ciągu 30 minut metodą grawitacyjną lub z użyciem objętościowej pompy infuzyjnej. Delikatnie masować worek podczas infuzji produktu Yescarta, aby zapobiec tworzeniu się grudek materiału komórkowego. Po podaniu w infuzji całej zawartości worka, przepłukać zestaw do infuzji roztworem chlorku sodu (0,154 mmol sodu/ml) z tą samą prędkością infuzji, aby upewnić się, że produkt Yescarta został podany w całości. Instrukcja dotycząca postępowania, przypadkowego narażenia oraz usuwania tego produktu, patrz punkt 6.6 ChPL. **4.3 Przeciwwskazania:** Nadwrażliwość na substancję czynną lub którąkolwiek substancję pomocniczą wymienioną w punkcie 6.1 ChPL. Należy uwzględnić przeciwwskazania dotyczące chemioterapii limfodeplecyjnej. **4.4 Specjalne ostrzeżenia i środki ostrożności dotyczące stosowania:** Identyfikowalność: Należy bezwzględnie przestrzegać wymogów identyfikowalności produktów leczniczych stosowanych w zaawansowanych terapiach komórkowych. Aby zapewnić identyfikowalność nazwę produktu leczniczego, numer serii oraz imię i nazwisko leczonego pacjenta należy przechowywać przez okres 30 lat po upłynięciu terminu ważności produktu. Ogólne: Produkt Yescarta jest przeznaczony wyłącznie do stosowania autologicznego i nie wolno podawać go innym pacjentom. Przed infuzją: tożsamość pacjenta musi być zgodna z danymi identyfikacyjnymi na worku infuzyjnym i kasecie z produktem Yescarta. Nie należy podawać infuzji produktu Yescarta, gdy informacja na etykiecie dotyczącej danego pacjenta nie jest zgodna z danymi pacjenta, dla którego przeznaczony jest produkt. Pacjentów należy monitorować codziennie przez pierwsze 10 dni po infuzji w celu wykrycia przedmiotowych i podmiotowych objawów potencjalnego CRS, zdarzeń neurologicznych i innych toksyczności. Lekarze powinni rozważyć hospitalizację pacjenta przez pierwsze 10 dni po infuzji lub w momencie wystąpienia pierwszych objawów przedmiotowych/podmiotowych CRS i (lub) zdarzeń neurologicznych. Po pierwszych 10 dniach po infuzji o monitorowaniu pacjenta zdecyduje lekarz. Należy poinformować pacjentów o konieczności pozostawania w pobliżu kwalifikowanego ośrodka leczniczego przez co najmniej 4 tygodnie po infuzji oraz o konieczności uzyskania natychmiastowej pomocy medycznej w razie wystąpienia przedmiotowych lub podmiotowych objawów CRS lub neurologicznych działań niepożądanych. W zależności od stopnia nasilenia danego działania niepożądanego należy rozważyć monitorowanie parametrów życiowych i czynności narządów. Powody odroczenia leczenia: Ze względu na zagrożenia związane z leczeniem produktem Yescarta podanie infuzji należy wstrzymać, jeśli u pacjenta występuje którykolwiek z następujących stanów: utrzymujące się ciężkie działania niepożądane (w szczególności działania dotyczące płuc, serca lub niedociśnienie), w tym związane z wcześniejszymi chemioterapiami; czynne, niekontrolowane zakażenie; czynna choroba przeszczep przeciwko gospodarzowi (ang. *graft-versus-host disease*, GVHD). Badania serologiczne: Przed pobraniem komórek do wytworzenia produktu Yescarta, należy wykonać badania przesiewowe na obecność HBV, HCV i HIV (patrz punkt 4.2). Oddawanie krwi, narządów, tkanek i komórek: Pacjenci leczeni produktem Yescarta nie powinni być dawcami krwi, narządów, tkanek ani komórek do przeszczepienia. Choroba współistniejąca: Pacjenci z czynnym zaburzeniem OUN lub z zaburzeniami czynności nerek, wątroby, płuc lub serca mogą być bardziej podatni na skutki działań niepożądanych opisanych poniżej i wymagają szczególnej uwagi. Pierwotny chłoniak ośrodkowego układu nerwowego (OUN): Brak doświadczenia związanego ze stosowaniem produktu Yescarta u pacjentów z pierwotnym chłoniakiem OUN. W związku z tym nie określono stosunku ryzyka do korzyści leczenia produktem Yescarta dla tej populacji. Zespół uwalniania cytokin: U prawie wszystkich pacjentów wystąpił CRS któregoś stopnia. W przypadku stosowania produktu Yescarta bardzo często stwierdzano ciężkie przypadki CRS, w tym reakcje zagrażające życiu i prowadzące