OFFICIAL JOURNAL OF THE POLISH SOCIETY OF CLINICAL ONCOLOGY

Oncology

IN CLINICAL PRACTICE

2020, Vol. 16, Number 5

ISSN 2450–1654

VIA MEDICA

Oncology in Clinical Practice 2020, Vol. 16, Number 5, 207–300

ONCOLOGY
IN CLINICAL PRACTICE

Official Journal of the Polish Society of Clinical Oncology

https://journals.viamedica.pl/oncology_in_clinical_practice

Oncology in Clinical Practice (ISSN 2450–1654) is published six times a year by
VM Media sp. z o.o. VM Group sp.k.
ul. Świętokrzyska 73, 80–180 Gdańsk, Poland
Phone: (+48 58) 320 94 94, fax: (+48 58) 320 94 60
e-mail: redakcja@viamedica.pl,
http://www.viamedica.pl, wap.viamedica.pl

19-0214.015.001

Editorial Address
Klinika Nowotworów Płuca i Klatki Piersiowej
Narodowy Instytut Onkologii im. Marii Skłodowskiej-Curie — Państwowy Instytut Badawczy
ul. Roentgena 5, 02–781 Warszawa, Poland
Phone: (+48 22) 546 21 69
e-mail: sekretariat4@pib-nio.pl

Indexed in Index Copernicus (ICV 2019 = 89.65), Ulrich's Periodicals Directory and CAS.

According to the statement of the Polish Ministry of Science and Higher Education publication in the journal has been awarded with 20 points.

Editorial policies and author guidelines are published on journal website: http://journals.viamedica.pl/oncology_in_clinical_practice

The journal "Oncology in Clinical Practice" is financed under Contract No. 790/P-DUNdem/2019 by the funds of the Minister of Science and Higher Education for the science promotion activities.

Ministerstwo Nauki i Szkolnictwa Wyższego

ONCOLOGY
IN CLINICAL PRACTICE

Official Journal of the Polish Society of Clinical Oncology

https://journals.viamedica.pl/oncology_in_clinical_practice 2020, Vol. 16, Number 5

GUIDELINES FOR DIAGNOSTIC AND THERAPEUTIC MANAGEMENT IN MALIGNANT NEOPLASMS

EXPERTS' OPINION

ORIGINAL ARTICLES

Professor Krzysztof Krzemieniecki Award for the best case report accepted for publication

Case Report Contest Policies

This policy defines the scope, requirements and regulations regarding **The Krzysztof Krzemieniecki Award** for the best case report published in "Oncology in Clinical Practice" (OCP) Fifth Edition.

1. The aim of the contest is to encourage submission of quality case reports related to oncological practice and to promote them in the scientific deliberations.
2. All respective manuscripts submitted to OCP between June 1st, 2020 and May 31st, 2021 and accepted for publication will qualify.
3. Manuscripts should be prepared in line with Authors' guidelines and should be submitted only through the manuscript system available at Journal's website: https://journals.viamedica.pl/oncology_in_clinical_practice
4. All submitted manuscripts will be evaluated during the peer review process and authors will be informed about their qualification for publication in OCP. Accepted papers will be evaluated by the Contest Committee based upon fulfillment of the Contest criteria as well as practical significance, originality, applicability and addressing of current/critical concerns.
5. The first author of the winning paper will be eligible for a prize of gross 1000,00 Euro gross (one thousand euro).
6. Results will be announced during the XXIV National Congress of The Polish Society of Clinical Oncology and subsequently at the Journal website.
7. Winner will be notified via email.
8. Contest Committee may exclude a paper from participation in case of potential conflict of interest or ask submitting author for adequate clarifications.
9. The Sponsor at any stage and in any respect, will not participate in the evaluation of entries and selection of a winning paper.
10. The award amount shall be paid based on the copyright transfer agreement to the paper.
11. These Regulations are the sole and exclusive document defining the principles and conditions for the Contest. In all matters not regulated, decisions are made by The Organizer.

