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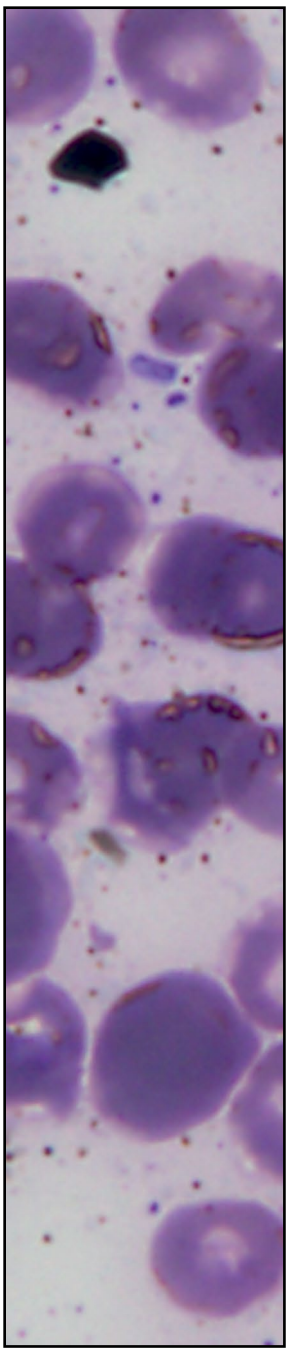

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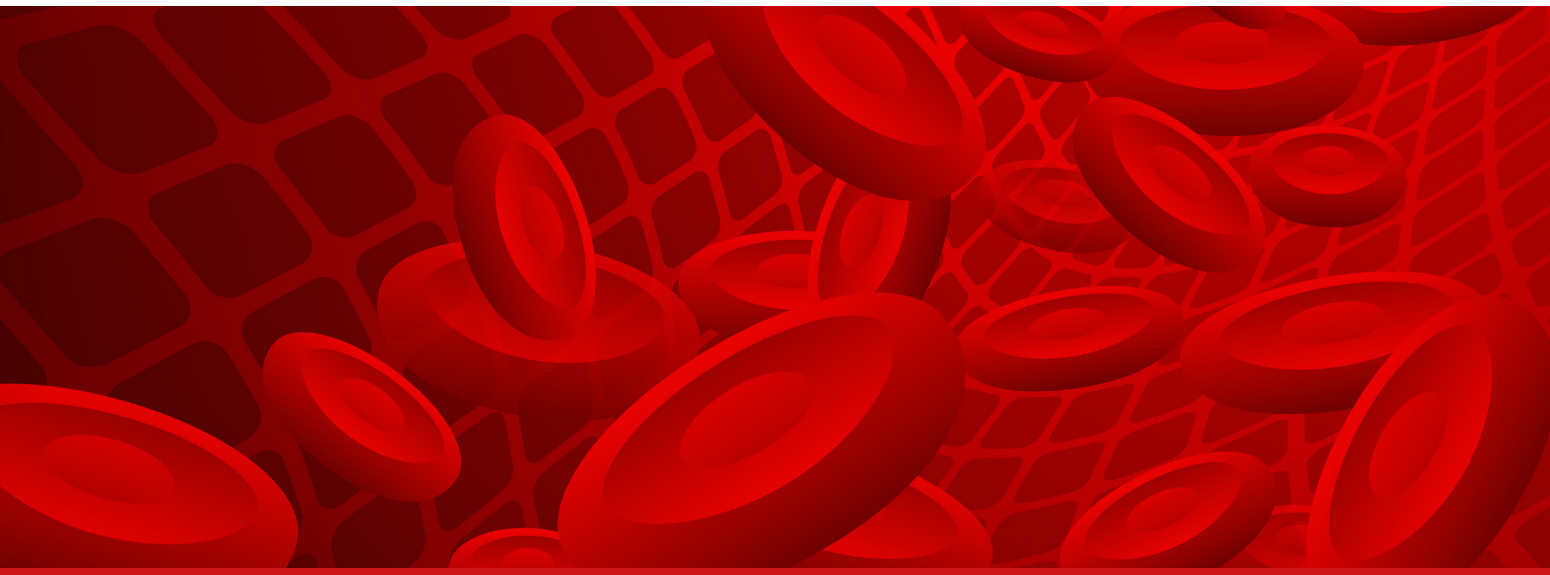

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JAK and *STAT* gene mutations and JAK-STAT pathway activation in lympho- and myeloproliferative neoplasms

Michał Łączak, Martyna Kuczyńska, Joanna Grygier, Dominika Andrzejewska, Wiktoria Grochowska, Hanna Gulaczyk, Krzysztof Lewandowski

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Abstract

Lympho- and myeloproliferative neoplasms are a very heterogeneous group of haematological malignancies originating from a haematopoietic stem cell (HSC). In most of them, the neoplastic transformation is a result of the acquisition of molecular defects by HSC or progenitor cells, impairing their proliferation, differentiation and maturation. Herein, the role of the Janus kinase--signal transduction and transcription activation (JAK-STAT) signalling pathway in the normal and neoplastic lympho- and myelopoiesis is presented. Particular attention is paid to the molecular aberrations of the JAK and STAT genes and their impact on JAK and STAT signalling pathway function and the mutation-driven mechanism of the lymphoid and myeloid cells neoplastic transformation. In the Authors' opinion, its early identification allows to incorporate the molecularly targeted drugs, including JAK-STAT pathway signalling inhibitors, to the therapeutic algorithm used and to improve the treatment results of lymphoid- and myeloid neoplasms.

Key words: lympho- and myeloproliferative neoplasms, Janus tyrosine kinases, signal transducer and activator of transcription proteins, molecular aberrations, malignant transformation

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Introduction

Haematopoiesis is a multi-stage and hierarchical process in which all types of blood cells are formed from marrow multi-potential stem cells. Each blood cell is derived from a multi-potential hematopoetic stem cell (HSC) [1]. The process of haematopoiesis is initiated by the division of HSCs; as a result, one daughter cell undergoes further differentiation, while the other remains in the pool of stem cells, ensuring self-renewal of the HSC population. The process of self-renewal and differentiation of HSC into daughter cells requires a suitable bone marrow microenvironment, composed of adipocytes, endothelial cells, osteoblasts and fibroblasts. Also, cells resulting from haematopoiesis — osteoclasts and bone marrow macrophages [2–5], play an important role in this process. The capacity for self-renewal is the basic feature that distinguishes stem cells from their later developmental stages. The divisions of daughter cells lead to the formation of more and more mature forms, up to extremely differentiated forms without the ability to divide. Regardless of their ability for self-renewal, haematopoietic stem cells can differentiate in many ways. According to the current state of knowledge, the process of haematopoiesis takes place within 11 major cell lines (Figure 1). HSC differentiation is a multi-stage process. During the first stage, HSC is transformed

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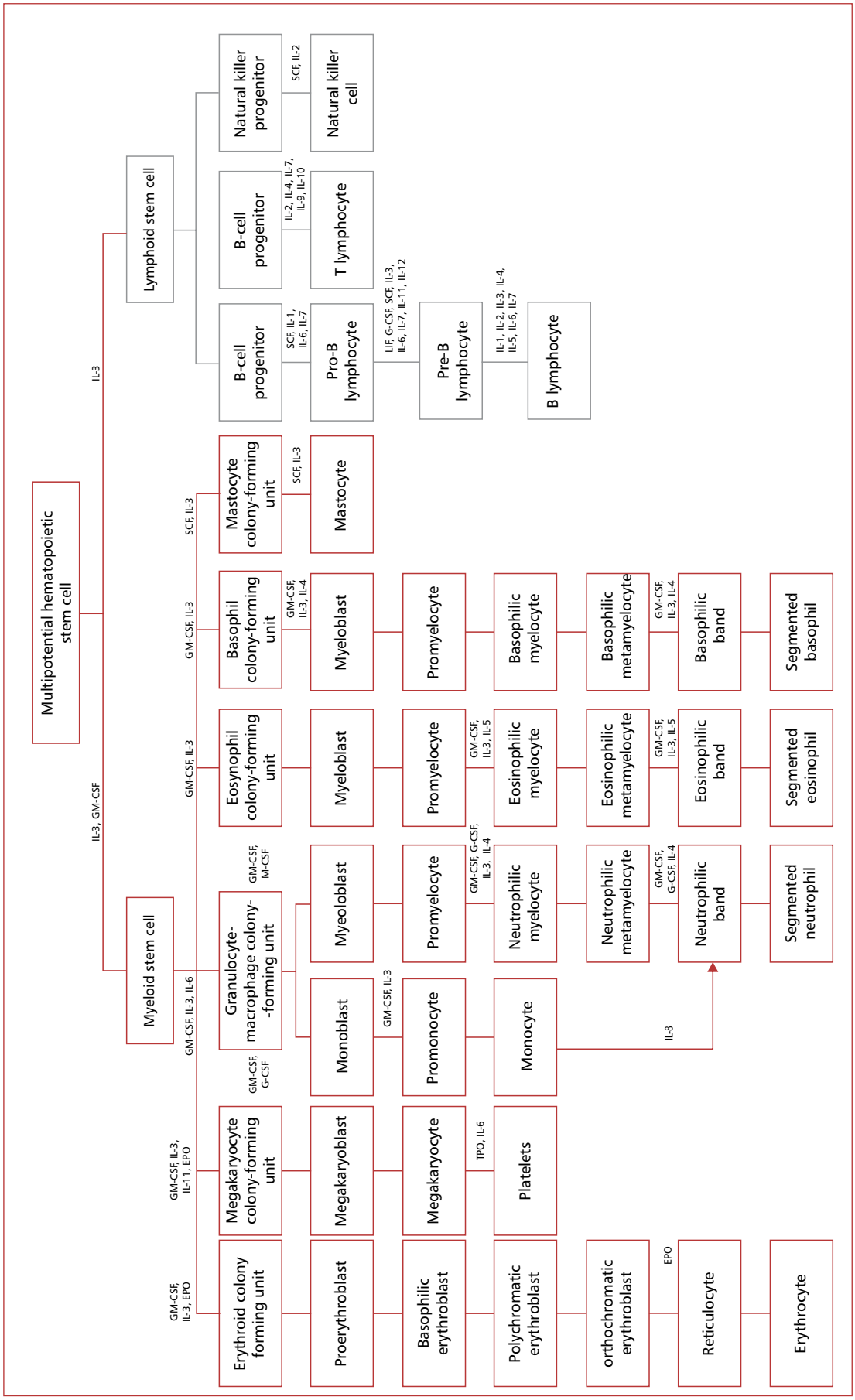

Figure 1. A schematic diagram of haematopoiesis; GM-CSF — granulocyte-macrophage colony-stimulating factor; G-CSF — granulocyte colony-stimulating factor; M-CSF — macrophage colony-stimulating factor; SCF — stem cell factor; EPO — erythropoietin; TPO — thrombopoietin; LIF — leukaemia inhibitory factor; IL-1 — interleukin-1; IL-2 — interleukin-2; IL-3 — interleukin-3; IL-4 — interleukin-4; IL-5 — interleukin-5; IL-6 — interleukin-6; IL-7 — interleukin-7; IL-8 — interleukin-8; IL-9 — interleukin-9; IL-10 — interleukin-10; IL-11 — interleukin-11

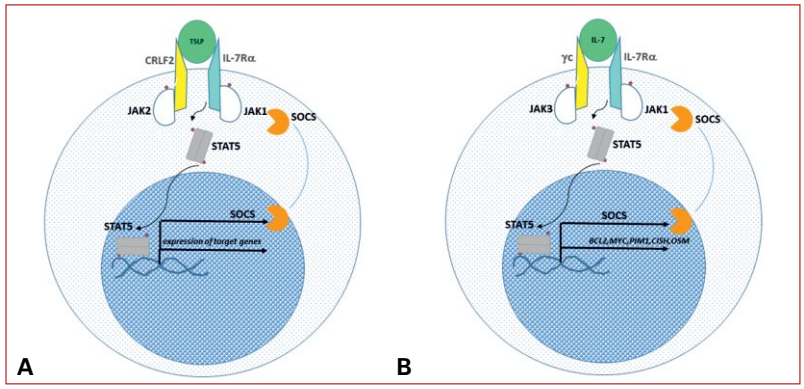

Figure 2. The mechanism of cell signaling induced by thymic stromal lymphopoietin (TSLP) (**A**) and interleukin-7 (IL-7) via the IL-7 receptor (**B**). Explanation of the abbreviations used; TSLP — thymic stromal lymphopoietin; IL-7Rα — interleukin-7 receptor subunit alpha; CRLF2 — cytokine receptor-like factor 2; JAK — Janus tyrosine kinase; STAT5 — signalling and transcription protein; SOCS — the suppressor of cytokine signalling; BCL2 — B-cell lymphoma 2 protein; *MYC* — *MYC* proto-oncogene; *PIM1* — *PIM-1* proto-oncogene; CISH — cytokine-inducible SH2-containing protein; OSM — the oncostatin *M* gene encoding the sequence of a pleiotropic cytokine from the interleukin-6 family

into a multipotent progenitor (MPP), followed by the formation of common lymphoid progenitors (CLP) and common myeloid progenitors (CMP). The intensity of haematopoiesis within individual cell lines is controlled by the coordinated action of cytokines, transcription and epigenetic factors, and growth factors [6, 7]. A common precursor for all myelopoiesis is CFU-GEMM (colony-forming unit of granulocyte, erythrocyte, macrophage, megakaryocyte). Myelopoiesis stem cells gradually lose their ability to renew themselves, while retaining their multidirectional differentiation function. Under the influence of the cytokine game and the influence of the growth factors, CFU-GEMM transforms into targeted progenitor cells, and then the precursors of the erythropoietic line — units that form large, early erythroid colonies (BFU-E), erythroid colony-forming units (CFU-E), eosinophilopoietic line — units forming eosinophilic colonies (CFU--E), basophilopoietic line — basophilic colony forming units (CFU-Baso), mast cell line — mast cell colony-forming units (CFU-mast), monocytopoietic line — monocytic colony forming units (CFU-M), megakaryopoietic line — mast cell colony-forming units (CFU-mast) and neutrophilopoietic line — granulocytic colony-forming units (CFU-G).

In the process of lymphopoiesis, the ability to differentiate precursor cells is fully preserved. The mechanism of proliferation and differentiation of B, T, and NK lymphocytes from lymphopoietic precursor cells (CLP) also depends on the number of environmental factors, including growth factors, cytokines, kinases (JAK, Kit-L), and surface molecules, such as Notch-1. Notch-1 which in cooperation with GATA-3 is involved in the process of T lymphocytes differentiation via the receptor $\alpha\beta$, plays an extremely important role in this process. The receptor for interleukin-7 (IL-7) (CD127, IL-7Rα) [8, 9] plays a special role in the early stages of B- and T-lymphocyte proliferation and differentiation. Its activation by IL-7 leads to phosphorylation of JAK1 or JAK3 kinase, and the activation of JAK1-STAT5 or JAK3-STAT5 signal transduction pathway. It should be noted that activation of IL-7R simultaneously leads to the signal transduction along the PI3K-AKT and Ras-MAPK [10, 11] pathways. In the case of the IL-7 receptor, the JAK1 kinase is structurally and functionally related to the IL-7R subunitα, and the JAK3 kinase to the γc subunit of the receptor [12]. The mechanism of cell signalling induced by thymic stromal lymphopoietin (TSLP) and interleukin-7 (IL-7) via the IL-7 receptor is shown in Figure 2.