do zgonu, a czas do ich wystąpienia wynosił od 1 do 12 dni (patrz punkt 4.8). O leczeniu CRS decyduje lekarz na podstawie występujących u pacjenta objawów klinicznych oraz zgodnie z algorytmem postępowania w CRS przedstawionym w tabeli 1. W leczeniu CRS o nasileniu umiarkowanym lub ciężkim, związanego z podaniem produktu Yescarta, stosowano terapię opartą na inhibitorze receptora interleukiny 6 (IL-6), takim jak tocilizumab. Ośrodek, w którym odbywa się infuzja produktu Yescarta, musi przystępując do niej dysponować co najmniej 1 dostępną do podania dawką tocilizumabu dla każdego pacjenta. Ośrodek, w którym odbywa się leczenie, musi mieć dostęp do dodatkowej dawki tocilizumabu w ciągu 8 godzin od podania każdej z uprzednich dawek. Pacjentów należy codziennie monitorować w kwalifikowanej placówce klinicznej przez co najmniej 10 dni po infuzji pod kątem objawów przedmiotowych i podmiotowych CRS. Po upływie pierwszych 10 dni od infuzji o monitorowaniu pacjentów zdecyduje lekarz. Należy poinformować pacjentów o konieczności pozostawania w pobliżu kwalifikowanej placówki klinicznej przez co najmniej 4 tygodnie po infuzji oraz o konieczności uzyskania natychmiastowej pomocy medycznej w razie wystąpienia objawów przedmiotowych lub podmiotowych CRS. Opracowano algorytmy postępowania mające na celu łagodzenie niektórych objawów CRS występujących u pacjentów przyjmujących produkt Yescarta. Obejmują one stosowanie tocilizumabu lub tocilizumabu z kortykosteroidami w przypadkach CRS o nasileniu umiarkowanym, ciężkim lub zagrażającym życiu, jak przedstawiono w tabeli 1. Pacjentów, u których wystąpi CRS w stopniu 2. lub wyższym (np. niedociśnienie, brak odpowiedzi po podaniu płynów lub hipoksja wymagająca podania dodatkowego tlenu), należy monitorować z wykorzystaniem ciągłej telemetrii kardiologicznej oraz pulsoksymetrii. U pacjentów z ciężkim CRS należy rozważyć wykonanie echokardiogramu w celu oceny czynności serca. W przypadku ciężkiego lub zagrażającego życiu CRS należy rozważyć wdrożenie intensywnej terapii w ramach leczenia wspomagającego. Produktu Yescarta nie należy podawać pacjentom, u których występuje czynne zakażenie lub choroba zapalna, dopóki stany te nie ustąpią. Wiadomo, że CRS jest związany z niewydolnością narządów docelowych (np. wątroby, nerek, serca i płuc). Ponadto w przebiegu CRS może dojść do nasilenia występujących zaburzeń tych narządów. U pacjentów z istotnymi klinicznie zaburzeniami kardiologicznymi należy stosować standardy opieki w stanach krytycznych oraz rozważyć takie środki, jak echokardiografia. W celu rozpoznania CRS należy wykluczyć inne przyczyny ogólnoustrojowej odpowiedzi zapalnej, w tym zakażenie. W razie wystąpienia gorączki neutropenicznej należy dokonać oceny pod kątem zakażenia i zastosować antybiotyki o szerokim spektrum działania, płyny oraz leczenie podtrzymujące zgodnie z istniejącymi wskazaniami medycznymi. W przypadku pacjentów z ciężkim lub nieodpowiadającym na leczenie CRS należy rozważyć możliwość wystąpienia limfohistiocytozy hemofagocytarnej/zespołu aktywacji makrofagów (ang. *haemophagocytic lymphohistiocytosis/macrophage activation syndrome*, HLH/MAS). Produkt Yescarta nadal rozprzestrzenia się i utrzymuje w organizmie po podaniu tocilizumabu oraz kortykosteroidów. Nie zaleca się stosowania antagonistów czynnika martwicy nowotworu (ang. *tumour necrosis factor*, TNF) w leczeniu CRS związanego z zastosowaniem produktu Yescarta. **Tabela 1: Algorytm postępowania w zespole uwalniania cytokin (CRS).