Contest Organizer:
VM Media sp. z o.o. VM Group sp. k., seated at 73 Swietokrzyska Street, 80-180 Gdansk, Poland (Register of Entrepreneurs kept by the District Court for Gdansk, Commercial Division VII of the National Court Register under KRS No 0000266430, VAT Reg. No PL 583-28-39-187).

Patronage NOVARTIS

Breast cancer

Editors:
Jacek Jassem, Maciej Krzakowski

Authors:
Jacek Jassem[1], Maciej Krzakowski[2], Barbara Bobek-Billewicz[3], Renata Duchnowska[4], Arkadiusz Jeziorski[5], Wojciech Olszewski[6], Elżbieta Senkus-Konefka[1], Hanna Tchórzewska-Korba[7], Piotr Wysocki[8]

[1]Department and Clinic of Oncology and Radiotherapy, Medical University of Gdańsk, Poland
[2]Department of Lung and Chest Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[3]Department of Radiology and Imaging Diagnostics, Maria Skłodowska-Curie National Research Institute of Oncology, Branch in Gliwice, Poland
[4]Oncology Clinic, Military Institute of Medicine, Warsaw, Poland
[5]Department of Oncological Surgery, Medical University of Lodz, Poland
[6]Department of Pathology and Laboratory Diagnostics, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[7]Department of Rehabilitation, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[8]Department and Clinic of Oncology, Jagiellonian University *Collegium Medicum*, Krakow, Poland

Key words: breast cancer, diagnostics, treatment, recommendations

Table of contents

Translation: dr n. med. Dariusz Stencel
Oncology in Clinical Practice 2020, Vol. 16, No. 5, 207–260. DOI: 10.5603/OCP.2020.0038, copyright © 2020 Via Medica, ISSN 2450–1654

According to the authors and editors, this report contains the most justified principles of diagnostic and therapeutic procedures prepared considering the scientific value of evidence and category of recommendations. These principles should always be interpreted in the context of an individual clinical situation. The recommendations do not always correspond to the current reimbursement rules in Poland. In case of doubt, the current possibilities of reimbursement of individual procedures should be established. The quality of the evidence and recommendations categories were defined according to the following criteria:

1. *The quality of scientific evidence*
   I — Scientific evidence obtained from well-designed and conducted randomized clinical trials or meta-analyses of randomized clinical trials
   II — Scientific evidence obtained from well-designed and conducted prospective observational studies (non-randomized cohort studies)
   III — Scientific evidence obtained from retrospective observational studies or case-control studies
   IV — Scientific evidence obtained from clinical experiences and/or experts, opinions
2. *Category of recommendations*
   A — Indications confirmed unambiguously and absolutely useful in clinical practice
   B — Indications probable and potentially useful indications in clinical practice
   C — Indications determined individually

Epidemiology, etiology and primary prevention

Breast cancer is the most common malignancy in women in Poland (18,529 cases in 2017; 19.7% of all malignancies; standardized incidence rate 53/100,000) [1]. The number of breast cancers in men is around 140 cases per year. Breast cancer in Poland is the second cause of cancer-related mortality among women after lung cancer (6,670 deaths in 2017; 17.4% of all deaths; standardized mortality rate 15/100 000).

The cause of the majority of breast cancer remains unknown. The most important risk factor is sex, then older age, and the following: carriage of mutations of some genes (primarily *BRCA1* and *BRCA2*), family history of breast cancer, especially at younger age, early menarche, late menopause, late age at first full-term birth, long-term hormonal replacement therapy, especially containing estrogen-gestagen combination, long-term hormonal contraception (to a small extent), overweight and obesity in the postmenopausal period, exposure to ionizing radiation (radiotherapy to the chest area before the age of 30 years) and some benign breast proliferative diseases (atypical hyperplasia, preinvasive breast lobular carcinoma). The individual risk of developing breast cancer can be estimated with the use of various methods, of which the Gail scale is best known. It takes into account the age at which the first menstrual period occurred, the number of previously performed breast biopsies, the presence of atypical hyperplasia in the biopsy sample, the age at which the first live birth occurred, breast cancer in first-degree relatives and age. This method is helpful in the qualification of women at high risk of developing breast cancer for preventive actions.