Cytokines involved in the process of lympho- and myelopoiesis

Cytokines and growth factors are glycoproteins that regulate the proliferation and differentiation of progenitor cells. They also control some functions of mature blood cells [13]. Cytokines are primarily survival factors and act as growth factors in higher concentrations. It should be kept in mind that survival is regulated by so-called "deadly cytokines" which include tumour necrosis factor-alpha (TNF alpha). The most important factors acting on haematopoietic stem cells include: Kit ligand (KL) — the Steel factor, FMS-like ty-

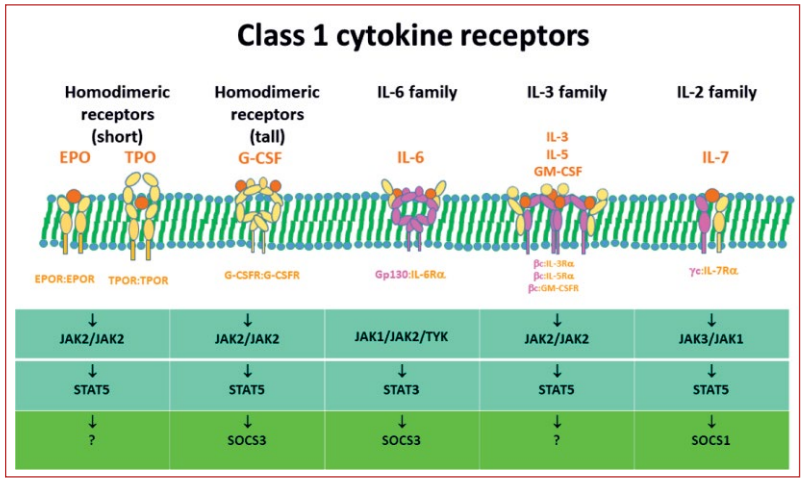

Figure 3. Cytokine-initiated cell signalling via the JAK-STAT pathway involved in haematopoiesis (acc. to [14]); IL — interleukin; IL-xR — the receptor for a specific (x) type of interleukin; GM-CSF — granulocyte-macrophage colony-stimulating factor; GM-CSFR — granulocyte-macrophage colony-stimulating factor receptor; G-CSF — granulocyte colony-stimulating factor; G-CSFR — granulocyte colony-stimulating factor receptor; EPO — erythropoietin; EPOR — erythropoietin receptor; TPO — thrombopoietin; TPOR — thrombopoietin receptor; Gp130 — glycoprotein 130; βc — common beta chain; JAK — Janus tyrosine kinase; TYK — tyrosine-protein kinase TYK; STAT — signal transducer and activator of transcription protein; SOCS — suppressor of signal transduction by cytokines

rosine kinase 3 ligand (FLT-3 ligand), as well as factors affecting multi-potential progenitor cells: the granulocyte-macrophage colony-stimulating factor (GM-CSF) — the factor that stimulates the formation of granulocytes and macrophages; the granulocyte colony-stimulating factor (G-CSF), interleukin 3 (IL-3) and interleukin 6 (IL-6). The factors involved in the maturation and differentiation of targeted precursor cells include granulocytic growth factor (G-CSF), macrophage growth factor (M-CSF), interleukin 5 (IL-5), erythropoietin (EPO), and thrombopoietin (TPO) (Figure 3) [14].

The FLT3 receptor (fms-like tyrosine kinase 3) and the FLT3 ligand play a key role in the functioning of haematopoiesis. FLT3 plays an important role in initiating the expansion of early progenitor cells, and its proper function is essential not only for the maintenance of the HSC population but also for the growth of targeted common myeloid progenitor cells (CMP), granulocyte-monocyte progenitor (GMP) and macrophages and megakaryocyte-erythroid progenitor (MEP) cells [15–17].

The role of the JAK-STAT signalling pathway in lympho- and myelopoiesis

The course of both lympho- and myelopoiesis is dependent on the undisturbed interaction between cytokines and cytokine receptors on the surface of haematopoietic stem cells, as well as the Janus receptor-tyrosine kinase function, Janus tyrosine kinase-STAT protein interactions and STAT dimers/monomers binding the promoter sequences of target genes.

During the first stage, the binding of the cytokine/growth factor to the target receptor leads to its dimerization/oligomerization and the recruitment of JAK molecules. The JAK recruitment results in their phosphorylation by autophosphorylation and/or transphosphorylation mediated by another JAK molecule or another kinase from the tyrosine kinase family. An activated Janus kinase mediates phosphorylation of the target tyrosine molecule within the receptor. In this way, a docking site for the STAT molecules is created [18]. After the binding of the STAT molecule to the receptor, tyrosine phosphorylation occurs. The abovementioned process initiates the reorientation of STAT proteins and their homo- or heterodimerization. STAT dimerization is the result of an interaction between the SH2 domain on one STAT molecule and phosphorylated tyrosine on the other STAT molecule. After phosphorylation, STAT dimers translocate to the nucleus, bind to the target gene sequence via the DNA-binding domain, and initiate the process of transcription of target genes. The activity of STAT (except for STAT2) is controlled by serine-threonine kinases (e.g. ERK, p38, mTOR). The regulation of STAT activity is possible by serine phosphorylation in the transactivation domain (within the conserved PSMP motif) [19–21]. The conceptual structure of STAT3 and STAT5B molecules is shown in Figure 3 [14].

Proteins involved in the signal transduction along the JAK-STAT pathway

Janus tyrosine kinases

Cell signal transmission within the cell is complex and involves basic life functions, including cell cycle coordination. These processes involve protein kinases involved in the phosphorylation of several amino acids, including serine, threonine, histidine and tyrosine. So far, there have been distinguished two types of protein kinases — serine-threonine kinases and tyrosine kinases. The network of protein kinases is formed by mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases (ERK), epidermal growth factor receptor (EGFR), Src kinase proto-oncogene (src), Abelson tyrosine-protein kinase (ABL), focal adhesion kinase (FAK) and Janus family kinase [22]. The JAK family consists of the JAK1, JAK2, JAK3 and TYK2 kinases. The NH2 portion of the JAK kinases contains two essential motifs: the Src homology domain (SH2) and the FERM domain (Four-point-one, Ezrin, Radixin, Moesin). The first one consists of the JH3 region and the JH4 domain. The FERM domain is involved in the binding of the kinase to the receptor, as well as to other proteins. It is also involved in the regulation of the catalytic activity of JAK kinase [23]. A characteristic feature of the Janus family kinases is the presence of domains with a high degree of homology: JH1 and JH2 [24]. The functional domain is the JH1 domain containing the YY motif within the activation loop. The JH2 domain (kinase-like domain), structurally similar to functional kinases, has no measurable biological activity, possibly due to the loss of formations responsible for the catalytic activity of the kinase and its binding to nucleotides [25–27]. However, the JH2 domain has a regulatory function concerning JAK2 and JAK3 kinase activity [28–31]. The detailed mechanism of this interaction has been discovered thanks to the studies of the mutant form of JAK2 V617F kinase in patients with Philadelphia negative myeloproliferative neoplasms. It has been shown in them that the presence of the *V617F* mutation abolishes the inhibitory effect of the JH2 domain on JH1, which results in the constant constitutive activity of JAK2 V617F kinase in neoplastic cells [32].

STAT proteins

STAT (signal transducer and activator of transcription) family proteins are intracellular transcription factors mediating several cellular processes, including proliferation, differentiation, and their programmed death (apoptosis). STAT proteins are one of the major cell signal transducers in response to the action of various types of agonists on cell receptors, including growth factors, cytokines, and other protein ligands [33].

Some of them, including STAT5, have been shown to play a key role in cytokine-induced proliferation, self-renewal and survival of HSC [34, 35]. The normal function of both JAK1 and JAK2 has also been shown to play a key role in maintaining HSC homeostasis. The *in vivo* experimental model showed that the deletion of Jak1 leads to changes in the self-renewal of HSC and disrupts the process of cell differentiation in both the lymphoid and myeloid lineage [36]. The knock-out of the *Jak2* gene leads to even greater consequences. Its deletion leads not only to ineffective haematopoiesis but also to the death of the embryo [37, 38].

The STAT family includes seven proteins — STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6. Each protein contains an N-terminal region, a coiled-coil domain, DNA binding domain, linker region, Src homology 2 domain and C-terminal transactivation domain [39, 40]. All STAT proteins share a common, highly conserved N-terminal region sequence. It consists of a hydrophobic core that participates in the attachment of STAT dimers to DNA [41]. The coiled-coil domain consists of approximately 180 amino acids making up the 4 α-helices. They form a hydrophobic surface that allows STAT proteins to interact with other proteins [42]. The SH2 domain is responsible for the process of combining the STAT protein with phosphotyrosine of the activated JAK tyrosine kinase [21]. The structure of the DNA binding domain allows STAT proteins to bind to specific DNA sequences and thus act as transcription factors. Each STAT protein participates in different cellular processes. And so, STAT1 is involved in the expression of genes that determine the viability and survival of cells, as well as in the response of cells to pathogens, including *Candida sp.* [43, 44]. STAT2 is involved in signalling through the IFN-α/β receptor. In response to the binding of the JAK tyrosine kinase with the interferon, there is formed STAT1-STAT2 heterodimer, to which the p48 [interferon regulatory factor 9 (IRF9)] also binds. The resulting complex called ISGF3 (interferon stimulated gene factor 3) functions as a transactivator because it cannot bind to the DNA structure itself [45, 46]. It plays a key role in the processes of growth of cellular differentiation, apoptosis and cell migration, as well as in the process of DNA methylation and nuclear chromatin modification

[47–49]. STAT4, STAT5A and STAT5B proteins play a slightly different role. STAT4 participates in the process of maturation and development of Th1 cells. It also plays an important role in the initiation of interferon-gamma synthesis, as well as the control of the expression of certain transcription factors [50, 51]. A different role is played by the STAT5A and STAT5B proteins, which show 90% homology in terms of the amino acid sequence. They participate in the control of cell growth and division, their specialization, and, at the end of their life, in their apoptosis [52]. STAT6 mediates signalling with the participation of IL-4 and IL-13. It plays an important role in the process of the immune response of T lymphocytes and the formation of type 2 (Th2) lymphocytes [53].

STAT proteins are activated by phosphorylation of a single tyrosine at 701 position by activated JAK kinase [54]. As a result of tyrosine phosphorylation, individual molecules of the STAT protein form dimers through the interaction between the SH2 domain and the C-terminal transactivation domain [55]. In the case of the STAT1, STAT3, STAT4, STAT5A and STAT5B proteins, these are homodimers. In some cases, however, the formation of heterodimers (e.g. STAT1 and STAT2 as well as STAT1 and STAT3) occurs [56].

Phosphorylated STAT1 homodimers or STAT1/STAT2 heterodimers are translocated to the nucleus with the participation of importin-α5. This phenomenon has not been confirmed in the case of STAT1 monomers. STAT1 dimers bind to two 5α importin molecules, due to the presence of lysine-rich fragments of nuclear localization signals (NLSs) in the DNA binding domain. The presence of leucine at position 407 in the DNA binding domain is crucial for STAT1 protein transport into the nucleus. Its role is confirmed by the lack of STAT1 binding to importin-α5 in the presence of the Leu407Ala STAT1 mutation [57, 58]. The transport of STAT3 to the nucleus is independent of tyrosine phosphorylation. It is postulated that STAT3 is transported to the nucleus with the participation of importin-α3 [59]. According to some authors, STAT1 and STAT3 are transferred to the nucleus with the participation of importin-α5 and importin-α7, and not importin-α3 [60]. It has been shown that STAT proteins can be recirculated, returning to the cytoplasm after their previous phosphatase-mediated dephosphorylation [61].

Studies on the *Drosophila* model have shown the existence of a non-classical JAK-STAT signalling pathway. They confirmed the presence of non-phosphorylated STAT proteins bound to the heterochromatin protein 1 (HP1) in the cell nucleus. The activation of STAT by phosphorylation results in the detachment of STAT from HP1, as a result of which HP1 detaches from heterochromatin, disrupting its structure [62]. The non-phosphorylated STAT1, STAT3, STAT5 proteins can also move between the cytoplasm and the cell nucleus without the participation of transport proteins and cellular energy transfer processes [63]. Non-phosphorylated STAT proteins affect gene transcription in a different way than phosphorylated STAT proteins. They bind to specific DNA sequences as monomers [64]. It has been postulated that they play an important role in type 1 interferon-initiated cell signalling, transcription and mRNA translation of interferon target genes [65].

JAK2 gene mutations

JAK2 V617F is the most common mutation in the Philadelphia negative MPNs. Its presence leads to the abnormal autoinhibition of Janus kinase 2 and the constitutive activation of the JAK-STAT signalling pathway. This mutation is present in over 90% of PV cases, 35–45% of ET cases, and 35–45% of PMF cases. Due to the different picture and clinical course of various *JAK2* V617F positive MPNs, their role and importance in the pathogenesis of this group of diseases are currently being investigated [66]. An attempt at answering this question is the theory of the relationship between the content of the mutated allele and the course of the disease process. The variant allele fraction (VAF) expresses the mean content of the pathological variant in the haematopoietic cell population. In most patients with ET, the variant allele fraction is low, namely 25% or lower. In patients with PV, the content of the abnormal allele is usually high (50% or higher). The most common defect in the Janus kinase 2 gene is the *JAK2* V617F mutation resulting from a sequence change at position nt1849 G→T in exon 14 of the *JAK2* gene. Its presence leads to the conversion of valine to phenylalanine within the JAK2 kinase pseudokinase domain sequence. Its occurrence leads to inhibition of the kinase autoinhibition process, which results in constitutive activation of JAK2 kinase, regardless of changes in the conformation of the receptor as a result of interaction with the agonist. Constitutive activation of JAK2 results in excessive JAK-STAT signalling, initiating transcription of target genes involved in the proliferation and differentiation of myeloid precursor cells. The presence of *JAK2* V617F was confirmed in haematopoietic stem cells (HSCs) and myeloid progenitor cells [31, 67–69]. Some

reports confirm the presence of the mutations also in the cells of the lymphoid lineage [70, 71]. The presence of mutations that interfere with the auto-inhibition of JAK2 kinase leads to an over-activation of the JAK2-STAT signalling pathway via a range of cellular receptors, including the erythropoietin receptor (EPOR), the granulocyte colony-stimulating factor receptor (GCSFR), and the thrombopoietin receptor (myeloproliferative leukaemia protein; MPL). Many data indicate that the heterogeneity of MPN, both in terms of clinical and laboratory manifestation, as well as a different clinical course, is the result of the coexistence of other defects, modifying the course of the disease in individual cases. The clinical manifestation of the disease also appears to include the sequence of "acquiring" specific genetic defects by proliferating bone marrow stem cells, as well as the presence of germline defects preceding the transformation [72, 73].

Exon 12 mutations in the *JAK2* gene also play an important role in the pathogenesis of MPNPh–. Their presence also disrupts the JAK2 kinase auto-inhibition process. Most defects lead to amino acid substitutions within positions 536–547. Contrary to the *JAK2* V617F mutation, their occurrence was described only in patients with PV (approx. 3%). Therefore, testing for their presence should be limited to patients who meet the PV criteria who are *JAK2* V617F negative [74], and occasionally in patients with primary myelofibrosis [75]. In patients with the mutation present within exon 12 of the *JAK2* gene, the leukocyte and platelet counts are lower, as compared to patients with the *JAK2* V617F mutation. This suggests that in PV patients, the presence of mutations within exon 12 leads to increased proliferation of erythroid precursors only. The mechanism of this phenomenon is unknown [76].

Genetic defects with the presence of *JAK2* and lymphoproliferative neoplasms

One of the manifestations of neoplastic transformation of lymphocyte cells in patients with malignant non-Hodgkin's lymphoma is the presence of gene mutations of the proteins in the JAK-STAT signalling pathway. Interestingly, their occurrence is typical of T-cell lymphomas. Thus, in patients with cutaneous T-cell lymphomas, several genetic aberrations involving the *JAK1*, *JAK3*, *STAT3* and *STAT5B* genes have been described, significantly altering the JAK-STAT pathway of cell signalling. These include both auto-activating point mutations and fusion genes involving Janus kinases. So far, the presence of *JAK1*, *JAK2* and *JAK3* mutations has been confirmed in patients with acute lymphoblastic leukaemia. In adult patients with T-ALL, their frequency was estimated at 16% [77].

The presence of the *JAK1* gene mutation has been confirmed sporadically, most often in people carrying *JAK3* or *IL7R* defects. Interestingly, these mutations were heterozygous in nature and concerned the regions coding for the FERM, SH2, pseudokinase and kinase domains [77].

Another typical example of the first group of disorders is the mutations of the Janus 2 kinase gene leading to amino acid changes at position R683 (R683S, R683G and R683E). Their presence leads to a decrease in the stability of the JAK2 protein with a simultaneous significant increase in the kinase activity [78].

A typical example of a fusion defect is described in the case of a CD4 positive T-cell lymphoma t(9;13;16) (p24;q34;p11) leading to the formation of the *ATXN2L-JAK2* fusion gene and a chimeric ATXN2L-JAK2 protein responsible for constitutive signalling downstream along the JAK-STAT pathway [79]. Another example of changes affecting cell signalling via the JAK-STAT pathway is the confirmation of the presence of the STAT3-JAK2 fusion gene resulting from t(9;17) (p24.1;q21.2) in 4 out of 5 patients with CD4 positive indolent T-cell lymphoproliferative disorder of the gastrointestinal tract (GI TLPD) [80].