** Nasilenie CRS[a]: Stopień 1.: Objawy wymagają zastosowania tylko leczenia objawowego (np. gorączka, nudności, zmęczenie, ból głowy, ból mięśni, złe samopoczucie). *Tocilizumab:* W razie braku poprawy po 24 godzinach postępować jak w stopniu 2. *Kortykosteroidy:* N/A. Stopień 2.: Objawy wymagają umiarkowanej interwencji, po której ustępują. Zapotrzebowanie na tlen wynoszące mniej niż 40% FiO_2 lub hipotensja odpowiadająca na podanie płynów lub małej dawki jednego leku wazopresyjnego, lub toksyczność narządowa 2. stopnia[b]. *Tocilizumab:* Podać tocilizumab[c] w dawce 8 mg/kg dożylnie przez 1 godzinę (nie przekraczać dawki 800 mg). W razie potrzeby podawać tocilizumab co 8 godzin, gdy brak odpowiedzi na dożylne podanie płynów lub rośnie zapotrzebowanie na tlenoterapię. Nie podawać więcej niż 3 dawki w ciągu 24 godzin; maksymalna całkowita liczba dawek to 4 dawki, jeśli brak poprawy klinicznej dotyczącej objawów przedmiotowych i podmiotowych CRS lub jeśli brak odpowiedzi na drugą lub kolejną dawkę tocilizumabu, należy rozważyć zastosowanie alternatywnych sposobów leczenia CRS. *Kortykosteroidy:* Leczenie jak w stopniu 3., gdy brak poprawy w ciągu 24 godzin od podania tocilizumabu. Stopień 3.: Objawy wymagają poważnej interwencji, po której ustępują. Zapotrzebowanie na tlen przekraczające lub równe 40% FiO_2 lub hipotensja wymagająca podania dużej dawki leku wazopresyjnego albo zastosowania wielu leków wazopresyjnych, lub toksyczność narządowa 3. stopnia, albo hipertransaminazemia 4. stopnia. *Tocilizumab:* Leczenie jak w stopniu 2. *Kortykosteroidy:* Podać metyloprednizolon dożylnie w dawce 1 mg/kg dwa razy na dobę lub równoważną dawkę deksametazonu (np. 10 mg dożylnie co 6 godzin). Kontynuować podawanie kortykosteroidów do momentu, gdy zdarzenie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a następnie stopniowo zmniejszać dawkę. Gdy brak poprawy, zastosować leczenie jak w stopniu 4. (poniżej). Stopień 4.: Objawy zagrażające życiu. Pacjent wymaga zastosowania respiratora lub ciągłej hemodializy żylno-żylnej, lub toksyczność narządowa 4. stopnia (z wyjątkiem hipertransaminazemii). *Tocilizumab:* Leczenie jak w stopniu 2. *Kortykosteroidy:* Podawać metyloprednizolon dożylnie w dawce dobowej 1000 mg przez 3 dni, gdy jest poprawa – stosować leczenie jak powyżej. Rozważyć podanie innych leków immunosupresyjnych, gdy brak poprawy lub pogorszenie stanu. N/A = brak/nie dotyczy. *(a) Lee et al 2014. (b) Leczenie neurologicznych działań niepożądanych – patrz Tabela 2. (c) Szczegółowe informacje można znaleźć w charakterystyce produktu leczniczego tocilizumabu.* Neurologiczne działania niepożądane: U pacjentów leczonych produktem Yescarta bardzo często obserwowano ciężkie neurologiczne działania niepożądane, które mogą zagrażać życiu lub prowadzić do zgonu (patrz punkt 4.8). Pacjenci z zaburzeniami OUN w wywiadzie, takimi jak drgawki lub niedokrwienie naczyniowo-mózgowe, mogą być narażeni na większe ryzyko. Zgłaszano przypadki zakończonego zgonem i ciężkiego obrzęku mózgu u pacjentów leczonych produktem Yescarta. Pacjentów należy monitorować pod kątem objawów przedmiotowych i podmiotowych neurologicznych działań niepożądanych (Tabela 2). Po infuzji pacjentów należy monitorować w kwalifikowanej placówce opieki zdrowotnej co najmniej codziennie przez pierwsze 10 dni pod kątem objawów przedmiotowych i podmiotowych toksyczności neurologicznej. Po tych pierwszych 10 dniach o monitorowaniu pacjenta zdecyduje lekarz. Pacjentów należy poinformować o konieczności pozostania w pobliżu kwalifikowanej placówki klinicznej przynajmniej przez 4 tygodnie po infuzji i natychmiastowego zwrócenia się po pomoc medyczną, jeśli wystąpią objawy przedmiotowe lub podmiotowe toksyczności neurologicznej. W zależności od stopnia nasilenia danego działania niepożądanego należy rozważyć monitorowanie parametrów życiowych i wydolności narządów. Pacjentów, u których wystąpi toksyczność neurologiczna stopnia 2. lub wyższego, należy monitorować z wykorzystaniem ciągłej telemetrii kardiologicznej oraz pulsoksymetrii. W razie ciężkiej lub zagrażającej życiu toksyczności neurologicznej należy wdrożyć intensywną terapię wspomagającą. Należy rozważyć podanie leków przeciwdrgawkowych niemających działania uspokajającego w ramach profilaktyki przeciwdrgawkowej zgodnie ze wskazaniami klinicznymi dla działań niepożądanych 2. lub wyższego stopnia. Opracowano algorytmy postępowania mające na celu złagodzenie neurologicznych działań niepożądanych występujących u pacjentów przyjmujących produkt Yescarta. Obejmują one stosowanie tocilizumabu (w razie współistniejącego CRS) i (lub) kortykosteroidów w przypadku umiarkowanych, ciężkich lub zagrażających życiu neurologicznych działań niepożądanych, jak przedstawiono w tabeli 2. **Tabela 2: Algorytmy postępowania dotyczące objawów neurologicznych działań niepożądanych.