The possibilities of primary breast cancer prevention are limited. Modifiable risk factors include avoiding overweight and obesity, physical activity, restriction of alcohol consumption, avoiding smoking, and reducing the use of hormone replacement therapy (HRT). The management of very high-risk groups is presented in the section on genetic counseling.

Recommendations

— To reduce the risk of breast cancer it is recommended:
- avoiding overweight and obesity (II, B);
- physical activity (II, B);
- restricting of alcohol consumption (II, B);
- avoiding smoking (III, B);
- reducing the use of hormone replacement therapy (I, A).

Screening tests

Screening mammography (MMG) is the only effective method for early detection of breast cancer. A properly planned and conducted screening program reduces breast cancer-related mortality, with the greatest reduction in the risk of death in the 50–70 age group. A recent British study showed a beneficial effect of screening MMG started at the age of 40 [2], but its role in this age group remains debatable. This goal can be achieved provided that a large portion of the target population is included, these tests are linked with the cancer registry, they are performed according to the guidelines, and their quality and effectiveness are under systematic control. A prerequisite for a rational screening program is the ability to monitor and evaluate it in accordance with international quality standards [3]. Routine MMG (both screening and diagnostic) are performed in 2 basic projections (oblique and upper-lower).

Table 1. Categories of final conclusions according to the BIRADS system (concerns MMG, USG and MR)

Category	Description	Comments
0	Incomplete evaluation	Additional imaging tests necessary
1	The normal breast histology	The probability that the lesion is malignant 0%
2	Benign lesion	The probability that the lesion is malignant 0%
3	Probable benign lesion	The probability that the lesion is malignant exceeds 2%. The standard management is observation and control tests. BIRADS 3 in MMG and US is currently an indication for a follow-up after 6 months and if the change is stable — after the next 6 months, then after 12 months and the next 12 months
4	Suspicious abnormality	The probability that the lesion is malignant ranges between 2% and 95%; microscopic verification recommended In MMG and US, category 4 is divided into 3 subcategories: 4A — probability of malignant lesion > 2% to ≤ 10% 4B — probability of malignant lesion > 10% to ≤ 50% 4C — probability of malignant lesion > 50% to ≤ 95%
5	Malignant lesion	The probability that the lesion is malignant exceeds 95% Necessary microscopic verification. The negative result of microscopic examination does not exclude the malignant character of the lesion and does not exempt from the necessity of performing an operation. If induction systemic therapy is anticipated, histopathological diagnosis is required based on material from a core needle biopsy, a vacuum assisted or open biopsy. It allows to determine morphological features of the tumor (differentiation grade, HR and HER2 expression), which determine the choice of treatment method
6	Breast cancer diagnosed on the basis of a previously performed biopsy	Breast cancer confirmed in a biopsy performed prior to the assessed breast examination (MMG, US or MRI). The category is reserved for examinations performed between percutaneous biopsy and surgery. *ATTENTION! The radiologist does not evaluate the change in BIRADS 6 — only states that the lesion from which cancer has been confirmed is visible on the image

Abbreviations developed in the text

The American College of Radiology (ACR) has developed the Breast Imaging Reporting and Data System (BIRADS, 5th edition, 2013) [4], which should be the standard not only for all MMG reports, but also ultrasound (US) and breast magnetic resonance examination (MR) (Table 1). In the description of breast imaging, there should be additional information about the breast structure, and in the MR examination also the information about stromal contrast enhancement. According to the ACR system, there are four types of breast structure (breast density), taking into account the ratio of adipose tissue to fibrous-glandular tissue:

— type A — fatty structure; the breast is almost entirely composed of adipose tissue (type 1 in older versions);
— type B — presence of scattered areas with a density of fibrous-glandular tissue;
— type C — breasts unevenly "dense", with areas of dense tissue that may obscure small focal lesions;
— type D — very "dense" breasts, MMG sensitivity decreased.