STAT gene mutations

The presence of mutations of genes encoding proteins from the STAT family may lead to the loss of function or its duration, regardless of the gain of function. So far, the presence of the STAT mutation has been confirmed mainly in patients with lymphoproliferative diseases. Most often they concern the sequences coding the SH2 domains of STAT3 and STAT5 genes and are of gain-of-function character. In individual cases, the presence of mutations of the mentioned genes within the DNA binding domain and the coiled-coil domain was confirmed. Their detailed overview is presented in Figure 4 [81]. The most common of these defects is the STAT5B N642H mutation. Its presence has been confirmed in peripheral T-cell_lymphoma (PTLC) [82, 83–85]. The presence of the defect stabilizes the formed STAT dimer which leads to an increase in the number of phosphorylated tyrosines and hyperactivation of STAT5B [86]. The reason for starting the research on the role of STAT protein

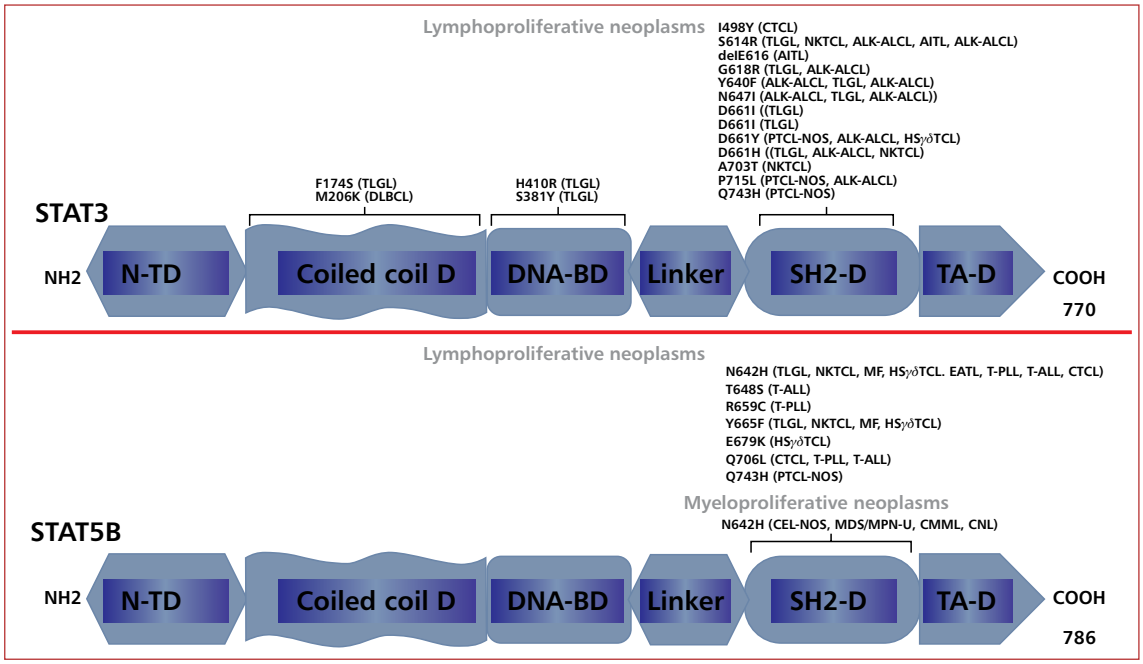

Figure 4. The most common location of STAT3 and STAT5B mutations in patients with lymphoid and myeloid neoplasms (acc. to [81]). Explanation of the abbreviations used: N-TD — NH2 terminal domain; coiled-coil; D — coiled--coil domain; DNA-BD — DNA binding domain; SH2-D — Scr homology domain 2; TAD — transactivation domain; TLGL — T-cell large granular lymphocytic leukaemia; CTCL — cutaneous T-cell lymphoma; NKTCL — natural killer/T-cell lymphoma; ALK-ALCL — ALK-negative anaplastic large cell lymphoma; HS $\gamma\delta$TCL — hepatosplenic T-cell lymphoma $\gamma\delta$; PTCL-NOS — peripheral T-cell lymphoma, not elsewhere classified, MF — mycosis fungoides; GF — granuloma fungoides; EATL — enteropathy-associated T-cell lymphoma; T-PLL — T-cell prolymphocytic leukaemia; T-ALL — T-cell acute lymphoblastic leukaemia; DLBCL — diffuse large B-cell lymphoma; CEL-NOS — chronic eosinophilic leukaemia not otherwise specified; MDS/MPN-U — myelodysplastic/myeloproliferative neoplasms unclassified; CMML — chronic myelomonocytic leukaemia; CNL — chronic neutrophilic leukaemia

defects in oncogenesis was to demonstrate the constitutive activity of STAT1, STAT3 and STAT5 in acute myeloid leukaemia cells [87, 88]. It also turned out that constitutive activation of STAT1 plays a key role in promoting the growth of leukaemic cells, and STAT5 in myeloid or lymphoid differentiation of leukaemic cells [89]. In the latter case, the presence of the *STAT5A* mutation leads to the formation and cellular accumulation of a stable tetramer as a consequence of the development of multilineage leukaemia [90]. Ser725 and Ser779, which are the phosphorylation sites of the STAT5A protein, seem to play a key role in this process [91]. The N-terminal fragment of STAT5A/B seems to play a similar role in the neoplastic transformation of B lymphocytes [92]. According to current data, STAT5A and STAT5B act as proto-oncogenes, participating in the processes of regulation of HSC proliferation and survival [93, 94]. STAT5A and STAT5B promote the transcription of many anti-apoptotic genes (Mcl-1, Bcl-2, Bcl-xl), D-type cyclins (D1, D2 and D3), receptor protein chains and cytokines [93, 95–97].

However, it is worth remembering that the activation of STAT5A/B may also be the result of excessive activity of upstream mutant tyrosine kinases, including JAK2 V617F, BCR-ABL, FLT3--ITD, and KIT D816V [100].

STAT gene mutations in lymphoproliferative neoplasms

In the case of the *STAT3* gene, the described defects lead to an increase in the gain of function. The presence of the *STAT3* mutation has been confirmed in T-LGL leukaemia, chronic NK lymphoproliferative disorder (CLPD-NK) and ALK-negative anaplastic large cell lymphoma [99, 100]. The STAT5A and STAT5B proteins participate in the activation of the transcription of anti-apoptotic genes. The STAT5A/B overexpression may contribute to the disruption of the natural mechanisms of programmed cell death [81].

It is postulated that STAT5 mutations may be solely responsible for the neoplastic transformation of defective cells [93, 100, 101]. Autoactivating molecular defects concerns the *STAT5B* gene much more often than *STAT5A*. The cause of this phenomenon is unknown. Most of them are located within the SH2 STAT5B domain coding sequence. Their presence leads to the stabilization of the dimer form of STAT proteins [102]. The presence of *STAT5B* defects has been confirmed in NK cell/T-lymphocyte neoplasms [86], as well as in various forms of peripheral T cell lymphomas [103]. One of the recently published studies on the driver mutation confirmed the key importance of the *STAT6* mutation also in patients with B-cell lymphoma. Their occurrence was confirmed using an integrated whole-genome analysis [104]. Mutations in the *STAT3*, *STAT5B*, *JAK1*, *JAK2*, and *PTPN1* genes have also been documented in patients with classic Hodgkin's disease, affecting cell signalling via the JAK-STAT pathway. Mutations of the gene encoding the STAT6 protein, present in approximately 1/3 of patients, functionally related to SOCS1, the main inhibitor of JAK-STAT signalling, seem to be of particular importance in this regard [105].

Defects involving STAT genes in myeloproliferative neoplasms

In recent years, data confirming the potential importance of the presence of *STAT* gene mutations also in the pathogenesis of MPN have been presented. Current data confirmed the activation of the JAK-STAT pathway in patients with chronic myeloid leukaemia and MPNPh– as a result of the presence of defects other than *STAT* gene defects. In the first case, excessive activation of the JAK-STAT pathway is the result of mainly constitutive activation of the BCR-ABL tyrosine kinase. In the case of MPNPh–, the STAT over-expression is the result of the presence of mutations in the *JAK2* genes that reduce the auto-inhibition of the kinase, mutations in the *MPN* gene responsible for autoactivation of the MPL receptor, or mutations in the calreticulin (*CALR*) gene leading to a change in the location of the CALR protein and the formation of the auto-activating MPL receptor protein complex. However, the occurrence of *STAT5B* N642H mutations associated with eosinophilia in MPNs [chronic eosinophilic leukaemia not elsewhere specified (CEL-NOS), hypereosinophilic syndrome] has been reported recently. The presence of this mutation has also been confirmed in patients with myelodysplastic syndrome/unclassified MPN (MDS/MPN-U), atypical form of chronic myeloid leukaemia (aCML) and chronic myelomonocytic leukaemia (CMML) [82]. The presence of the *STAT5B* N642H mutation was also confirmed in a patient with chronic neutrophilic leukaemia with progressively increasing leukocytosis and severe infectious complications. In the described case, its occurrence was accompanied by the presence of *CSF3R*T618I, *ASXL1*G942 fs defects [85].

Other gene defects responsible for the disruption of JAK-STAT signalling in patients with lympho- and myeloproliferative neoplasms

IL-7 receptor mutations alpha

Most of the described defects of the gene encoding the IL-7 subunit α of the IL-7 receptor are auto-activating mutations. Their presence has been confirmed in approximately 19% of patients with T-ALL and 2–3% of patients with the precursor form of B-ALL [106–111].

CRLF2 receptor mutations

One of the described defects of the *CRLF2* gene is the gain-of-function type Phe232Cys mutation. Its presence leads to receptor dimerization and cytokine-independent proliferation of defective cells [112, 113].

Calreticulin gene mutations

For the first time, the presence of mutations within *CALR* was independently confirmed by Klampf et al. and Nangalia et al. in 2013 [114, 115]. CALR is a protein with a pleiotropic function, 46 kDa in size. The cellular location of calreticulin is the endoplasmic reticulum. Its presence has also been confirmed in the cytosol and the cell nucleus. The main biological function of CALR is the binding of calcium ions in the endoplasmic reticulum (ER) of muscle cells, in which it also plays a regulatory role in the process of muscle contraction [116]. Other functions of CALR include regulation of calcium-dependent cell signalling, electrical conductivity, cell differentiation and division, as well as the processes of adhesion and apoptosis. The CALR structure consists of 3 domains: N-terminal globular domain, proline-rich domain (P domain) and highly negatively charged C-terminal domain. The proline-rich domain and the C-terminal domain are mainly involved in the binding of proteins to calcium ions. While the N-terminal domain, together with the proline-rich domain, performs chaperone functions due to their proximity which enables

interaction with glycosylated and non-glycosylated proteins [117]. They are responsible for the quality control of the folding of most of the produced proteins in the lumen of the endoplasmic reticulum. A disruption of this process may be important in the pathogenesis of the Philadelphia negative MPNs. The specific KDEL amino acid sequence present within the C-terminal domain is responsible for the correct localization of the CALR protein in the ER lumen (protein retention due to the correct sequence of the localization domain) [118, 119].

The incidence of *CALR* gene defects in patients with ET is 25%, and in patients with PMF it is 35% [115]. Mutations of the *CALR* gene in patients with MPNPh– take the form of insertions or deletions located within exon 9 of the *CALR* gene, at the end of the coding sequence from the 3' side. Their occurrence leads to a change of the reading frame within the 3'-terminal codons by –1 or +2. The genetic changes result in the replacement of the KDEL motif (ER retention signal) on the C-terminal part of the protein with a different mutant amino acid sequence [114, 115]. The two most common types of molecular defects in the *CALR* gene are a 52-nucleotide deletion (del52, a type-I mutation) and a 5-nucleotide insertion (ins5, a type-II mutation) [66]. The presence of the *CALR* gene mutation, as well as the *JAK2* and *MPL* gene defects, leads to the transformation of the malignant myeloid stem cell and its excessive proliferation as a result of the activation of the JAK-STAT pathway. Presently, the mechanism of aberrant JAK-STAT pathway activation in these cases has been described. It postulates the formation of the CALRmut-MPL protein complex already within the ER, with the subsequent placement of the complex in the cell membrane of the precursor cells already in the form with the originally activated MPL receptor [120]. The proposed mechanism for activating the JAK-STAT pathway was confirmed by laboratory tests. These studies demonstrated the activating properties of the mutant CALR protein on MPL, resembling that of a natural agonist. The activity of the CALRmut protein is MPL dependent, as both the activation of STAT5 induced by both CALR mutants and the cytokine-dependent growth of Ba/F3 cells require the presence of MPL [121]. The presented mechanisms of MPL-CALR interactions explain why mutations of the *CALR* gene lead to disease symptoms only in patients with ET and PMF. This phenomenon is the result of the possible occurrence of the described interactions only in progenitor cells of the megakaryocytic lineage expressing MPL.

Thrombopoietin receptor gene mutations (*MPL*)

Under physiological conditions, *MPL* plays a key role in the regulation of thrombopoietin-mediated megakaryopoiesis. The TPO-dependent activity of *MPL* enables the maintenance of a constant pool of megakaryopoietic progenitor cells in a feedback mechanism [122, 123]. The importance of the proper functioning of the MPL-TPO interaction is confirmed by clinical observations made in patients with thrombocytopenia and/or bone marrow failure due to the presence of TPO or MPL defects [124, 125]. MPL is expressed in haematopoietic stem cells and cells of the platelet-forming lineage. MPL is not necessary for the development of other cell lines [126].

The MPL protein belongs to the type I family of integrin receptors. Its ligand is thrombopoietin [127, 128]. MPL consists of 3 domains. The extracellular domain is made of two cytokine receptors. Each of them has two fibronectin type III-like domains [129]. The extracellular domain is responsible for the binding of thrombopoietin. The transmembrane domain is essential for anchoring the receptor to the membrane. The role of the intracellular domain is signalling by JAK2 kinases. This domain contains two box motifs that are responsible for the binding of JAK tyrosine kinases. MPL is present on the cell surface as a monomer or an unstable dimer [128, 129].

The presence of a mutation in the *MPL* gene encoding the receptor for thrombopoietin was confirmed in 5% of primary bone marrow fibrosis and 1% of essential thrombocythemia patients. Their presence, as in the case of the *JAK2* V617F mutation, leads to cytokine-independent activation of the JAK-STAT pathway. The most common mutations in the *MPL* gene in patients with MPNPh– are those located within the W515L/R hotspot. In individual cases, the presence of other mutations, including W515A/G has also been described [130]. Most of the described defects in the *MPL* gene were identified within exon 10 which encodes the receptor's transmembrane domain [131]. Their presence leads to constitutive activation of the receptor without the participation of an agonist, and thus the thrombopoietin receptor-JAK-STAT signalling pathway [132, 133]. The importance of the presence of defects located at position 505 in patients with MPNPh– is currently under evaluation. Their occurrence has been described in both familial and neoplastic thrombocythemia [132].

Molecularly targeted therapy and mutations of *JAK* and *STAT* genes

The use of Janus 2 kinase inhibitors (ruxolitinib, fedratinib) in the therapy of Philadelphia negative myeloproliferative neoplasms is currently the standard treatment for this group of diseases. Interestingly, the therapeutic effect of reducing the severity of cytokine symptoms and splenomegaly is obtained regardless of the presence of the *JAK2* gene mutation. This phenomenon is explained by the inhibition of excessive activation of the JAK-STAT signalling pathway in patients with MPNPh–, irrespective of the presence of the Janus kinase 2 defect.

Experience with the use of JAK inhibitors is at an early stage in clinical trials. In an ongoing phase 2 clinical trial (CELTIC-1, NCT0402108), the overall response rate was assessed as 38% [134] in patients with relapsed refractory PTCL. Cerdulatinib, a competitive inhibitor of the SYK and JAK family kinases, is at a similar stage of Phase 2A research. In the evaluation of 38 patients with resistant PTCL and 22 patients with CTCL, the response rate was 35% [135]. Another drug also evaluated in Phase 2 clinical trials (NCT 03598959) in patients with extranodal natural killer/T-cell lymphoma (ENKTCL) is Tofacitinib (a JAK3 inhibitor) [136]. A molecule that may find potential application in the treatment of NK/T-cell lymphomas is a selective JAK3 inhibitor defined by the acronym PRN371. It exhibits potent inhibitory activity against JAK3 by binding to Cys909 at the ATP binding site [137]. Experimental studies evaluating the effect of JAK-STAT inhibitors on γ/δ T lymphoma cells are also at the preliminary phase [138].

Summary

The presence of genetic defects within genes encoding components of the JAK-STAT signalling pathway is one of the basic mechanisms leading to the transformation of neoplastic precursor cells of lympho- and myelopoiesis. Currently, the assessment of the presence of *JAK2* gene mutations is a standard in the diagnosis of myeloproliferative neoplasms. Perhaps the assessment of mutations of other *JAK* and *STAT* genes will soon become a standard for the assessment of lympho- and myeloproliferative neoplasms, and the confirmation of their presence will be a requirement (criterion) for the diagnosis of their specific form. Demonstrating the presence of the mutations within the *JAK* or *STAT* genes will probably also change the therapeutic approach in neoplastic lympho- and myeloproliferative diseases in the nearest future, with the increasing use of inhibitors of the Janus family kinases and STAT proteins.