** Stopień 2.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Gdy brak poprawy w ciągu 24 godzin po podaniu tocilizumabu: podać deksametazon dożylnie w dawce 10 mg co 6 godzin, jeśli pacjent nie przyjmuje już innych kortykosteroidów. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. *Bez współistniejącego CRS:* Podawać deksametazon dożylnie w dawce 10 mg co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. Stopień 3.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Dodatkowo z pierwszą dawką tocilizumabu podać deksametazon dożylnie w dawce 10 mg, powtarzając co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. *Bez współistniejącego CRS:* Podawać deksametazon dożylnie w dawce 10 mg co 6 godzin. Kontynuować podawanie deksametazonu, dopóki zdarzenie nie zostanie zakwalifikowane jako zdarzenie stopnia 1. lub mniejszego, a wówczas stopniowo zmniejszać dawkę. Stopień 4.: Rozważyć podanie leków przeciwdrgawkowych bez działania uspokajającego (tj. lewetyracetam) w ramach profilaktyki przeciwdrgawkowej. *Współistniejący CRS:* Podać tocilizumab jak w tabeli 1 dla CRS stopnia 2. Wraz z pierwszą dawką tocilizumabu podać metyloprednizolon dożylnie w dobowej dawce 1000 mg oraz kontynuować podawanie metyloprednizolonu dożylnie w dobowej dawce 1000 mg przez 2 kolejne dni; w przypadku poprawy stosować leczenie jak powyżej. Gdy brak poprawy, rozważyć podanie metyloprednizolonu 1000 mg dożylnie 3 razy na dobę lub alternatywne leczenie[a]. *Bez współistniejącego CRS:* Podawać metyloprednizolon dożylnie w dawce dobowej 1000 mg przez 3 dni, gdy poprawa – stosować leczenie jak powyżej. Gdy brak poprawy, rozważyć podanie metyloprednizolonu 1000 mg dożylnie 3 razy na dobę lub alternatywne leczenie[a]. *(a) Alternatywne leczenie obejmuje (między innymi): anakinrę, siltuksymab, ruksolitynib, cyklofosfamid, immunoglobuliny podawane dożylnie* (*ang. intravenous immunoglobulin,* IVIG) *oraz globulinę anty-tymocytarną* (*ang. anti-thymocyte globulin,* ATG). Zakażenia i gorączka neutropeniczna: Podczas stosowania produktu Yescarta bardzo często obserwowano ciężkie zakażenia (patrz punkt 4.8). Pacjentów należy monitorować pod kątem objawów przedmiotowych i podmiotowych zakażenia przed, w trakcie i po podaniu produktu Yescarta w infuzji oraz zastosować odpowiednie leczenie. Należy zastosować profilaktykę przeciwbakteryjną zgodnie z obowiązującymi standardowymi wytycznymi. Po infuzji produktu Yescarta u pacjentów stwierdzano gorączkę neutropeniczną (patrz punkt 4.8) i może ona występować jednocześnie z CRS. W przypadku wystąpienia gorączki neutropenicznej należy ocenić, czy nie jest to zakażenie, i zastosować antybiotyki o szerokim spektrum działania, płyny oraz leczenie objawowe zgodnie ze wskazaniami medycznymi. Reaktywacja HBV: U pacjentów leczonych lekami skierowanymi przeciwko limfocytom B może dojść do reaktywacji HBV, w niektórych przypadkach prowadzącej do piorunującego zapalenia wątroby, niewydolności wątroby i zgonu. Przed pobraniem komórek do wytworzenia produktu Yescarta należy przeprowadzić badanie przesiewowe w kierunku HBV, HCV i HIV. Przedłużająca się cytopenia: Po chemioterapii limfodeplecyjnej i infuzji produktu Yescarta u pacjentów przez kilka tygodni może występować cytopenia. Odnotowano bardzo częste występowanie przedłużającej się cytopenii stopnia 3. lub wyższego, w tym małopłytkowość, neutropenię i niedokrwistość, po infuzji produktu Yescarta. Należy monitorować wyniki badań morfologii krwi po infuzji produktu Yescarta. Hipogammaglobulinemia: U pacjentów leczonych produktem Yescarta może wystąpić aplazja limfocytów B, prowadząca do hipogammaglobulinemii. Hipogammaglobulinemię