The regimen of control tests recommended for women without the symptoms of cancer and no additional risk factors for breast cancer is presented in Table 2. Women from families at high risk of breast cancer and carriers of mutations associated with breast cancer should be covered by the care program as outlined in the next part of this chapter.

Table 2. Scheme of prophylactic examinations in women without symptoms and additional risk factors

Women age	Palpation as part of a routine physical examination	Mammography
20–39 years	Every 36 months	No
40–49 years	Every 12 months	No[1]
50–69 years	Every 12 months	Every 24 months
From 70 years	Every 12 months	No[2]

[1]In the group of women aged 40 to 49 years, an accurate family history should be collected and information on symptoms and risk factors should be provided, and the decision on commencement of MMG (performed every 12–24 months) should be made a subject to discussion of potential benefits and risks with the patient

[2]In the group of women over 70 years of age, the decision to perform screening MMG should be made individually

Recommendations

— Screening mammography should be routinely performed every 2 years in women aged 50–69 years (I, A).
— Lesions detected in MMG and requiring further diagnostics should be identified in specialized centers performing at least 5,000 tests annually per radiologist (in centers specialized in breast cancer diagnostics within so-called breast units or in centers performing at least 5,000 tests per year per radiologist), in close cooperation with a radiologist, surgeon, oncologist and pathologist (III, B).
— The scope of further diagnostic tests should correspond to the level of risk of breast cancer diagnosis (III, B).
— Women from families at high risk of breast cancer and carriers of mutations associated with breast cancer should be included in a healthcare program tailored to their individual risk (III, B).

Genetic counseling

Breast cancer-associated with inherited genes mutations affects 5–10% of patients. In this group, the morbidity risk depends on the degree of mutant gene penetrance expressed by the breast cancer incidence in women being carriers of this mutation. This is the basis to distinguish the syndromes with the highest, high and moderate hereditary predisposition to breast cancer.

The highest genetic predisposition syndrome is most often associated with the presence of mutations in suppressor genes with high penetrance: *BRCA1* and *BRCA2* [5–7]. The carrier state of these mutations in the general population of breast cancer patients is only 3–5%, but it is associated with an over 10-fold higher risk of developing the malignancy, corresponding to the risk of breast cancer for the rest of life between 56% and 84% [5–7]. As a result of more frequent use of multigene tests in women with a family history of breast cancer, other mutations associated with the risk of cancer are detected and numerous variants of unknown clinical significance [8]. Other suppressor genes whose mutations are associated with high penetrance include *TP53* (Li-Fraumeni syndrome), *CDH1*, *PTEN,* and *PALB2*. High predisposition is accompanied by a situation in which genetic tests fail to detect hereditary mutations, but there is a strong aggregation of cancer cases in the family. The genes mutations associated with an increased risk of breast cancer also include *ATM*, *NBN*, *NF1*, *CHEK2* and *STK11* [5–7].

Qualification rules for genetic testing

The family history is the most important factor in the qualification for genetic testing for the carrier state of *BRCA1* and *BRCA2* genes mutations. However, there is impossible to get reliable data in about 10% of breast cancer patients due to lack of information about relatives or too few family members. In assessing the individual risk of breast cancer and the likelihood of carrier state for mutations, various computer programs are used: Gail, Claus, Cuzick-Tyrer, BRCAPRO, BOADICEA, Myriad 1 and 2 or Manchester [9]. In most countries, the threshold in the qualification for testing for mutations in the *BRCA1* and *BRCA2* genes is the probability of their occurrence at the level of 10%.