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Conflict of interest

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Acute promyelocytic leukemia: from genetic lesions identification to molecularly targeted therapy

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Abstract

Acute promyelocytic leukemia (APL) differs from other types of acute myeloid leukemia both in terms of the spectrum of clinical symptoms, as well as cytogenetic and molecular background. Fast diagnosis of APL enables highly effective targeted therapy initiation and avoiding of serious organ/ /tissue damage (including fatal bleeding into the central nervous system). In the initial diagnostic process the most important is the rapid identification of the presence of specific cytogenetic and molecular changes involving the retinoic acid receptor alpha (RARA) gene located on the 17q21 chromosome. In patients with APL, alongside the most commonly observed translocation t(15;17) (q24;q21) leading to the formation of the PML-RARA fusion, several dozen variant cases have also been identified as a result of other translocations involving RARA gene with different clinical symptomatology and variable sensitivity to the targeted therapy with all-trans retinoic acid and arsenic trioxide. The paper presents the recent data concerning the epidemiology, symptomatology and accurate diagnostics methods useful for early identification of APL and immediate initiation of the molecularly targeted therapy

Key words: acute promyelocytic leukemia, epidemiology, clinical manifestation, laboratory changes, karyotype anomalies, molecular defects, targeted therapy, all-trans retinoic acid, arsenic trioxide, measurable residual disease, epidemiology, prognosis

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Introduction

In the last few years, significant progress has been made in understanding the mechanisms of leukemic transformation of hematopoietic stem cells (HSC). It has been shown that this is a stepwise process, leading to transformation of HSC to the leukemic stem cells (LSCs), as well as the disruption of the mechanisms of normal hematopoiesis. The aforementioned changes resulted in increasing peripheral blood cytopenia, leading to an increased risk of severe infections due to granulocytopenia, bleeding complications due to thrombocytopenia/coagulopathy and changes related to infiltration by leukemic cells of other organs [1]. The mechanism of transformation is different for each cytogenetic-molecular subtype of acute myeloid leukemia (AML). However, it is known that AML cells exhibit features of a hierarchical organization with the presence of a different sub-populations of the unmaturated cells corresponding to LSCs. For this reason, in most cases of AML there is a phenotypic and molecular diversity of individual subpopulations of leukemic cells [2]. One of the best-known types of AML is acute promyelocytic leukemia (APL) in which the primary mechanism

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Table 1. Incidence of acute promyelocytic leukemia in the pediatric population by country (the percentage of all diagnosed acute myeloid leukemias) (sources [6, 7])

Percentage [%]	Country
< 5	Saudi Arabia, the Netherlands, Germany, Switzerland, Sweden
5–10	India, Israel, Japan, South Korea, Malawi, United States, Thailand, Turkey, Hungary, United Kingdom
10–15	Bolivia, Chile, Egypt, Finland, France, Malaysia, Oman, Poland, Russia, Serbia, Singapore, Taiwan, Ukraine
15–20	Argentina, Australia, Belarus, Brazil, China, Czech Republic, Greece, Spain, Iran, Canada, Mexico, Nepal, Nigeria, South Africa
> 20	Sudan, Tunisia, Iraq, Cuba, Nicaragua, Pakistan, Venezuela, Italy

of leukemic transformation is reciprocal, balanced translocation, involving the retinoic acid receptor alpha subunit (*RARA*) gene located on chromosome 17. The most common partner gene for *RARA* is the acute promyelocytic leukemia protein (*PML*) gene located on chromosome 15. The result of this translocation is the formation of *PML-RARA* fusion, leading to the inhibition of transcription of *RAR* target genes and disruption of the function of the *PML* gene as the regulator of homeostasis and tumor growth suppressor [3].

Epidemiology of APL

The incidence of APL varies with the age of patients and geographic region [4]. In the pediatric population, the prevalence of APL as a subtype of AML varies between countries. A detailed summary of the incidence of APL is presented in Table 1 [5–7]. So far, the reason for such significant differences in the frequency of APL in children in individual countries, as compared to all forms of AML, has not been established (Table 1).

In adults, AML account for 70–80% of all leukemias. Approximately 10% of all AML are APL cases [6, 7].

Influence of race and ethnicity on the incidence of APL

The influence of race and ethnicity on the incidence of APL is under discussion. There are observed differences in incidence between populations, but there is no clear evidence that one race is more likely to suffer from APL than another. In Canada, the incidence (expressed by the incidence rate, IR) of APL is extremely low. The IR of this form of AML is only 0.083/100,000 inhabitants per year. It is not known why the IR is so low. In Canada, healthcare is easily accessible in large agglomeration centers and all new cases of APL are accurately reported. Therefore, it is not possible that the incidence is underrated [8].

The IR of APL in the United States is higher than in Europe (0.22/100,000 inhabitants per year vs. 0.12/100,000 inhabitants per year, respectively) [9, 10]. The IR values for individual European countries are different. In Spain it is 0.257/100,000, in the Netherlands 0.174/100,000, in Sweden 0.145/100,000, and in Poland 0.023/100,000 inhabitants per year [9]. The low IR for Poland, as compared to other countries, may result, among others, from low availability of modern diagnostic techniques, especially genetic and molecular ones.

The incidence of APL has been shown to be high in the Hispanic populations (descendants of Hispanics) in the United States and Latin America. According to Douer et al. [11], APL in Hispanics accounts for as much as 37.5% of all acute myeloid leukemias, while in non-Hispanics only for 6.5%. A higher incidence of APL in Latin Americans was also confirmed by Yamamoto et al. For both Hispanic women and men, the IR values for APL were higher than for non-Hispanics, but the values for Hispanic women were significantly higher [10].

According to Matasar et al. [12], there are no significant differences in the incidence of APL throughout the whole life. Ethnic and racial differences in the incidence of APL are manifested primarily in particular age groups. Thus, in the 0–19 age group, the Hispanics have 1.94 times higher IR than non-Hispanic whites and 2.59 times higher IR than Africans and African Americans. In the 20–44 age group, the Hispanics have the highest IR value. This value is 1.55 times higher, as compared to non-Hispanic whites, 2.2 times higher than in the case of Africans and African Americans, and 1.67 times higher than that of Asians. In the 45–64 age group, the IR value does not differ statistically between particular races. In the +65 age group, non-Hispanic white people have an IR of 1.85 times higher than that of the Hispanics. The people of African and African American origin have the lowest IR for APL throughout their whole life. The differences in the IR values for white non-Hispanics, Hispanics, and Asians are not statistically significant. However, the presented results do not make it possible to unequivocally state that race is a factor particularly predisposing to APL [12].

Changes in the incidence of APL over the years

The progress in the field of diagnostic methods used in the recent years has clearly influenced the frequency of the APL diagnosis. According to Zhang et al. [5], the frequency of APL in the years 1992––2006 increased by 5.5% each year, as compared to the previous year. It was only after 2006 that the increase in the incidence reached a relative plateau [13]. It should be mentioned that no increase in the incidence of APL was observed during this period in Canada. The cause of it remains unknown [8].

The assessment of the effect of gender on the incidence of APL has also been the subject of comprehensive analyzes. Their results have shown that gender is not related to the incidence of APL [9, 10, 12]. However, it should be mentioned that in the 16–34 and 35–54 age group in Sweden, the rate of APL in women was much higher than in men. The situation was different in the oldest age group (+75 years of age), where the IR for men was higher than for women. A similar analysis which did not consider age showed that the incidence rate among women was higher (0.179/100,000 per year) than among men (0.113/100,000 per year) [14].

Interestingly, APL is most common among middle-aged people. According to a study by Venkitachalam et al. [15], 40% of new cases in the years 2000–2014 concerned people in the 40–59 age group, while in a study by Chen et al. in the years 1975–2014, the greatest number of new cases was observed in two age groups: 30–44 and 45–59 years (25,46% and 23,85%, respectively). Young adults (20–39 age group) suffer from APL more often than people over 60 years of age (32% and 28% of all cases in 2000–2014, respectively). People over 60 years of age account for approximately 28% of all new APL cases [13, 15].

Incidence of secondary APL

In most cases, the development of secondary acute promyelocytic leukemia occurs as a result of prior chemo- and/or radiotherapy. According to Duffield et al. [16], secondary APL accounts for 14% of all newly diagnosed APL. Most often, secondary APL develops as a result of treatment of other malignant neoplasms or as a result of chronic immunosuppression. Secondary APL usually develops within 3 years from the discontinuation of treatment. In the clinical picture, the morphology of promyelocytes and their immunophenotype do not differ from those found in *de novo* APL [16].

Clinical symptoms of acute promyelocytic leukemia

APL can occur at any age. However, the largest group of patients are middle-aged adults. People over 60 years of age are diagnosed with the disease less frequently. The disease occurs infrequently in patients who are younger than 10 years. In this group of patients, however, there have been described cases with atypical variants of genetic rearrangement, rarely seen in adulthood [17].

APL is characterized by a dynamic, severe and rapidly deteriorating clinical condition [18]. Common symptoms include infectious complications, hemorrhagic episodes, and weakness due to neutropenia, thrombocytopenia, and anemia, respectively. However, these symptoms are not typical of APL and may occur also in other types of AML. Other common symptoms include periodontal lesions, proliferative neutropenic ulcers of the oral mucosa with erosions and painful aphthae, impaired cellular immunity and activation of latent viral infections (i.e. the herpes zoster virus). Leukemic transformation of hematopoietic progenitor cells resulted in almost complete block of myeloid progenitors cells maturation and profound neutropenia, and thrombocytopenia in the peripheral blood. In their typical form, single blasts can also be present in the peripheral blood [17]. In the microgranular form of APL, usually associated with leukocytosis, their presence in the blood is typical. Depending on the morphology of peripheral blood cells, the following forms of the disease are distinguished with their characteristic clinical and laboratory symptoms:

"Typical" (hypergranular) form (AML-M3)

The typical form accounts for 60–70% of cases and is characterized by peripheral blood pancytopenia with distinct leukopenia which distinguishes this form from the hypogranular form. These laboratory changes may be accompanied by general symptoms, such as weakness and fatigue or weariness, as well as pale skin and symptoms of infection or hemorrhagic diathesis in the physical examination [19].

Hypogranular or microgranular form (AML–M3v)

The hypogranular form, unlike the previous one, is also accompanied by leukocytosis in the peripheral blood [20]. Patients with hyperleukocytosis may develop the symptoms of leukostasis as a result of clumping of leukemic blasts cells in

Table 2. Comparison of selected clinical morphological subtypes of acute promyelocytic leukemia according to the French–American–British (FAB) classification system of hematological diseases (source [20])

„Typical" form (ca. 60–70% of all cases)	„Microgranular" form (ca. 30–40% of all cases)
Morphological subtype AML–M3	Morphological subtype AML–M3v
Hypergranular promyelocytes	Hipogranular/microgranular promyelocytes
Leukopenia	Leukocytosis
Low risk of associated DIC syndrome	High risk of associated DIC syndrome

*DIC syndrome — disseminated intravascular coagulation syndrome

Table 3. Groups at risk of an unfavorable acute promyelocytic leukemia outcome (source [20])

Risk category	Stratification criteria	
	WBC [G/L]	PLT [G/L]
Low risk	≤ 10	≥ 40
Intermediate risk	≤ 10	< 40
High risk	> 10	–

WBC — white blood cells (total number of leukocytes in the blood); PLT — platelets (the number of thrombocytes in the blood)

within the small vessels and microcirculation. The result of this process are blood perfusion symptoms, especially affecting the central nervous system (CNS), and resulting in headaches and dizziness, tinnitus, focal symptoms or disturbed consciousness. They can also lead to abnormal pulmonary blood flow manifested by shortness of breath, and, in the severe form, respiratory failure. The presence of hyperleukocytosis is also associated with a higher 4-week mortality rate (24%), compared to the patient population without hyperleukocytosis (9%) [21]. Rare symptoms of this form of APL include ischemic low-flow (veno-occlusive) priapism (Table 2) [22].

The clinical picture of APL also includes disseminated intravascular coagulation (DIC) syndrome. The pathophysiology of DIC in APL patients is complex and involves many mechanisms of hemostasis. The occurrence of DIC symptoms in the course of APL may lead both to the thrombosis [23], and hemorrhage. DIC syndrome is the result of nonspecific activation of plasma blood coagulation caused by the release of azurophilic cytoplasmic granules from disintegrating leukemic cells with intrinsic thromboplastic activity [24]. One of the most important inducers of DIC is the tissue factor (TF) released in large amounts from leukemic blasts. The risk of developing DIC is highest in patients with the hypogranular or microgranular variant of APL (M3v) [17]. DIC is especially common in high-risk patients with the leukocyte count ≥ 10 G/L at diagnosis, and in patients +60 years of age [19, 20, 25] (Table 3).

The presence of increased symptoms of hemorrhagic diathesis (Latin: *diathesis haemorrhagica*) is typical for APL. In the clinical picture, it is manifested, among others, by small ecchymoses (disseminated mucocutaneous purpura), hemorrhages and extensive bruises, or bleeding from the mucous membranes, especially from the nose (Latin: *epistaxis*) and gums. Less frequently, there can be observed bleeding from the gastrointestinal tract and genital tract (*menorrhagia*) [26].

The activation of plasma fibrinolysis mechanisms caused by the release of urokinase (u-PA) and tissue type (t-PA) plasminogen activators (PA) and lysosomal enzymes by the leukemic cells is also important. The activation of fibrinolysis accompanying the DIC symptoms may also lead to the occurrence of central nervous system complications (e.g. hemorrhagic stroke). Their occurrence often makes it impossible to undertake a decision regarding the core treatment, having an adverse effect on the prognosis in this group of patients at the same time [27–29]. It is generally accepted that in these cases, due to the high risk of potential threat to life, it is necessary to implement urgent diagnostic and therapeutic procedures and initiate anti-leukemia treatment with vitamin A derivatives, before the final diagnosis of APL is made.

In untreated cases, severe hemorrhagic diathesis occurs due to secondary coagulopathy and abnormal platelet hemostasis ("consumptive coagulopathy") in which severe thrombocytopenia and hypofibrinogenemia play a key role [20, 28]. In the cases of APL complicated with fully decompensated DIC syndrome, the occurrence of hemorrhagic complications in the lungs and CNS often occurs even before the diagnosis and initiation of anti-leukemic treatment [19, 30, regardless of the profile of changes in the blood coagulation tests [31].

The rapid APL progression is associated with a high incidence of early deaths (ED). In APL, ED is defined as death within the first 30 days from the moment of diagnosis [19, 32]. In the past, before the introduction of targeted therapy, patients who were untreated or on corticosteroids died within the first 30 days of diagnosis. The median survival time was less than one week [33]. Presently, de-

spite the development of a therapy protocol based on all-trans retinoic acid (ATRA), arsenic trioxide (ATO) and anthracyclines, it is still a significant problem. It has been shown that nearly one third of patients diagnosed with APL dies within the first month, ca. 70–77% of which die within the first 7 days from the moment of diagnosis, and 30% within the same day. This makes ED the major cause of treatment failure. Despite the use of treatment with plasma derivatives and platelet concentrate, the incidence of early hemorrhagic deaths during induction therapy has not improved significantly in the recent years. It amounts to 20% and is comparable to that from 20 years ago [34]. An exception in this regard are patients treated in specialized clinical centers, where the mortality remains at the level of approximately 3–10%. It probably results from easier availability of rapid genetic diagnostics and pharmacotherapy. The studies carried out so far show particular effectiveness of therapy in patients receiving treatment according to the guidelines aimed at reducing bleeding and infectious complications or the differentiation syndrome (DS). The best results are achieved with rigorous adherence to current standards of care for supportive care and monitoring of blood parameters for platelet count, fibrinogen blood concentration, and international normalized ratio (INR) and activated partial thromboplastin time (APTT) [14, 32].

The occurrence of infectious and/or hemorrhagic complications in the fully developed phase of the disease, in the pre-diagnosis period and shortly after appropriate treatment, may lead to premature death. The main cause of death in this period are most often massive hemorrhages into the critical organs (56–69%), the most common of which are intracranial and intracerebral hemorrhages (64%), and less frequently pulmonary hemorrhages (32%) [7, 29, 35]. Also in these cases, most deaths occur within the first week after the diagnosis.

12–27% of patients die due to opportunistic infections within the respiratory tract, mainly bacterial and fungal, often associated with septic shock [14]. In this group, death occurs within 3 to 39 days from the introduction of treatment; the median survival time is 21 days. For these reasons, empiric and based on germ sensitivity test results antibiotic therapy is recommended in all cases as the general standard of care in the APL management. A rare cause of ED is multiple organ failure (6.9%) due to severe infectious and/or the DS [32, 36]. The clinical picture is rarely dominated by severe thrombotic/infarction complications (e.g. limb ischemia, myocardial ischemia, acute myocardial infarction, pulmonary embolism or cerebral venous thrombosis) as a complication of coagulopathy associated with the overexpression of proteins that promote the abnormal blood clotting, including TF or cancer procoagulant (CP) [23].

The occurrence of thromboembolic complications in patients with APL is a contraindication to invasive procedures (including central venous cannulation, lumbar puncture or bronchoscopy) [37]. In the event of thrombosis within the vascular catheters, they should be removed prior to the diagnosis of APL, immediately after the diagnosis of the disease [29].

The DS, formerly known as the retinoic acid syndrome (RAS), is also mentioned as a rare cause of early death. It represents a complex set of clinical symptoms occurring within one to two weeks after the initiation of the ATRA treatment. The data on its prevalence vary. This syndrome may affect up to 24–26% of patients, and according to some studies, up to one third of patients with APL [26]. The latest reports indicate that the risk of this syndrome exists in nearly half of the patients undergoing ATRA/ATO treatment [38]. In the course of DS, the interview and physical examination include fever, dyspnea, acute respiratory failure, hypotension, peripheral edema, and weight gain. The radiographic examination usually reveals pulmonary edema with interstitial infiltrates, and pleural and/or pericardial effusion [38]. Multiple organ failures may occur in the course of the syndrome, including liver failure, acute renal failure, and congestive heart failure [19, 20]. These symptoms may be accompanied by hyperleukocytosis. It should be noted that severe course of DS is the cause of 15–18.5% of ED [32].