obserwowano bardzo często u pacjentów leczonych produktem Yescarta. Należy monitorować stężenie immunoglobulin po leczeniu produktem Yescarta oraz stosować leczenie obejmujące profilaktykę zakażeń, profilaktykę antybiotykową oraz immunoglobulinową terapię zastępczą. Reakcje nadwrażliwości: Po infuzji produktu Yescarta mogą wystąpić reakcje alergiczne. Ciężkie reakcje nadwrażliwości, w tym reakcja anafilaktyczna, mogą być spowodowane obecnością dimetylosulfotlenku (ang. *dimethylsulfoxide*, DMSO) lub resztkowej gentamycyny w produkcie Yescarta. Wtórne nowotwory złośliwe: U pacjentów leczonych produktem Yescarta mogą rozwinąć się wtórne nowotwory złośliwe. Pacjentów należy monitorować przez całe życie pod kątem wtórnych nowotworów złośliwych. W razie pojawienia się wtórnego nowotworu złośliwego należy skontaktować się z firmą w celu uzyskania instrukcji dotyczących pobrania od pacjenta próbek do badań. Zespół rozpadu guza (ang. *tumour lysis syndrome*, TLS): Rzadko obserwowano występowanie TLS, który może mieć ciężką postać. W celu zminimalizowania ryzyka wystąpienia TLS pacjenci ze zwiększonym stężeniem kwasu moczowego lub dużą łączną masą guza powinni otrzymywać allopurynol lub inne leczenie profilaktyczne przed infuzją produktu Yescarta. Pacjentów należy monitorować pod kątem przedmiotowych i podmiotowych objawów TLS, a objawy należy leczyć zgodnie ze standardowymi wytycznymi. Wcześniejsze leczenie terapią anty-CD19: Doświadczenie związane ze stosowaniem produktu Yescarta u pacjentów, którzy wcześniej otrzymali leczenie celowane przeciwko CD19, jest ograniczone. Nie zaleca się stosowania produktu Yescarta, jeśli u pacjenta nastąpił nawrót choroby bez ekspresji CD19 po uprzedniej terapii anty-CD19. Substancje pomocnicze: Ten produkt leczniczy zawiera 300 mg sodu na worek infuzyjny, co odpowiada 15% maksymalnego dziennego dopuszczalnego spożycia sodu, wynoszącego 2 g dla osoby dorosłej, zgodnie z zaleceniami WHO. Oczekuje się, że pacjenci zostaną wpisani do rejestru pacjentów, na podstawie którego prowadzona będzie obserwacja w celu lepszego poznania długoterminowego bezpieczeństwa stosowania i skuteczności produktu Yescarta. **4.8 Działania niepożądane:** Podsumowanie profilu bezpieczeństwa: Dane dotyczące bezpieczeństwa opisane w tym punkcie odzwierciedlają skutki narażenia na produkt Yescarta w badaniu fazy I/II ZUMA-1, w którym 108 pacjentów z nawrotowym/opornym chłoniakiem nieziarniczym (NHL) z limfocytów B otrzymywało limfocyty CAR-T w zalecanej dawce w zależności od masy ciała. Mediana okresu obserwacji wyniosła 27,4 miesiąca. Do najbardziej poważnych i najczęstszych działań niepożądanych należały CRS (93%), encefalopatia (58%) oraz zakażenia (39%). Ciężkie działania niepożądane wystąpiły u 56% pacjentów. Do najczęstszych działań niepożądanych należały: encefalopatia (22%), zakażenia nieswoistymi patogenami (16%), zakażenia bakteryjne (6%), gorączka neutropeniczna (6%), zakażenia wirusowe (5%) i gorączka (5%). Najczęściej występujące działania niepożądane stopnia 3. lub wyższego obejmowały encefalopatię (31%), zakażenia nieswoistymi patogenami (19%), CRS (11%), zakażenie bakteryjne (9%), afazję (7%), zakażenie wirusowe (6%), stan majaczeniowy (6%), hipotensję (6%) i nadciśnienie (6%). Tabelaryczna lista działań niepożądanych: Działania niepożądane zgłaszane w badaniach klinicznych oraz po wprowadzeniu produktu do obrotu przedstawiono poniżej. Działania te są wymienione według klasyfikacji układów i narządów oraz częstości występowania. Częstości występowania określone są w następujący sposób: bardzo często (≥ 1/10), często (≥ 1/100 do < 1/10), niezbyt często (≥ 1/1000 do < 1/100), rzadko (≥ 1/10 000 do < 1/1000) i bardzo rzadko (< 1/10 000). W obrębie każdej grupy o określonej częstości występowania działania niepożądane wymieniono zgodnie ze zmniejszającym się nasileniem. **Tabela 3: Działania niepożądane związane ze stosowaniem produktu Yescarta.** *Zakażenia i zakażenia pasożytnicze:* Bardzo często: zakażenia nieswoistymi patogenami, zakażenie wirusowe, zakażenie bakteryjne. Często: zakażenie grzybicze. *Zaburzenia krwi i układu chłonnego:* Bardzo często: leukopenia, neutropenia, niedokrwistość, małopłytkowość. Często: koagulopatia. *Zaburzenia układu immunologicznego:* Bardzo często: zespół uwalniania cytokin, hipogammaglobulinemia. Często: nadwrażliwość, histiocytoza hemofagocytarna. *Zaburzenia metabolizmu i odżywiania:* Bardzo często: odwodnienie, zmniejszone łaknienie, hipofosfatemia, hiponatremia, zmniejszenie masy ciała. Często: hipokalcemia, hipoalbuminemia. *Zaburzenia psychiczne:* Bardzo często: majaczenia, lęk. Często: bezsenność. *Zaburzenia układu nerwowego:* Bardzo często: encefalopatia, ból głowy, drżenie, zawroty głowy, afazja. Często: ataksja, neuropatia, drgawki, dyskalkulia, mioklonie. Niezbyt często: obrzęk rdzenia kręgowego, zapalenie rdzenia kręgowego, porażenie czterokończynowe. *Zaburzenia serca:* Bardzo często: tachykardia, arytmia. Często: zatrzymanie akcji serca, niewydolność serca. *Zaburzenia naczyniowe:* Bardzo często: niedociśnienie, nadciśnienie. Często: zakrzepica, zespół przesiąkania włośniczek. *Zaburzenia układu oddechowego, klatki piersiowej i śródpiersia:* Bardzo często: kaszel, duszność, hipoksja, wysięk opłucnowy. Często: obrzęk płuc. *Zaburzenia żołądka i jelit:* Bardzo często: biegunka, nudności, wymioty, zaparcia, ból brzucha, suchość w jamie ustnej. Często: dysfagia*. *Zaburzenia skóry i tkanki podskórnej:* Często: wysypka. *Zaburzenia układu mięśniowo-szkieletowego i tkanki łącznej:* Bardzo często: zaburzenia ruchowe, ból kończyn, ból pleców, ból stawów, ból mięśni. *Zaburzenia nerek i dróg moczowych:* Często: niewydolność nerek. *Zaburzenia ogólne i stany w miejscu podania:* Bardzo często: zmęczenie, gorączka, obrzęk, dreszcze. *Badania diagnostyczne:* Bardzo często: zwiększone stężenie aminotransferazy alaninowej, zwiększone stężenie aminotransferazy asparaginowej. Często: zwiększone stężenie bilirubiny. W tabeli 3 ujęto wyłącznie przypadki cytopenii, które spowodowały (I) wystąpienie nowych lub zaostrzenie istniejących następstw klinicznych, (II) wymagały leczenia lub (III) modyfikacji aktualnego leczenia. * Dysfagię zgłaszano w ramach toksycznego działania na układ nerwowy i encefalopatii. Opis wybranych działań niepożądanych: *Zespół uwalniania cytokin:* CRS wystąpił u 93% pacjentów. U jedenastu procent (11%) pacjentów wystąpił CRS stopnia 3. lub wyższego (ciężki, zagrażający życiu oraz prowadzący do zgonu). Mediana czasu do wystąpienia CRS wynosiła 2 dni (zakres od 1 do 12 dni), a mediana czasu trwania 7 dni (zakres od 2 do 29 dni). U dziewięćdziesięciu ośmiu procent pacjentów (98%) doszło do ustąpienia CRS. Najczęściej występujące objawy przedmiotowe i podmiotowe związane z CRS obejmowały gorączkę (83%), niedociśnienie (44%), tachykardię (24%), hipoksję (23%) i dreszcze (20%). Ciężkie działania niepożądane, które mogą być związane z CRS, obejmowały: ostre uszkodzenie nerek, migotanie przedsionków, tachykardię komorową, zatrzymanie akcji serca, niewydolność serca, zespół przesiąkania włośniczek, niedociśnienie, hipoksję i HLH/MAS. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Neurologiczne działania niepożądane:* Neurologiczne działania niepożądane wystąpiły u 67% pacjentów. U trzydziestu dwóch procent (32%) pacjentów wystąpiły działania niepożądane stopnia 3. lub wyższego (ciężkie lub zagrażające życiu). Mediana czasu do wystąpienia działań niepożądanych wynosiła 5 dni (zakres od 1 do 17 dni). Mediana czasu trwania wynosiła 13 dni (zakres: od 1 do 191 dni). U większości pacjentów neurologiczne działania niepożądane ustąpiły, z wyjątkiem 4 pacjentów, u których niepożądane działania neurologiczne utrzymywały się do momentu zgonu; zgony te były spowodowane innymi przyczynami. Najczęstszymi objawami przedmiotowymi i podmiotowymi związanymi z neurologicznymi działaniami niepożądanymi były: encefalopatia (58%), ból głowy (40%), drżenie (31%), zawroty głowy (21%), afazja (18%) i stan majaczeniowy (17%). Zgłaszano ciężkie działania niepożądane, w tym encefalopatię (22%), afazję (4%), stan majaczeniowy (4%) i drgawki (1%), u pacjentów, którym podawano produkt Yescarta. Inne neurologiczne działania niepożądane były zgłaszane rzadziej w badaniach klinicznych i obejmowały dysfagię (5%), zapalenie rdzenia kręgowego (0,2%) i porażenie czterokończynowe (0,2%). W okresie po wprowadzeniu produktu do obrotu zgłoszono wystąpienie obrzęku rdzenia kręgowego, w kontekście neurotoksyczności. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Gorączka neutropeniczna i zakażenia:* Gorączkę neutropeniczną zaobserwowano u 36% pacjentów po infuzji produktu Yescarta. Zakażenia wystąpiły u 39% pacjentów w badaniu ZUMA-1. Zakażenia stopnia 3. lub wyższego (ciężkie, zagrażające życiu lub prowadzące do zgonu) wystąpiły u 26% pacjentów. Zakażenia nieswoistymi patogenami oraz zakażenia bakteryjne i wirusowe stopnia 3. lub wyższego wystąpiły u, odpowiednio, 19%, 9% i 6% pacjentów. Najczęstszym miejscem zakażenia były drogi oddechowe. Wskazówki dotyczące monitorowania i leczenia – patrz punkt 4.4. *Przedłużająca się cytopenia:* Neutropenia (w tym gorączka neutropeniczna), niedokrwistość i małopłytkowość stopnia 3. lub wyższego wystąpiły u, odpowiednio, 80%, 45% i 40% pacjentów. Przedłużająca się (utrzymująca się w dniu 30. lub pojawiająca się w dniu 30. lub później) neutropenia, małopłytkowość i niedokrwistość stopnia 3. lub wyższego wystąpiła u, odpowiednio, 26%, 24% i 10% pacjentów. Utrzymująca się neutropenia, małopłytkowość i niedokrwistość stopnia 3. lub wyższego po dniu 93. wystąpiła u, odpowiednio, 11%, 7% i 3% pacjentów. Wskazówki dotyczące leczenia – patrz punkt 4.4. *Hipogammaglobulinemia:* W badaniu ZUMA-1 hipogammaglobulinemia wystąpiła u 16% pacjentów. Łącznie 33 (31%) ze 108 pacjentów otrzymywało dożylne leczenie immunoglobulinami w momencie przeprowadzania analizy po 24 miesiącach. Wskazówki dotyczące leczenia – patrz punkt 4.4. Immunogenność: Immunogenność produktu Yescarta oceniano za pomocą testu immunoenzymatycznego (ELISA) wykrywającego przeciwciała wiążące FMC63, z których wywodzą się przeciwciała anty-CD19 CAR. U trzech pacjentów przed leczeniem produktem Yescarta badanie przeciwciał anty-FMC63 dało wynik dodatni. Wpływ tych przeciwciał na skuteczność lub bezpieczeństwo stosowania nie był dostrzegalny. Specjalne grupy pacjentów: Doświadczenie dotyczące stosowania produktu Yescarta u pacjentów w wieku 75 lat i starszych jest ograniczone. Na ogół bezpieczeństwo stosowania oraz skuteczność były podobne jak w przypadku pacjentów w wieku ≥ 65 lat i < 65 lat leczonych produktem Yescarta. Wyniki były zgodne z wynikami uzyskanymi w grupach pacjentów z oceną w skali Eastern Cooperative Oncology Group (ECOG) 0 i 1 oraz według płci. Zgłaszanie podejrzewanych działań niepożądanych: Po dopuszczeniu produktu leczniczego do obrotu istotne jest zgłaszanie podejrzewanych działań niepożądanych. Umożliwia to nieprzerwane monitorowanie stosunku korzyści do ryzyka stosowania produktu leczniczego. Osoby należące do fachowego personelu medycznego powinny zgłaszać wszelkie podejrzewane działania niepożądane za pośrednictwem *Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, PL-02 222 Warszawa, tel.: +48 22 49 21 301, faks: +48 22 49 21 309, strona internetowa: https://smz.ezdrowie.gov.pl.* **7. PODMIOT ODPOWIEDZIALNY POSIADAJĄCY POZWOLENIE NA DOPUSZCZENIE DO OBROTU:** Kite Pharma EU B.V., Tufsteen 1, 2132 NT Hoofddorp, Holandia. **8. NUMER POZWOLENIA NA DOPUSZCZENIE DO OBROTU:** EU/1/18/1299/001. **DATA WYDANIA PIERWSZEGO POZWOLENIA NA DOPUSZCZENIE DO OBROTU I DATA PRZEDŁUŻENIA POZWOLENIA:** Data wydania pierwszego pozwolenia na dopuszczenie do obrotu: 23 sierpnia 2018. **DATA ZATWIERDZENIA LUB CZĘŚCIOWEJ ZMIANY TEKSTU CHARAKTERYSTYKI PRODUKTU LECZNICZEGO:** 04/2021. Szczegółowe informacje o tym produkcie leczniczym są dostępne na stronie internetowej Europejskiej Agencji Leków http://www.ema.europa.eu.