The pathogenesis of DS is not fully understood. It has been suggested that its occurrence is related to the mechanism of action of the drug and the induction of the process of blast cell differentiation. Maturing cells release pro-inflammatory cytokines responsible for triggering the systemic inflammatory response (including fever and hypotension). One of the effects of their action is an increase in the capillary permeability, leading to the systemic capillary leak syndrome (SCLS) [26]. An important role is also attributed to the regulation of the expression of adhesion molecules (including CD13), promoting the adhesion of leukocytes to capillary endothelial cells, thus initiating a local inflammatory reaction. The ability of migrating leukocytes to infiltrate tissues and the potential risk of vascular occlusion by blood cell aggregates are the basis for the development of organ

dysfunction (including respiratory failure or renal dysfunction) [38].

Symptoms of organ infiltration with leukemic cells

In patients with APL, the CNS may also be affected by the leukemic process, but its exact incidence is unknown. The presence of infiltration is rarely seen within the CNS *de novo*. The infiltration of the CNS is much more frequent in the case of extra-medullary disease [39]. The presence of neurological symptoms (such as headaches and dizziness, nausea, vomiting, visual disturbances, photophobia, phonophobia, dysarthria, convulsions, hemiplegia, unstable gait, disturbance of consciousness) is an indication for head imaging (CT contrast, MRI) to rule out the CNS bleeding. If such bleeding is excluded, APL treatment should be immediately initiated, preferably with the use of drugs penetrating the CNS.

If infiltration of the CNS is suspected, the presence of neurological symptoms at the time of diagnosis or recurrence of the disease and the results of diagnostic imaging may be an indication for performing a lumbar puncture for diagnostic and therapeutic purposes [39]. However, it is debatable if it can be performed during coagulopathy. Current guidelines allow it in high-risk patients with hyperleukocytosis during remission. It allows to avoid the development of secondary bleeding complications and minimizes the risk of implantation of leukemia cells into the CNS [40].

Morphological diagnostics of acute promyelocytic leukemia

Based on the morphological image of leukemia cells, two variants of the disease can be distinguished according to the French–American–British (FAB) classification systems of hematologic diseases. The classic form — hypergranular (M3), which accounts for 60 to 70% of APL cases, and the hypogranular — microgranular variant form (M3v) [1, 2, 17].

The diagnosis of the hypergranular form is usually not difficult. Leukemia cells are characterized by an irregular cell nucleus and numerous, large basophilic granules and/or the presence of linearly arranged peroxidase-positive granules (Auer rods) in the cytoplasm. The diagnosis of the hypogranular form of APL is more difficult. Leukemia cells are characterized by the absence or presence of small, dispersed granules. The presence of Auer rods is also sporadic. There can be found atypical promyelocytes with bilobed nucleus in the blood smear. In the hypergranular APL form, the white blood cell count is lowered and in the hypogranular variant form, it is increased [7, 17, 41]. There has also been distinguished a version of the disease with the presence of atypical basophils, the presence of which was confirmed in one third of the patients with APL. This form of the disease is associated with a high risk of bleeding and increased mortality [42]. Depending on the different genetic variants, as compared to the classic type of APL, the morphological picture of leukemic cells is similar (with some exceptions) [17].

In cytochemistry, APL cells show a strong positive reaction to the presence of myeloperoxidase (MPO) and a positive reaction with Black Sudan B (SBB) [17]. According to the available data, 100% of APL cells show a strong MPO expression and a strong positive reaction with SBB. In 63% of cases, leukemia cells show a positive reaction to the presence of esterases (Es-chl) and in 45% of cases — a positive reaction with Schiff's reagent [43].

The most common changes in the laboratory tests in patients with APL are leukopenia and thrombocytopenia [17, 44]. In the presence of DIC syndrome, the prothrombin time and the activated partial thromboplastin time are prolonged [44].

When compared to other types of AML, laboratory test results in APL are more likely to show increased levels of fibrin/fibrinogen degradation products (FDP), D-dimers, and decreased levels of fibrinogen. In patients with APL, however, no differences in total iron binding capacity (TIBC) and transferrin levels were found, and they were within the normal range. Compared to patients with other types of AML, however, the TIBC values, blood transferrin levels, triglycerides and cholesterol levels are higher [45].

Changes in the immunophenotype of blasts in acute promyelocytic leukemia

Leukemic cells in APL are characterized by a strong expression of CD13+ and CD33+ and the lack of expression of CD34 and HLA class II antigen [7, 46]. In the hypogranular form of APL, there is a weak expression of HLA class II and CD34+. On the other hand, the expression of CD2+ is more frequent. In approximately 10% of patients, regardless of the form of APL, there can be observed the expression of CD117+, CD15+, CD56+ and CD64+ [17, 25, 47]. The expression of the CD2+ antigen is often accompanied by the expression of CD34+. The CD2+ expression carries a higher risk of death and a lower 5-year overall survival.

The presence of the CD34 antigen on leukemic blasts is also associated with poor prognosis [25].

According to the latest recommendations, the use of CD9+/CD11b+/HLA class II (–) antigens in the diagnosis of APL enables the diagnosis of APL with sensitivity amounting to 85% and specificity amounting to 95% . In turn, the combination of CD34+/CD117+/HLA class II (–) antigens enables the diagnosis of APL with the sensitivity of 39% and the specificity of 92%. Similarly, the diagnostic use of a CD11b+/HLA class II (–) antigen combination enables the diagnosis of the disease with the sensitivity and specificity equal to 80% [47]. Another study, where the cut-off was 20%, showed that the combination of CD117+/CD13+/ /CD56+/CD64+ antigens and MPO enables the diagnosis of APL with the sensitivity of 91.7% and the specificity of 93.1%, as compared to other types of AML without the expression of the HLA antigen class II [48].

Genetic basis of APL

In most cases, APL results from a reciprocal, balanced translocation of t(15;17)(q24;q21) involving the promyelocytic leukemia protein (*PML*) gene on chromosome 15q24 and the retinoid acid receptor alpha unit (*RARA*) gene on chromosome 17q21 [49]. In the case of the *PML* gene, the translocation area covers 35 kb and 9 exons, with the sequences 7a, 7b, 8a and 8b within exons 7 and 8, respectively. For the *RARA* gene, the size of the translocated material is 7.5 kb. In this case, 9 exons of the *RARA* gene are translocated, with the start codon within exon 2 and the stop codon within exon 9, encoding the sequence of 6 domains (A, B, C, D, F) of the RARA protein [50, 51]. In the case of the *RARA* gene, the break point cluster region is in intron 2. Within the *PML* gene, three break point cluster regions (bcr) have been identified: bcr1 in intron 6, bcr2 in exon 6 and bcr3 in intron 3. The cases with a break point cluster region within bcr1 and bcr3 account for 90–95% of all the cases reported for APL patients [52]. About 30% of APL patients also show other chromosomal abnormalities, including trisomy 8 [53]. The coexistence of other molecular defects with the t(15;17)(q24;q21) translocation have also been confirmed, among others, the FMS-like tyrosine kinase 3 (*FLT3*) gene mutation detected in 30–33% of APL patients (including *FLT3*-ITD in 13% of cases), *WT1* mutation in about 14% and *NRAS* in about 7–10% [54, 55].

So far, 17 new variant translocations involving the *RARA* gene have been detected in patients with APL. Their detailed molecular characteristics and the sensitivity to ATRA and ATO treatment are presented in Table 4 [52, 56, 57].

Proceedings in the event of suspicion of APL

According to the latest recommendations, if APL is suspected, treatment with ATRA should be started immediately. At the same time, molecular tests should be performed to confirm the presence/absence of the *PML-RARA* fusion and/or the translocation t(15;17). The presence of leukopenia or leukocytosis in the variant form of disease, the identification of promyelocytes in the peripheral blood smear, and blood coagulation disorder with the clinical and laboratory features of consumption coagulopathy, makes the diagnosis of APL very probable [56, 57].

The administration of ATRA induces the maturation and differentiation process of leukemic cells by changing the conformation of the *PML-RARA* fusion protein. A direct connection of ATRA to the RARA protein leads to the release of corepressors and cofactors of the process of the activation of the vitamin A-dependent gene(s) transcription process. Their result is the induction of the promyelocyte differentiation, a decrease in promyelocytes content in the bone marrow and in the peripheral blood, with a significant reduction in the severity of coagulopathy. The recommended daily dose of ATRA is 45 mg/m^2 of body surface area, administered orally in two equally divided doses. In patients with hepatic and/or renal insufficiency, the daily ATRA dose should be reduced to 25 mg/m^2 body surface area [58].

Remission-inducing treatment

It is possible to use one of several remission induction schemes in patients with APL. One of them is based on chemotherapy with the use of full doses of anthracyclines (TxA) in combination with ATRA.

The use of combination therapy is very effective and allows to achieve complete remission of the disease even in 90–95% of patients. Moreover, it has been shown that the use of the combination therapy with ATRA + TxA significantly reduces the frequency of relapses, as compared to monotherapy with ATRA or TxA alone [59]. Combination therapy has also been shown to be more effective than the crossover method [60]. An alternative protocol for the induction treatment is the AIDA regimen (ATRA + idarubicin). In this regimen,

Table 4. Clinical and cytogenetic and molecular characteristics of atypical forms of acute myeloid leukemia (sources [52, 56, 57])

Variant of the fusion with *RARA*	Translocation/ /chromosomal variant	Gene function	Clinical symptoms DIC	Sensitivity to ATRA	Sensitivity to ATO
ZBTB16-RARA	t(11;17)(q23;q21)	*ZBTB16* expression is associated with a number of cellular processes including differentiation, apoptosis, proliferation	Yes	Resistant	Resistant
NPM1-RARA	t(5;17)(q35;q21)	*NPM1* regulates the activity of many tumor growth suppressors, including MDM2, p53, and ARF. Participates in ribosome biogenesis, regulates protein synthesis by interacting with c-myc	Yes	Sensitive	No data
STAT5B-RARA	der(17)	Factor involved in signaling the JAK-STAT path, participates in the stimulation of cytokine and hormone dependent growth and differentiation	Yes	Resistant	Resistant
IRF2BP2-RARA	t(1;17) (q42;q21)	IRF2BP2 protein (interferon regulatory factor 2 binding protein 2) is responsible for the regulation of the transcription of genes dependent on type I interferon	In some cases	Sensitive	Sensitive
FIP1L1-RARA	t(4;17)(q12;q21)	Encodes a component of the complex involved in mRNA trimming and polyadenylation (mRNA processing)	No	Sensitive	No data
BCOR-RARA	t(X;17)(p11;q21)	*BCOR* interacts with the BTB-POZ domain of the BCL-6 protein causing the repression of its functions	In some cases	Sensitive	Insensitive
TBLR1-RARA	t(3:17) (q26:q21)	*TBLR1* gene (transduction β-like 1 X-linked receptor 1) plays a role in the activation of transcription, and the proteins expressed as a result of its action are an important element of the nuclear corepressor receptor (N-CoR) and histone deacetylase 3 (HDAC 3)	No data	Resistant	Sensitive
STAT3-RARA	der17	Factor involved in the JAK-STAT pathway signaling, participates in the stimulation of cytokine and hormone dependent growth and differentiation	No data	Resistant	No data
NUMA1-RARA	t(11;17)(q13;q21)	*NUMA1* is involved in the mitotic process by interacting with microtubules during the formation of the mitotic spindle	Yes	Sensitive	No data
PRKAR1A-RARA	t(17;17)(q21;q24), del(17)(q21;q24)	One of the main factors of type 1 protein kinase A, the main mediator of signal transmission via cAMP	No	Sensitive	Sensitive
NABP1-RARA	t(2;17)(q32;q21)	*NABP1* (nucleic acid binding protein 1) plays an important role in the response to DNA damage and the processes of maintaining genome stability	No	Sensitive	No data
GTF2I-RARA	t(7;17) (q26;q21)	*GTF21* (general transcription factor IIi) plays an important role in the processes of transcription, signal transmission by growth factors, and the function of the immune system	Yes	resistant	resistant
FNDC3B-RARA	t(3;17) (q26;q21)	Fibronectin type III domain containing 3B gene probably participates in the positive regulation of adipogenesis	No data	Sensitive	Sensitive
ADAMTS17- -RARA	Comment: *ADMTS17* gene (ADAM metallopeptidase with thrombospondin type 1 motif 17) is localized on chromosome 15q26.3	Unknown	No data	Sensitive	No data
TFG-RARA	t(3;14;17) (q12;q11;q21)	*TFG* gene participates in the trafficking from endoplasmic reticulum to the Golgi regulator	No	Sensitive	No data
Unknown *RARA*	t(4;17)(q12;q21)	–	No data	Resistant	No data
NUP98-RARA	der17	*NUP98* codes the nuclear pore complex (NPC) protein, regulates the nuclear-cytoplasmic transport of proteins and mRNAs	Yes	No data	No data

ATRA is administered at a dose of 45 mg/m^2/day and idarubicin (IDA) 12 mg/m^2 intravenously on days 2, 4, 6 and 8 of the treatment cycle. The administration of ATRA is continued until complete remission is achieved in the peripheral blood and bone marrow. The occurrence of side effects requires a reduction in the ATRA dose to 25 mg/m^2/day. Similarly, in patients +70 years old, it is recommended to reduce the number of IDA administrations to 3 days — i.e. on days 2, 4 and 6 of the treatment cycle [61, 62]. Recently, ATO has been applied in the induction treatment of APL. In lower concentrations, the drug induces the process of differentiation of leukemic cells, and in high concentrations, it induces the process of their apoptosis. The mechanism of action of ATO differs from that of ATRA. ATO binds directly to the PML fragment of the PML-RARA fusion protein, the consequence of which is the attachment of the ubiquitin-like protein SUMO to PML, initiating the process of SUMOylation and subsequent degradation of the PML-RARA protein. Theoretically, the use of ATO may eradicate clonogenic leukemia initiating cells (LIC) by stimulating their differentiation process. It should be remember that LIC constitute a small subpopulation of leukemic blasts, able to relapse even in remission of the disease [7]. The aforementioned properties of ATO have become the reason for an increase in its use in the treatment of patients with a relapse of the disease, as well as patients with the refractory form of APL. Monotherapy with ATO makes it possible to achieves a similar percentage of complete remissions as in the case of combined ATRA and TxA therapy [57]. The effectiveness of the induction treatment of combination therapy with ATRA and IDA was also compared with that of combination therapy with ATRA + ATO. In the latter case, the drugs were used in the following doses: ATO 15 mg/kg bw intravenously and ATRA 45 mg/m^2/day orally. It turned out that this therapy significantly improved the overall survival of patients with APL. The hematological toxicity also turned out to be low, having a direct impact on the risk of neutropenic infections. However, combination therapy turned out to be associated with a higher incidence of symptoms of hepatotoxicity, as compared to the standard ATRA and TxA induction regimen. The use of combined ATRA + ATO therapy is also possible in the elderly with comorbidities, including cardiac conditions which are a contraindication to the use of anthracyclines [63].

Consolidation treatment [64]

The most common form of consolidation treatment is the administration of 2–3 cycles of treatment with TxA + ATRA [57]. Its application enables molecular remission (MR) in 90–99% of patients. Autologous hematopoietic cell transplantation or reinduction with ATO may be considered in patients in whom the *PML-RARA* transcript is still detectable in PCR after the consolidation treatment [65].

Post-remission maintenance treatment

Obtaining MR with the consolidation treatment does not protect against relapse. To prevent relapse in patients treated with the ATRA + + TxA regimen, two cycles of the ATO + ATRA combination treatment are recommended. If, for some reason, this is not possible, the maintenance therapy consists of a triple therapy: mercaptopurine (MP), methotrexate (MTX) and ATRA [23]. Each maintenance treatment cycle begins between 2 and 4 weeks after the last cycle of consolidation therapy, as soon as the neutrophil count increases ≥ 1,0 G/L, and the platelet counts is ≥ 100 G/L. The treatment with ATRA + ATO lasts 12 weeks and the treatment regimen is as follows:

- ATO: 0.15 mg/kg/day intravenously, 5 days a week, with a 2-day break. The treatment is continued for 4 weeks followed by a 2-week break;
- ATRA: 45 mg/m^2/day, orally, in two divided doses for 2 weeks daily, followed by a 2-week break. In order to avoid the symptoms of pseudotumor cerebri in people under 20 years of age, the ATRA dose should be reduced to 25 mg/m^2/day.