Działania niepożądane można zgłaszać za pośrednictwem: Departamentu Monitorowania Niepożądanych Działań Produktów Leczniczych Urzędu Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Al. Jerozolimskie 181C, PL-02 222 Warszawa, tel.: +48 22 49 21 301, faks: +48 22 49 21 309, https://smz.ezdrowie.gov.pl lub za pośrednictwem przedstawiciela podmiotu odpowiedzialnego w Polsce: Gilead Sciences Poland Sp. z o.o., tel.: +48 22 262 87 02 lub e-mail: DrugSafety.Poland@gilead.com.

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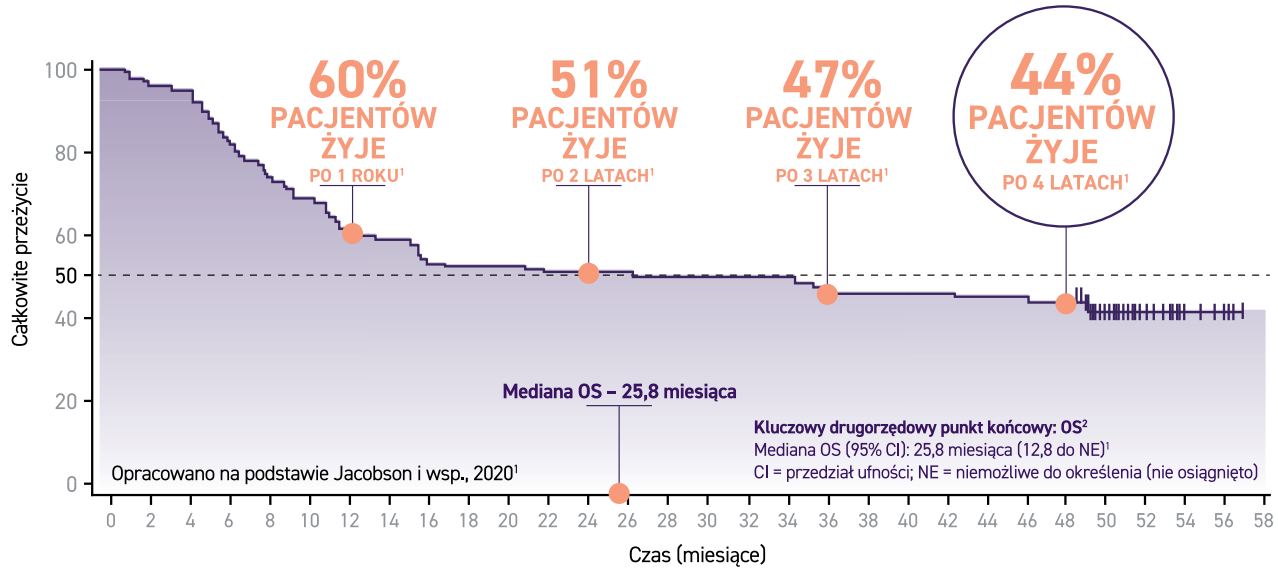
4 LATA TEMU TAKIE PRZEŻYCIA BYŁY MARZENIEM

DZIŚ SĄ
RZECZYWISTOŚCIĄ

4-LETNIE CAŁKOWITE PRZEŻYCIE (OS) WYNIOSŁO 44%[1]
w badaniu rejestracyjnym Zuma-1



Pacjenci narażeni	101	97	93	80	74	69	61	60	54	53	53	51	51	50	50	50	50	50	47	47	47	46	46	45	44	28	16	6	1	0
(Pacjenci odcięci)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(15)	(27)	(37)	(42)	(43)

* Wartości szacunkowe wg Kaplana-Meiera dla 3-letniego i 4-letniego wskaźnika OS wyniosły, odpowiednio, 47% i 44%.

Piśmiennictwo: 1. Jacobson C et al. Poster presented at: American Society for Hematology Annual Meeting (Virtual); December 5-8, 2020. 2. Locke FL et al. Lancet Oncol 2019; 20(1): 31-42.

PL-YES-0007. Data przygotowania: 06.2021

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