Treatment of APL relapse

Relapse after complete remission is usually defined as hematological, extra-medullary, or molecular disease reoccurrence. A hematological relapse should be diagnosed when the number of blasts or atypical promyelocytes in the marrow is > 20% or when the number of blasts or atypical promyelocytes in two bone marrow samples taken one week apart is > 5%. The confirmation of APL relapse in these cases is the presence of the *PML-RARA* fusion. An extra-medullary relapse should be diagnosed when the leukemic infiltrates and/or leukemic cells are found in the cerebrospinal

fluid or other tissues or organs. Extra-medullary APL relapse should also be confirmed by immunophenotyping and/or molecular tests. A molecular APL relapse is defined as the reappearance of a *PML-RARA* positivity in two consecutive bone marrow samples taken at any time after the end of treatment. The persistence of a positive PCR result for *PML-RARA* after the end of treatment is the criterion of resistance to the treatment [20, 29]. In patients with persistent residual disease at the molecular level or molecular APL relapse, emergency treatment, including hematopoietic stem cell transplantation, should be introduced immediately. In these cases, ATO + ATRA therapy should be considered if ATRA + TxA/idarubicin were used as the first line of treatment. If ATO + ATRA was used as the 1st line of treatment, a treatment consisting of ATRA + TxA (idarubicin) can be introduced. Therapy with anti-CD33 monoclonal antibodies (GO, gemtuzumab ozogamicin) may also be considered.

Results of clinical trials in the treatment of acute promyelocytic leukemia

The introduction of ATRA to the treatment algorithms has significantly improved the long term outcome of APL patients. It is especially evident over the past two decades. Its use reduced the 5-year mortality from 82% to 36%. This was made possible thanks to the use of a combination therapy involving the administration of ATRA, cytarabine (Ara-C), ATO and anthracyclines [daunorubicin —(DNR) or IDA]. The use of combined therapy resulted in complete remissions (CR) rate 90–95%, and the cure rate exceeded 80%. Despite high CR rates, treatment-related mortality and relapse frequency were still high (10% and 20–30%, respectively). The aforementioned data necessitated the search for new drug combinations, the use of which will enable not only the achievement of CR, but also reduce the treatment related mortality and the frequency of APL relapses [66, 67].

Recently, the use of the aforementioned oral form of arsenic called the Realgar-Indigo naturalis formula (RIF) has been very popular. The results of its application were summarized by Sasijareonrat et al. in 2020 [68], based on a systematic review of MEDLINE and EMBASE databases. The meta-analysis included only the results of randomized and control group trials. The final analysis included 482 patients, 258 treated with RIF and 224 patients treated with the classic intravenous form of ATO. The chances of achieving CR were numerically higher in the group receiving the oral form of the drug, but this difference was not statistically significant. Also, the 30-day mortality, event-free survival (EFS), and overall survival (OS) rates were higher in the RIF-treated patients. However, these differences were not statistically significant. Interestingly, the risk of APL recurrence was higher in the group treated with the oral form of arsenic. Also in this case, the difference was not statistically significant. The incidence of side effects did not differ significantly, as well. These results may indicate that the use of ATRA in combination with the oral form of arsenic in patients with low to intermediate risk of an unfavorable APL course is at least as effective as intravenous administration of the drug [68].

A meta-analysis published in 2016 compared five previously used *de novo* treatment strategies of APL [69]. The efficacy of the following therapeutic strategies was assessed: ATO + ATRA, RIF with ATRA, ATRA + anthracycline, ATO and ATRA. The assessment was based on the results of 14 randomized controlled trials including 1407 newly diagnosed APL patients in the years 1998–2015 (ATRA + ATO, n = 537, RIF + ATRA, n = 117, ATRA + TxA, n = 297, ATRA, n = 346 and ATO, n = 110). 98 cases (7%) who had died before the 30th day from the initiation of treatment were excluded from the analysis. The analysis showed no significant differences between the assessed treatment regimens in terms of the percentage of the achieved CR. Taking into account the cases of early death and the percentage of achieved CRs, the most effective treatment method was the combination of ATO-ATRA and RIF-ATRA, followed by ATRA + TxA, and monotherapy with ATO. The lowest efficacy of treatment was confirmed with the use of ATRA as monotherapy. The evaluation of long-term treatment outcomes showed that the use of ATO-RIF in combination with ATRA improves OS and EFS, as compared to ATRA in combination with an anthracycline in patients with low and medium risk. On the basis of this observation, the authors of the study concluded that the treatment regimen of ATO in combination with ATRA and RIF in combination with ATRA may be considered optimal in the treatment of newly diagnosed cases of APL and should be recommended as the first-line therapy [69].

Abaza et al. [68] demonstrated that the combination of ATRA with ATO is better than the administration of ATRA in combination with chemotherapy in patients with newly diagnosed APL with low or moderate risk of an unfavorable course of the disease. It also turned out that adding GO to

the above-mentioned therapy regimen improved the effectiveness of treatment in high-risk and low-risk patients in the case of leukocytosis in the course of ATRA treatment (CR rate 96%). The mortality in this group of patients was estimated at 4%. It also turned out that as many as 45% of low-risk patients required the administration of GO or IDA due to leukocytosis during the ATRA treatment. After a mean follow-up of 47.6 months, the 5-year progression free survival (PFS), relapse free survival (RFS) and S were 85%, 96%, and 88%, respectively. This study confirmed the purposefulness of using ATRA and ATO with or without GO in patients with newly diagnosed APL.

Another meta-analysis conducted by Li et al. in 2017 [69], assessed the effectiveness of combination chemotherapy in patients with APL. The study group consisted of 7566 newly diagnosed patients, and the median follow-up time of the evaluated patients was 49.24 months (the follow-up ranged from 12 to 121 months). After the induction treatment, 89.77% of patients achieved CR in a mean period of 38.25 days. The mortality during the induction of remission was estimated at 6.34%. The CR rate after induction of ATRA, ATO and DNR was determined to be 96.16%, for ATRA-DNR it was 94.3%, for ATRA-DNR-cytarabine it was 92% and for ATRA--idarubicin it amounted to 91.2%. The total relapse rate was estimated at 14.4%. The analysis confirmed an inverse relationship between the number of leukocytes and the percentage of the obtained CRs. In this analysis, the 5-year OS and 5-year RFS were 86.41% and 75.42%, respectively [67].

It should be mentioned that the results of randomized clinical trials do not always reflect the results of treatment in real life conditions. Sobas et al. analyzed 283 unselected patients with APL treated in 20 Polish hematology centers in the years 2005–2017. All patients were treated according to the ATRA-based PETHEMA protocol regimen in combination with anthracycline chemotherapy. The 4-year OS rate was 67% and the EFS rate was 4%. The ED rate was estimated at 20.1%, and the pretreatment death rate was 3.5% (10 patients). The main causes of ED were hemorrhages (45.6%), infections (17.5%), and the DS (14%). Out of 273 treated patients, 214 (78.4%) achieved hematological remission. Two patients (0.7%) were resistant to the treatment. 47 (17.2%) patients could not be assessed due to ED. For 6 (3.7%) patients, response data could not be obtained. The conducted analyzes showed that the factors predisposing to ED and OS were: the patients' performance status assessed according to the Eastern Cooperative Oncology Group (ECOG) scale > 2, +60 years of age and the occurrence of bleeding before the start of treatment. An additional factor that had a negative impact on EFS was the number of white blood cells > 10 G/L. This study confirmed that the main factor influencing the end result of the APL treatment was early mortality [36]. The introduction of ATRA and ATO in the classic form of APL significantly improved the early results of the APL treatment. A problem which has not been solved in this group of patients is the relatively high frequency of disease relapses, especially among high-risk patients, and the high mortality in the case of relapse in patients previously treated with ATO. Another problem is the relatively frequent (20–25%) occurrence of the DS, directly related to the treatment used [38]. For these reasons, patients with risk factors predisposing them for relapse should be eligible for autologous stem cell transplantation (auto-SCT). Recently published registry data show that the post-transplant course of the disease in patients undergoing auto-SCT in the second complete hematological remission is better than in the case of allogeneic hematopoietic cell transplantation (allo-SCT), both in terms of leukemia free survival (LFS) as well as OS [70].

In some cases, achieving molecular remission in APL is difficult and requires additional treatment. An excellent example is obtaining MR in a young patient with the ider(17)(q10)t(15;17)(q22;q21) variant of the disease in whom CR was confirmed after one month of treatment with ATRA + ATO, with negative *PML-RARA* fusion test result after adjuvant DNR + cytosine arabinoside therapy [71].

Measurable residual disease assessment

Measurable residual disease (MRD) is a term that reflects the presence of persistent malignant cells after treatment that are not detectable by cytomorphological evaluation methods. MRD determines the number of leukemic cells in proportion to normal cells and is usually in the range of $1{:}10^4$ to $1{:}10^6$. By comparison, a standard disease assessment using morphological microscopic techniques detects one leukemia cell per 20 white blood cells [72]. The MRD assessment also concerns patients who achieved complete hematological remission defined as a decrease in the percentage of blast cells in the bone marrow smear < 5%, the absence of Auer rods/promyelocyte morphology; the absence of extramedullary leukemia foci and restoration of normal hematopoiesis in all hematopoietic cell lines [73].

One of the most widely used techniques to evaluate MRD is multicolor flow cytometry (MFC). The following characteristics of leukemia cells can be assessed by flow cytometry: side-scattered light (SSC), forward scatter (FSC) and cluster differentiation molecules (CD), as well as cytoplasmatic cluster differentiation molecules (cCD). It is possible thanks to the use of monoclonal antibodies coupled with various fluorescent dyes [74]. Distinguishing leukemia cells from normal hematopoiesis cells is possible due to the presence of an aberrant set of surface/cytoplasmic antigens on leukemic cells — the leukemia-associated immunophenotype (LAIP). If LAIP cannot be used, it is also possible to identify MRD by comparing the phenotype of the patient's marrow cells with the phenotype of normal marrow cells and selecting a population of cells with a different distribution of CD markers expression. After the identification of the patient-specific immunophenotype of APL cells, further monitoring of MRD is possible at any stage of the treatment [75]. The MFC method allows to obtain quick results with relatively low evaluation costs. The sensitivity of this method ranges from 10^{-3} to 10^{-5}. Unfortunately, the sensitivity of MRD monitoring with MFCs is often reduced due to the lack of differences in the expression of individual antigens on leukemia cells and their corresponding normal cells. Another problem affecting the reliability of the MFC MRD assessment is, among others, immunophenotypic instability of the leukemic clone which leads to the formation of several cell subpopulations with different phenotypes (resistance, disease relapse). This observation contributed to the use of a wide panel of antibodies in these cases. Despite its common use, the disadvantage of the MFC is also its lack of standardization in APL cases. It makes it impossible to compare MRD levels between particular laboratories, and to draw general conclusions about the relationship between the MRD levels and the risk of disease relapse.

The *PML-RARA* transcript assessment in total RNA originated from the bone marrow cells enables the diagnosis of molecular APL remission (molecular remission) [76]. MRD monitoring in acute myeloid leukemia — both at the cytometric and molecular level, is of great practical importance. The presence of MRD is an independent prognostic factor and correlates with the risk of disease relapse [72, 77, 78]. A high MRD level after the completion of induction therapy is associated with a poorer prognosis [79]. MRD assessment also makes it possible to recognize an imminent APL relapse and to initiate rescue therapy early [80]. Currently, attempts are being made at standardizing the MRD assessment using highly sensitive techniques including real-time polymerase chain reaction (RQ-PCR), reverse transcription polymerase chain reaction (RT-PCR), droplet digital PCR (ddPCR) and next generation sequencing (NGS) [76, 78, 81]. Unfortunately, the standardization of the above-mentioned methods has not been fully carried out so far. For this reason, their introduction into routine clinical practice remains a challenge. PCR and ddPCR tests are time-consuming (e.g. in the case of RT-PCR about two days), laborious and require appropriately trained personnel. However, molecular MRD assessment is widely used in laboratory monitoring of patients with APL. The preferred material for MRD monitoring is bone marrow mRNA. It has been shown that the analysis of peripheral blood mRNA samples is characterized by much less sensitivity [82]. Molecular MRD assessment is applicable in approximately 95% of APL cases positive for the *PML-RARA* fusion [82, 83]. Regular assessment of its presence is the basis of molecular monitoring of APL relapse after the end of treatment [77]. However, confirming its presence at the molecular level is not always associated with a rapid relapse of the disease. Some of the patients remain in long-term remission of the disease despite the detectable PML-RARA transcript [84]. MRD monitoring also enables making optimal therapeutic decisions, minimizing the possible risk of over-treatment and avoiding symptoms of excessive toxicity. The PETHEMA research group distinguished three groups at risk of an unfavorable course of the disease depending on the MRD level: high with MRD $\geq 0.1\%$, intermediate with MRD $< 0.1\%$ and $\geq 0.01\%$, low with MRD $< 0.01\%$. According to the guidelines of the National Comprehensive Cancer Network (NCCN Guidelines® from October 2020), a test confirming the achievement of molecular remission should be performed using PCR and the material obtained from the bone marrow biopsy after consolidation is completed. Post-treatment follow-up should also include the evaluation of MRD by the means of PCR every 3 months for 2 years to detect molecular APL recurrence. It particularly applies to high-risk patients over the age of 60, patients with long breaks in consolidation therapy, and those who do not tolerate maintenance therapy. Clinical experience shows that the risk of disease relapse in low-risk patients who are in molecular remission after the consolidation is complete is low. Therefore, MRD monitoring may not be necessary

in these cases. If the test result is positive, the test has to be repeated within 2–4 weeks. If the second test is also positive, molecular recurrence of APL should be recognized and appropriate treatment initiated. If the result of the second test is negative, it is recommended to monitor the presence of MRD every 3 months for 2 years. The test should be carried out in the same laboratory to ensure the same level of sensitivity [85]. MRD monitoring with NGS enables the examination of not only specific transcripts, but also potentially other *RARA* fusion transcripts [76]. The NGS method allows quantitative assessment of the MRD level, and thus an insight into the dynamics of MRD decrease/increase in patients with the classic form of APL and in the case of variant forms. Tests based on the detection of fusion show greater sensitivity, compared to tests based on the detection of specific fusion transcripts by means of PCR. It stems from the fact that leukemia cells have different expression levels and therefore different levels of certain transcripts. This can lead to an overestimation or an underestimation of the MRD level. Different patients may have different expression levels of certain fusion transcripts, which affects the sensitivity of the MRD assays [76]. However, the high cost of the NGS studies is also important [86]. An alternative method of quantifying the bcr1 and bcr3 transcripts of the *PML-RARA* fusion is the ddPCR. It shows a similar or higher sensitivity, as compared to the previously described PCR techniques. The main advantage of this method is absolute quantification and the ability to assess MRD when it is below the detection limit for other PCR methods [81].

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Adverse events of monoclonal antibodies use in therapy of hematological malignancies

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Abstract

Monoclonal antibodies given as monotherapy or combination therapy have emerged as effective treatment options for hematologic malignancies. By prolonging survival, mAbs reduced mortality and improved the clinical prognosis for patients with these diseases. However, despite the effective anticancer activity of mAbs, they induce adverse events. The most common side effects are infusion related reactions (IRR), associated with cytokine release within the first few hours after administration. IRR are usually mild to moderate and manifest in rash, fever, nausea, vomiting, dizziness, headache, hypotension or tachycardia. Other, common toxicities are cytopenias, increasing the risk of infections and bleeding. Most preventive strategies involve the use of glucocorticosteroids, acetaminophen, antihistamines, screening for antibodies against microorganisms and prophylaxis for infections. Cytokine release syndrome, cardiac, pulmonary, neurologic adverse effects occur less frequently. In cases of grade 1–2 toxicity, symptomatic management is recommended, but in more severe symptoms temporary or permanent discontinuation of therapy and use of glucocorticosteroids are recommended. In an effort to limit the incidence and severity of adverse events clinicians should know how to early recognize, precisely assess and timely manage.

Key words: monoclonal antibodies, hematological malignancies, adverse effects

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Introduction

Over recent years, tremendous progress in identifying therapeutic targets in hematological malignancies has been observed, leading to the discovery of new drugs with effectiveness proven in clinical trials [1]. Monoclonal antibodies (mAbs) bind to specific molecules on immune cells and activate various signaling pathways in the immune system. They may contribute to antibody-dependent cellular cytotoxicity (ADCC) though natural killer (NK) cells, antibody-dependent cellular phagocytosis (ADCP) though macrophages, or complement-dependent cytotoxicity (CDC) [2]. mAbs have revolutionized the treatment of hematological malignancies improving clinical outcomes. Nevertheless, this rapid development of therapy is accompanied by toxic effects, most of which are interdisciplinary in nature and a challenge for both hematologists and intensive care physicians [3]. Biomarkers of adverse events (AEs) induced by mAbs are not identified, therefore clinicians of all disciplines should be aware of the toxicity associated with mAbs therapy. This will increase the chances of effective outcomes optimization with the use of these agents in hematological malignancies.

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Rituximab

Rituximab (RTX) is a monoclonal antibody directed against the CD20 antigen present on B lymphocytes surface, indicated in the treatment of B-cell lymphoma, lymphoproliferative disorders and some autoimmune diseases [4]. Infusion related reactions (IRRs) are commonly reported AEs following the use of RTX. In clinical trials, they were reported in 77% of patients with non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL) [4]. Intravenous administration of RTX has been associated with reactions such as rash, fever, nausea, vomiting, dizziness, headache, hypotension and tachycardia. Therefore, the infusion should be performed slowly and the patient's condition should be monitored during and after completion of infusion [5]. To reduce the risk of RTX side effects, premedication should include glucocorticosteroids, paracetamol, and an antihistamine [4]. Most of the IRRs were recorded during the first RTX infusion within 1–2 hours of the infusion, and their incidence decreases with subsequent infusions. B-cell targeted therapies, including RTX, contribute to a B-cell reduction, hypogammaglobulinemia and an increased risk of infections [3, 6]. Hepatitis B virus (HBV) reactivation induced by combining RTX with chemotherapy has been reported, therefore, screening for chronic and previous HBV infection, including hepatitis B surface antigen (HBsAg) and antibodies against HBV should be performed. Other infections seen in studies were exacerbations of hepatitis C (HCV, hepatitis C virus), herpes virus infections, including herpes zoster virus (HZV), human herpes virus 3 (HHV-3), varicella zoster virus (VZV), as well as progressive multifocal leukoencephalopathy (PML). According to the European Conference on Infections in Leukemia, prophylaxis against *Pneumocystis jiroveci* pneumonia (PJP) is recommended in patients undergoing R-CHOP therapy (RTX, cyclophosphamide, doxorubicin, vincristine and prednisolone) [6]. For this reason, administration of immunoglobulins is also indicated in most patients receiving RTX and other anti-CD20 drugs in the treatment of B-cell neoplasms [3]. The mechanism of action of anti-CD19 therapy is similar to that of anti-CD20 antibodies, so their use also requires immunoglobulins substitution [3]. In studies involving NHL and CLL patients, combining RTX with chemotherapy was not associated with an increased incidence of infections, but with higher risk of grade 3–4 hematological complications — leukopenia, neutropenia and pancytopenia, compared to chemotherapy alone [4]. However, age ≥ 70 years was a risk factor for severe hematological AEs and bacterial infections in patients with previously untreated or relapsed/refractory (R/R) CLL treated with RTX in combination with chemotherapy. Late-onset neutropenia is also possible — up to 5 months after the end of therapy in patients treated with RTX [6].

Apart from IRR and infections, RTX can cause mucocutaneous complications, arrhythmias, renal and gastrointestinal dysfunction [5]. Decrease in the number of CD20+ lymphocytes can cause ileitis, as their presence in the gut is considered a protective factor and potentially preventing inflammation [7]. Therefore, therapy with anti-CD20 RTX may lead to dysregulation of T-reg lymphocytes and autoreactive stimulation of T lymphocytes, contributing to ileitis or exacerbation of inflammatory bowel disease (IBD).

An alternative to the intravenous form of RTX is subcutaneous RTX [5]. This new form of the drug has been approved by the US Food and Drug Administration (FDA) for the treatment of adults with follicular lymphoma (FL), a diffuse large B-cell lymphoma (DLBCL) and CLL. Compared to intravenous infusion, the advantage of this therapy is the reduction of the time of drug administration, time spent in the clinic and increased patient comfort [5]. It has been shown that AEs resulting from subcutaneously administered RTX in patients treated for FL, CLL or DLBCL were similar [5]. Due to lower costs compared to the original drugs, equivalent biological drugs biosimilars have become more and more widely used, including RTX biosimilar [8]. In a cohort study with NHL and CLL patients, the incidence and severity of AEs were similar for both formulations.

Obinutuzumab

Obinutuzumab is also an anti-CD20 antibody with the potential to overcome the mechanisms of RTX resistance. However, this drug is considered to be more toxic than RTX [9]. Irina Amitai et al. meta-analysis reported an increased risk of grade 3–4 AEs, including infections, IRR, thrombocytopenia and cardiac events after the use of obinutuzumab, compared to RTX [10]. The risk of infection is increased by the underlying disease, comorbidities that are common in the elderly, and the chemotherapy regimen used. Despite the high risk of infectious disease, there was no difference in the incidence of severe neutropenia (grade 3–4) between RTX and obinutuzumab. Obinutuzumab

in combination with venetoclax in CLL therapy is associated with grade 3–4 neutropenia with a frequency of 53–73% [11]. The development of thrombocytopenia and IRR is associated with the release of interleukins (ILs): IL-8, IL-6 and tumor necrosis factor alpha (TNF-α) as well as with cytokine release syndrome (CRS) [10]. Severe CRS, observed in patients treated with obinutuzumab, results in a higher rate of cardiotoxicity compared to RTX. In addition, the use of obinutuzumab with chemotherapy has been associated with the development of secondary hematological malignancies [10].

Brentuximab vedotin

Brentuximab vedotin (BV), mAb conjugated with monomethylated auristatin E (MMAE), a very potent anti-microtubule agent, directed against the CD30 receptor, is used in the treatment of classical Hodgkin lymphomas (cHLs) and peripheral T-cells lymphomas [12]. In the treatment of lymphoma patients, BV increased the risk of severe AEs in the form of peripheral sensory neuropathy, nausea, vomiting, and diarrhea. Peripheral neuropathy, a common side effect of BV (observed in about 60% of patients) manifested by numbness and tingling in the limbs, is induced by the toxic effect of MMAE on axonal microtubules [13]. It is also possible to develop neuropathy with immunological pathogenetic factors. Although the use of BV in combination with standard chemotherapy (ABVD: adriamycin, bleomycin, vinblastine, dacarbazine) in the first-line treatment in advanced cHL resulted in a high percentage of complete remission (CR), a high percentage of pulmonary toxic effects was also observed [14]. Studies show, that limiting co-administration of bleomycin with BV reduces the risk of pulmonary toxicity. However, BV combined with AVD resulted in a higher rate of neuropathy and neutropenia compared to BV-ABVD [15]. In order to prevent hematological complications, especially neutropenia associated with BV + ABVD therapy, administration of granulocyte colony stimulating factors (G-CSFs) is recommended. In addition, prevention of cytomegalovirus (CMV) infection by viral prophylaxis or plasma CMV PCR testing should be considered in patients receiving BV.

BV can be used in combination either with chemotherapy, or with immunotherapy. In phase II study with combination of two new drugs — BV and nivolumab — in the treatment of R/R HL patients, peripheral neuropathy was a troublesome side effect [16]. Neurological symptoms can be related to neuropathy, but also to PML. For this reason, in the case of neurotoxicity symptoms, in-depth differential diagnosis is essential. Exacerbation of neurotoxicity may lead to treatment discontinuation, so careful observation of behavior and neurological signs and symptoms in patients undergoing therapy is indicated [6].

Blinatumomab

Blinatumomab, as a bispecific mAb, binds to the CD3+ T-cell and the CD19+ B-cell, leading to T-cells activation as well as B-cells apoptosis and lysis [17]. The release of perforin and granzymes from granules in cytotoxic T-cells induces cytotoxicity of target B-cells and therefore is applicable in R/R therapy of acute lymphocytic leukemia (ALL). Antigen-antibody interaction stimulating T-cell activation induces toxic effects. The major, serious AEs of blinatumomab in R/R ALL therapy are neurological adverse events (NAEs) and CRS.

Antibody targeting to CD19 molecules is presumed to be the cause of neurotoxicity due to similar symptoms in patients treated with T-cells with chimeric antigen receptors cell therapy (CART) targeting CD19 [17]. The neurological toxicity observed with blinatumomab treatment is believed to be related to the production of neurotoxic cytokines and chemokines upon activation of T-cells, which leads to irritation of the neurendothelium.

It is assumed, that antibody targeting CD19 molecules causes neurotoxicity due to similar symptoms in patients treated with T-cells with CART targeting CD19 [17]. The neurological toxicity observed with blinatumomab treatment is believed to be related to the production of neurotoxic cytokines and chemokines upon T-cells activation, which leads to irritation of the neuroendothelium [18]. Neurotoxicity can manifest as nonspecific symptoms — headache, tremors, confusion or aphasia, convulsions or dementia. In the TOWER study with 405 R/R ALL patients, the proportion of NAEs of any grade was higher in the blinatumomab group than in the standard of care chemotherapy (SOC) group (61% vs. 50%, respectively). NAEs were more likely to cause discontinuation of therapy in the blinatumomab arm (6% vs. 1%). Occasionally, especially in the case of grade 1–2 toxicity, dexamethasone may reduce the severity of the neurological side effects without discontinuing blinatumomab treatment. In the case of grade 3 NAEs, treatment must be interrupted for at least 3 days, but grade 4 NAE is confirmed, blinatumomab should be permanently discontinued. An important strategy to prevent AEs associated with the use of

blinatumomab is to gradually increase the dose of blinatumomab from 9 μg/day in the first week of therapy to 28 μg/day from the second week to the end of treatment.

CRS manifests itself with fever, chills, fatigue, low blood pressure, and symptoms related to capillary leak syndrome (CLS). The incidence of any grade CRS was higher in R/R ALL patients than in MRD-positive ALL patients (16% vs. 3%) due to the difference in tumor burden between groups. Prophylactic management include dose modification, discontinuation of blinatumomab treatment, cytoreduction, and dexamethasone. Additionally, intravenous fluids and tocilizumab, an IL-6 inhibitor, can be used [19]. If grade 3 CRS is diagnosed, blinatumomab treatment may be resumed after discontinuation and administration of dexamethasone. In the case of grade 4 toxicity, treatment discontinuation is recommended [17]. Due to the mass production of cytokines such as IL-1β, IL-2, IL-6 and TNF-α, CRS is considered a risk factor for cardiac dysfunction and hemophagocytic lymphohistiocytosis.

The drug targeting CD19 molecules induces decrease of plasma cells and T lymphocytes count, as well as neutropenia [17]. Interestingly, the incidence of cytopenia and serious infections was lower in the blinatumomab group than in the chemotherapy arm (60% vs. 70% and 34% vs. 52%, respectively). Nevertheless, blinatumomab has been associated with catheter-related bloodstream infections, therefore patients receiving the drug should be carefully monitored for signs and symptoms of infection. Moreover, CRS, infections and febrile neutropenia were more common AEs of blinatumomab administered in second or later line of treatment compared to patients treated previously, while neurological toxic effects and neutropenia were more frequent during first-line treatment [19]. Also, serious AEs and treatment-related fatal events were more frequently reported in the group receiving blinatumomab as a second or subsequent line of therapy. However, these results may be due to the higher stage of the disease and worse prognosis of patients receiving drug in the later treatment lines.

Inotuzumab

Inotuzumab ozogamicin (InO) is a humanized anti-CD22 antibody combined with the alkylating agent calicheamicin used in the treatment of R/R ALL and NHLs. Release of calicheamicin into lysosomes in the cytoplasmic cell leads to double-stranded DNA cleavage and subsequent cell apoptosis [20, 21]. However, the direct toxic effect of the antibiotic is suspected of damaging liver cells, contributing to the inhibition of sinusoidal flow. Sinusoidal obstruction syndrome (SOS) is a serious complication after allogeneic stem cell transplantation (allo-SCT) and a symptom of hepatotoxicity [17]. It is believed that the higher incidence of SOS in pediatric patients (under 18 years of age) than in adults may be due to incomplete structural maturation of hepatic vessels in infancy. Earlier hematopoietic stem cell transplantation (HSCT), the use of other anticancer drugs and elevated levels of hepatic transaminases [aspartate aminotransferase (AST), alanine aminotransferase [ALT]) before HSCT are risk factors for SOS associated with the use of InO [22]. The clinical classification of SOS includes the level of total bilirubin, liver enzymes, serum creatinine, weight gain and the clinical progression rate [1]. Compared to hyperbilirubinemia and elevated transaminase level, severe SOS has less common and less specific symptoms, such as hypoxia, encephalopathy, and renal failure [17]. A liver biopsy is the best diagnostic method, but may result in bleeding due to thrombocytopenia. Prior to initiating mAb therapy, risk factors such as previous myeloablative conditioning, older age, and history of liver disease should be assessed. The risk of SOS can be reduced by using fractionated mAbs and extending the time between the last dose of InO and the HSCT [20].

Additionally, bilirubin and transaminase levels should be monitored during InO therapy. According to studies in which defibrotide improved survival rates in patients with veno-occlusive disease (VOD), it is believed that defibrotide may be an effective agent in the treatment of VOD [21]. In order to reduce the toxicity of the drug, it is worth avoiding nephrotoxic and hepatotoxic drugs such as azoles, prophylactically using ursodeoxycholic acid and considering combination therapy [1]. Consolidation therapy in the form of blinatumomab in the mini-HCVD-INO-blinatumomab regimen may extend the time interval between InO and allo-SCT and further reduce the risk of VOD [23]. While VOD is one of the frequently reported toxic effects of InO, the most frequently reported serious AEs during ALL and NHLs therapy were thrombocytopenia and neutropenia, with at least grade 3 events occurring in 29% and 48% of patients, respectively. Interestingly, the incidence of cytopenia was similar during CART therapy (CAR19/22). Neutropenia was reported more frequently in patients with a median age ≥ 60 years

or in patients with NHLs. Although the incidence of neutropenia in the treatment of R/R B-cell ALL was also high, infections were less frequently reported in this group of patients than in the group treated with chemotherapy. To prevent cytopenia, it is recommended to monitor complete blood count (CBC) before each InO cycle and react in the case of infection, sepsis or bleeding appearance.

Other AEs associated with InO treatment are QT prolongation and tumor lysis syndrome (TLS) [17, 21]. Prolonged QT interval may increase the risk of torsades de pointes and sudden cardiac death and therefore requires prompt intervention [1]. Electrolytes and concomitant use of drugs due to comorbidities may also affect the electrocardiogram, therefore special ECG monitoring is recommended when using drugs that prolong the QT interval [1].

Gemtuzumab ozogamicin

The addition of gemtuzumab ozogamicin (GO) to standard chemotherapy for acute myeloid leukemia (AML) resulted in an increase in event-free survival (EFS), relapse-free survival (RFS), but also reducing the risk of nausea, vomiting, diarrhea and grade 3–4 hepatotoxicity [24]. GO is a cytotoxic calicheamicin-conjugated recombinant humanized anti-CD33 mAb approved for the treatment of AML. Due to conjugation with calicheamicin, hepatotoxicity is a common AE of GO, as during InO therapy. VOD, a potentially fatal condition was reported more frequently in AML patients treated with GO compared to R/R B-cell NHL patients treated with InO (9% vs. 1%), but less frequently than in R/R ALL patients treated with InO [1]. However, the use of fractionated GO, reduced intensity of conditioning, and maintaining an interval of at least 60 days between the last administration of GO and HSCT may be responsible for a similar incidence of post-transplant VOD/SOS in the group treated with GO combined with chemotherapy compared to the group not treated with GO, which suggests the possibility of using HSCT in the consolidation therapy of AML patients previously treated with GO [25]. In randomized ALFA-0701 study, the main toxicity associated with the use of GO combined with chemotherapy (daunorubicin and cytarabine) was thrombocytopenia, lasting 45 days after starting treatment, reported in 20% of patients [26]. Treatment of GO is also associated with the risk of myelosuppression, IRR and TLS [27]. Prevention of TLS consists of hydration, use of hypouricemic drugs, renal replacement therapy and correction of electrolyte disturbances. It is important to monitor patient general condition, electrolytes blood levels, and initiate TLS treatment promptly as TLS-related ionic and metabolic abnormalities can result in renal failure, cardiac arrhythmias, and death. In order to increase stability and efficacy of calicheamicin-conjugated antibodies, GO and InO, in the treatment of hematological malignancies, modified conjugates were developed by direct binding of reduced calicheamicin thiol to modified cysteine on the antibody [28]. This is a promising form of therapy due to the fact that the altered drug has been proven to be less toxic in animal studies.

Daratumumab

Daratumumab is an anti-CD38 antibody used as monotherapy or in combination with traditional treatment regimens for multiple myeloma (MM) in adults [6, 29]. The most common AEs associated with newly diagnosed (NDMM) and relapsed/refractory multiple myeloma (RRMM) were IRRs, mostly mild, grade 1–2 and usually occurred during the first administration or within 4 hours of infusion completion [6, 29, 30]. Despite the well-known drugs used in premedication, such as glucocorticoids, antipyretics and antihistamines, according to Chari et al., montelukast, a leukotriene receptor antagonist can significantly reduce the risk of IRR. The use of subcutaneous daratumumab is associated with similar efficacy, safety and a lower IRR prevalence compared to the intravenous form [29, 31].

Other frequently reported AEs were diarrhea, cytopenia and respiratory infections [1, 29]. The hematotoxicity index may increase due to the synergistic effect of concomitantly administered drugs. VZV infection was a more frequent adverse effect in combination therapy than in monotherapy, but the incidence of neutropenia and all infections was similar in both groups [6]. In seropositive patients, infection prophylaxis should be considered.

Daratumumab may induce hematotoxicity via the cytopenia, but it may also disrupt the binding of red blood cells to CD38 [29]. This interaction can lead to general *in vitro* reactivity, a positive indirect Coombs test (ICT) and a delay in blood transfusion performance. Dithiothreitol (DTT) is added to the blood sample to reduce binding of daratumumab to blood cells, breaking extracellular binding to CD38. Another approach is to neutralize daratumumab with recombinant human anti-daratumumab antibodies, but their use is still limited. In a study

evaluating the efficacy and safety of daratumumab with bortezomib and dexamethasone (D-Vd) versus bortezomib and dexamethasone (Vd) in patients with MM first relapse, peripheral neuropathy (50 vs. 38% for DVd and Vd) and secondary malignancies (6% vs. 2% for DVd and Vd) were observed slightly more often.

Elotuzumab

Elotuzumab, mAb directed against signaling lymphocyte activation molecule family 7 (SLAM F7) in plasma cells is used in the treatment of RRMM. The most serious AEs (grade ≥ 3) following administration of elotuzumab with thalidomide and low dose of dexamethasone were fatigue, peripheral edema and IRR [31]. According to several studies, elotuzumab was less likely to cause lymphopenia in RRMM and was considered less toxic than daratumumab [32]. In therapy with thalidomide and dexamethasone, the most common side effects were fatigue, pulmonary edema and IRR [31]. Elotuzumab in combination with lenalidomide and dexamethasone (Elo-RD) in RRMM therapy reduced the risk of disease progression and death with a similar frequency of toxic effects [33]. The primary grade 3–4 AEs were lymphocytopenia (79%), neutropenia (36%), infections (33%), and thrombocytopenia (21%). The higher incidence of elotuzumab AEs such as infections and newly diagnosed malignancies is believed to reflect the longer duration of treatment. When elotuzumab was added to z pomalidomide (Elo-PD), therapeutic efficacy was demonstrated with a similar degree of toxicity to Elo-RD therapy. Neutropenia, pneumonia and infections were less frequent, while thrombocytopenia and cardiac events were slightly more frequent. Although elotuzumab is considered an effective treatment strategy for RRMM, there is a need for further studies on the efficacy and safety of combination therapies with various drugs.

Alemtuzumab

Alemtuzumab is mAb directed against the CD52 surface antigen, which is expressed on both normal and malignant B and T lymphocytes [34]. Its selective action has been used in the treatment of B-CLL, NHLs, T-cell prolymphocytic leukemia (T-PLL), mycosis fungoides (MF) and Sézary syndrome (SS) [35, 36]. Due to the induction of a deficiency of B and T lymphocytes, the drug is immunosuppressive, increasing the risk of infection. Immunodeficiency may appear up to 9 months after stopping treatment [6]. Grade 3 lymphocytopenia was the most common hematological toxicity (59%) of alemtuzumab in a phase II trial in adult patients with T-cell leukemia/lymphoma (ATL), a disease characterized by lymphocytosis [35]. However, infections were less common (14%), possibly due to PCP, antiviral, and antifungal prophylaxis. On the other hand, alemtuzumab + CHOP used in the treatment of peripheral T-cell lymphoma (PTCL) resulted in serious infections (40%), including CMV and EBV, despite the applied prophylaxis [37]. Due to the risk of severe immunosuppression and infectious complications, it is recommended to perform antibody screening and appropriate prophylaxis of diseases such as latent tuberculosis, HBV reactivation, HCV and opportunistic infections [6, 34, 36]. Further studies are needed to assess whether new drugs used in the prophylaxis of CMV, valganciclovir or letermovir and posaconazole against fungal infections can more effectively prevent the toxicity of alemtuzumab [37]. EBV reactivation and newly diagnosed DLBCL have also been reported in patients with PTCL treated with alemtuzumab, therefore vigilance should be exercised against the development of EBV + DLBCL as a comorbid condition.

The immune system, through the release of cytokines, can lead to a skin rash that can be prevented with antihistamines and paracetamol [34]. Glucocorticosteroids (GCS) are necessary for more severe events and IRRs. CRS with an increased concentration of TNF-α, INF-γ in the serum and IL-6 or infiltration of T lymphocytes in the heart may contribute to cardiotoxicity, especially in patients with MF/SS [38]. Despite these known complications, the use of alemtuzumab to prevent graft-versus-host disease (GvHD) may trigger autoimmune reactions such as pure red cell aplasia (PRCA) or autoimmune hemolytic anemia (AIHA) [39].

Mogamulizumab

Although alemtuzumab and BV appear to be effective therapeutic options for cutaneous T-cell lymphoma (CTCL), there are still studies ongoing on the use of antibodies with more satisfactory efficacy and less toxicity in the treatment of T-cell lymphoma [40]. One of the newest drugs is mogamulizumab, mAb directed against the CC chemokine receptor 4 (CCR4). The target of the drug's action is present on the surface of tumor cells in most ATL patients, some patients with other PTCL and CTCL subtypes, but also on the surface of effector T-reg cells, which have the greatest inhibi-

tory effect on the anti-tumor immune response. The IRR, major AE of mogamulizumab is thought to be due to a defucosylated Fc region on IgG1, which strongly activates NK cells and causes the release of cytokines and cytotoxic molecules [25, 41, 42]. Other common toxicities include rash and hematological complications, neutropenia and lymphopenia, considered as an expected effect related to the therapeutic target of mogamulizumab [25, 40, 42]. Serious dermatological AEs, such as Stevens-Johnson syndrome or toxic epidermal necrolysis, are rare but potentially life-threatening or even fatal. In patients undergoing allo-SCT, the use of mogamulizumab increases the risk of acute GvHD. Therefore, it is recommended to monitor T-reg counts and postpone transplantation for at least 50 days after the last administration of mogamulizumab. This is very important because allo-SCT is an important therapy for both advanced CTCL and ATLL. Serious toxicity with mogamulizumab also includes autoimmune AEs, mostly reversible with glucocorticosteroids. The use of mogamulizumab in patients with autoimmune diseases is relatively contraindicated due to the mechanism of action, T-reg cell depletion, and severe grade ≥ 3 AEs previously reported, including immune-related myositis, myocarditis, polymyositis, hepatitis, pneumonitis or Guillain-Barré syndrome [41].

Immune checkpoint inhibitors

Immune checkpoint inhibitors (ICIs) by blocking signaling pathways such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death protein-1 [PD(L)-1] molecules, allow anticancer and immune response [6]. Malignant hematological cells may also become targets for antibodies in cancer immunotherapy due to the expression of immune checkpoint molecules on their surface [43, 44]. The FDA has approved two molecules for the treatment of hematologic malignancies, e.g. nivolumab (anti-PD-1 mAb) for the treatment of R/R cHL and pembrolizumab (anti-PD-1 mAb) for the treatment of R/R cHL as well as R/R primary mediastinal large B-cell lymphoma (PMBL) [45]. These agents are also promising drugs in the treatment of MM, certain types of NHL, CLL, AML, and myelodysplastic syndromes (MDS) [45, 46]. However, ICIs mechanism of action, stimulation of the immune system and autoimmunity can lead to immune-related adverse events (irAEs) that can occur in any system and organ in the body, heart, lungs, skin or endocrine system. These toxic effects include myocarditis, pneumonia, kidney or liver toxicity.

The most common side effects of ICIs therapy of hematological malignancies are dermal toxicities. They manifest as rash, dermatitis, erythema nodosum, but can also cause skin necrosis or Stevens-Johnson syndrome. Combination therapy with nivolumab and other targeted drugs increased the risk of serious toxic effects. irAEs affect the balance of the endocrine system and can disturb the functioning of endocrine organs. Thyroid dysfunction was similar for nivolumab and pembrolizumab, and hypothyroidism (0–29% and 0–17%) was more common than hyperthyroidism (0–13% and 0–17%). In addition, hyperthyroidism usually progressed to organ failure and required constant hormone replacement therapy. Adrenal insufficiency and type 1 diabetes have also been observed. Gastrointestinal (GI) disorders, the most common of which was diarrhea, were more frequently caused by anti-CTLA-4 antibodies than by anti-PD(L)-1 antibodies. A rare but potentially dangerous complication of ICI therapy is cardiotoxicity and pulmonary toxicity, more common with anti-PD(L)-1 antibodies. Pneumonitis, which may manifest itself even 10–12 weeks after the initiation of ICIs therapy, is the leading cause of deaths associated with the use of ICIs in the treatment of hematological malignancies and solid tumors. In addition, some studies suggest that treatment with ICIs increases the risk of GvHD in patients with bone marrow malignancy undergoing HSCT.

Overexpression of PD-L1 on AML blasts and PD-1 on T lymphocytes has prompted the study of ICIs in some hematological neoplasms [46]. However, increased PD-L1 expression on leukemic blasts may be due to the use of interferon gamma (INF-γ) and induction chemotherapy. The use of ICIs in patients with AML after or before alloHSCT resulted in GvHD, but resolved in most of them after administration of glucocorticoids [47].

ICIs have been used in the treatment of leukemia, lymphoma, but also MM [48]. Compared to patients treated with lenalidomide and dexamethasone, the addition of the third drug, e.g. pembrolizumab to lenalidomide and dexamethasone in first line treatment of MM resulted in a similar frequency of any grade AEs (94% vs. 92%) and a higher rate of serious toxic effects (54% vs. 39%). In addition, the risk of death in the pembrolizumab group was higher than in the lenalidomide and dexamethasone group, but these patients were older and had a high cytogenetic risk. This study showed an unfavorable risk profile in patients with NDMM. However, other drug combination regimens, taking into account the patient's clinical

condition, may increase the efficacy of ICI therapy in hematological malignancies, including MM.

The results of studies with ICIs in the treatment of solid tumors have shown that these drugs cause serious bacterial, viral, fungal infections or PJP. However, it has not been clearly proven whether the use of glucocorticosteroids and infliximab to inhibit the development of irAEs increases the risk of infection [6]. Due to the use of ICIs in combination with chemotherapy, monoclonal antibodies or CART, an increased risk of therapy side effects is suspected. More research is needed on their effectiveness with the lowest possible toxicity.

Immunotherapy with ICIs may be continued in the case of mild grade 1 irAEs [1]. If the toxicity grade is higher, treatment should be discontinued until the toxic symptoms are resolved or permanently. The evaluation and management of irAEs treatment is based on the Common Terminology Criteria for Adverse Event (CTCAE) v5.0. Usually, the use of immunosuppressants like GCS as the first-line drug or infliximab for colitis and mycophenolate for hepatitis results in a reduction in the severity of the toxic effects of therapy [1, 6]. The management of AEs induced by ICIs and other antibodies used in the treatment of hematological malignancies is presented in Table 1.

Summary

Monoclonal antibodies are highly effective in the treatment of hematological malignancies, which is confirmed by the improvement of treatment outcomes and response rates in numerous studies. However, they can cause side effects manifested by infusion-related reactions as well as myelosuppression, infections and autoimmune diseases. While using monoclonal antibodies in hematological malignancies, clinicians should be aware of potential side effects, identify and treat toxic effects as early as possible to mitigate adverse effects of therapy and optimize treatment outcomes.

Conflict of interest

None.

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Table 1. The main adverse events of monoclonal antibodies (mAbs) used in therapy of hematological malignancies and prophylaxis and treatment suggestions

Side effect	Clinical manifestation	Prophylaxis and treatment suggestions
Infusion-related reactions	Fever, chills, hypotension, tachycardia, sore throat, cough, nausea and vomiting Severe reactions — bronchospasm, shortness of breath, hypoxia and hypertension	• The drug should not be administered to patients with hypersensitivity to the active substance or any of the excipients • Slow drug infusion under close clinical observation, including heart rate, blood pressure and temperature monitoring • The use of premedication — glucocorticosteroids, antipyretic drugs, antihistamines • The addition of montelukast may reduce the risk of adverse reactions of daratumumab therapy • Using an available subcutaneous form of the drug (rituximab/ /daratumumab) instead of the intravenous one reduces the risk of infusion reactions and is more comfortable for the patient • If toxicity occurs, interrupt the infusion and depending on the severity of the infusion-related reactions, discontinuation of the infusion or the use of glucocorticoids and antihistamines should be considered
Infections (especially: hepatitis B, C virus, *Pneumocystis jiroveci* pneumonia, herpes zoster, JC virus)	Pyrexia, asthenia, local symptoms, neurological symptoms of progressive multifocal leukoencephalopathy	• Screening for hepatotropic viruses and prophylaxis should be performed before starting treatment • Consider prophylaxis against pneumocystosis, herpes virus, screening for fungal infections if there are other risk factors such as concomitant use of fludarabine, alemtuzumab, immunosuppressants, or previous invasive fungal infection • Optionally immunoglobulins can be given • Asymptomatic or mild symptoms — clinical or diagnostic observations • Moderate symptoms — oral antibiotics, antifungal or antiviral medications • Severe symptoms — intravenous antibiotic, antifungal or antiviral intervention indicated

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Table 1 (cont.). The main adverse events of monoclonal antibodies (mAbs) used in therapy of hematological malignancies and prophylaxis and treatment suggestions

Side effect	Clinical manifestation	Prophylaxis and treatment suggestions
Tumor lysis syndrome	Symptoms resulting from hyperuricemia, hyperkalemia, hyperphosphatemia, renal failure, arrhythmias, convulsions	• Prophylaxis — allopurinol and rehydration • Treatment — hydration, use of hypouricemic drugs, renal replacement therapy, correction of electrolyte disturbances
Cytokine release syndrome	Pyrexia, chills, hypotension, tachypnea, fatigue, cardiotoxicity	• Mild symptoms — supportive treatment can be used • Severe symptoms — treatment discontinuation, intravenous fluids, vasopressors, glucocorticosteroids, and tocilizumab (an IL-6 inhibitor)
Hepatic veno-occlusive disease/ /hepatic sinusoidal obstruction syndrome	Painful ascites, jaundice, weight gain, hypoxia, edema, varicoses, encephalopathy	• Prophylactic administration of ursodeoxycholic acid in patients treated with gemtuzumab ozogamicin at high risk of hepatotoxicity • Optimization of intravascular volume with crystalloid or colloid solutions (e.g. albumin) without fluid overload, colloid solutions in the case of hypoalbuminemia • Avoidance of nephrotoxic and hepatotoxic drugs • High doses of glucocorticoids for moderate to severe cases • In patients with clinically significant liver disease, as well as prior hematopoietic cell transplantation, the benefits and risks of administration of inotuzumab/gemtuzumab should be balanced • Use lower, fractionated doses of both gemtuzumab and inotuzumab • Avoid conditioning regimens containing two alkylating agents • An interval of at least 3 months between discontinuation of gemtuzumab therapy and the hematopoietic cell transplant • Defibrotide in patients with hepatic veno-occlusive disease undergoing stem cell transplantation and optionally adding high doses of glucocorticosteroids
Cardiotoxicity	Arrhythmias, changes in blood pressure	• ECG monitoring • Serum electrolyte levels • Withdrawal of drugs that interfere with heart rhythm • Symptomatic treatment • Withdrawal of medications that affect heart function • Correction of electrolyte disturbances
Hematological toxicity — anemia, leukopenia, thrombocytopenia	Asthenia, infections, hemorrhage/bleeding	• Monitoring of complete blood count prior to drug administration and performing physical examination and clinical tests for signs and symptoms of infection, bleeding, hemorrhage, and other symptoms of myelosuppression • Dose delaying or permanent therapy discontinuation, and supportive care are recommended to control myelosuppression • Dithiothreitol may be helpful in preventing of daratumumab binding to white blood cells
Neurotoxicity	Headache, tremors, confusion or aphasia, convulsions or dementia, changes in sensation, movement	• Mild symptoms — observation • Moderate symptoms — discontinuation of therapy and glucocorticosteroids • Seizures grade ≥ 3 — treatment interruption and anti-epileptic drugs recommended • Mechanical hyperventilation, acetazolamide or mannitol
Autoimmune reactions	Selective red cell aplasia, autoimmune hemolytic anemia, immunological side effects in multiple organs, most commonly affecting the skin and thyroid gland	• In most cases the use of systemic steroids or other immunosuppressants such as infliximab or mycophenolate • Mild cutaneous toxic effects, grades 1–2 — topical emollients/ /steroids and/or antihistamines, grade 3 — oral or intravenous steroids and temporary treatment discontinuation, grade 4 is a life-threatening condition associated with symptoms such as erythema, purpura or epidermal detachment, requiring rapid administration of intravenous steroids and permanent discontinuation of therapy • Blood glucose, electrolytes, TSH, and fT4 tests before each infusion, • In the case of subclinical/symptomatic hypothyroidism, substitution with thyroid hormones should be considered • In symptomatic patients, especially with hyperthyroidism, treatment with beta-blockers should be initiated, carbimazole or steroids are rarely required

JC — John Cunningham; IL-6 — interleukin-6; ECG — electrocardiogram; TSH — thyroid-stimulating hormone; fT4 — free thyroxine

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