The most important clinical and ancestry-related features associated with increased probability of carrier state of *BRCA* genes mutations are:

— getting breast cancer before 40 years of age;
— multiple cases of breast and/or ovarian cancer in the family;
— other cancers in the same person, especially if one of them is ovarian cancer;
— bilateral breast cancer;
— breast cancer in men;
— triple negative breast cancer, i.e. the absence of estrogen receptor (ER) and progesterone (PgR) expression and the absence of *HER2* gene over-expression or amplification in premenopausal patients;
— medullary or atypical medullary cancer;
— Ashkenazic origin;
— confirmed presence of *BRCA* mutations in the family.

The groups of a high and very high risk of breast cancer defined on basis of clinical and ancestry-related features and molecular tests results (Table 3) constitute about 0.5–1% of the general population of women in Poland and about 15% of all breast cancer patients [5].

Carrying testing of *BRCA1*, *BRCA2*, *CHEK2* and *PALB2* gene mutations

Diagnostics and counseling regarding cancer prevention should be performed by genetic outpatient clinics employing a physician with specialization in the field of clinical genetics. The center ordering the test should provide the examined person with psychological care and have procedures allowing use of appropriate preventive measures toward the persons with a carrier state of mutations predisposing to cancer. As part of the National Program to Combat Cancer Diseases of the Ministry of Health (http://www.mz.gov.pl/wwwmz), basic screening tests include the 5 most common *BRCA1* mutations in the Polish population: p.Cys61Gly (c.181T>G, 300T/G), p.Gln1756Profs*74 (c.5266dup, 5382insC), p.Glu1346Lysfs (c.4035delA, 4153delA), p.Glu23Valfs (c.68_69delAG), and p.Ser1253Argfs (c.3756_3759delGTCT), 3 most common protein

Table 3. Risk groups for hereditary breast/ovarian cancer

The highest risk group		
More than 10-fold higher risk of disease compared to the general population[1]: — confirmed *BRCA1/2* gene mutation — aggravating family history: ≥ 3 cases of cancer in relatives of 1st and/or 2nd degree (including proband) — 1st degree relatives who have had metachronous or synchronous breast and ovarian cancer		
	Morbidity risk (%)[2]	
	Age	
	Up to 40 years	**Up to 80 years**
Women[3]		
Breast cancer:		
— *BRCA1*	21	56–84
— *BRCA2*	17	45–85
Ovarian/fallopian tube cancer:		
— *BRCA1*	5	36–62
— *BRCA2*	2	10–27
Men[4]		
Breast cancer:		
— *BRCA1*	No data	1,2
— *BRCA2*	No data	7
The high-risk group		
4–10-fold higher risk of disease compared to the general population[1] — without confirmed *BRCA1* gene mutation — aggravating family history: 2 cases of cancer in relatives of 1st and/or 2nd degree before the age of 50		

[1]One of the mentioned conditions

[2]Modified based on Levy-Lahad E. et al. [10]

[3]Increased risk of endometrial and cervical, peritoneal, colon, pancreatic, gastric cancer, ocular melanoma and **hematopoietic** and lymphoid **malignancies**

[4]Increased risk of prostate, colon, pancreatic, gastric cancer, ocular melanoma and **hematopoietic** and lymphoid **malignancies**

shortening mutations in *CHEK2* gene (1100delC; IVS + 1G>A; del 5395) and 2 mutations in *PALB2* gene (c.509_510 delGA; c.172_175 delTTGT).

As part of the National Program to Combat Cancer Diseases of the Ministry of Health the aforementioned tests can be carried out in:

— all patients with ovarian/fallopian tube/primary peritoneal cancer;
— all patients diagnosed with breast cancer;
— first- and second-degree relatives of patients with breast and/or ovarian cancer in the case when the marker mutation has not been established and it is not possible to start the diagnosis from a person with cancer.

Eligible for mutation testing in *CHEK2* and *PALB2* genes are individuals meeting the following criteria:

— all patients with breast cancer;
— first-degree relatives of breast cancer patients from families meeting the criteria of high and highest risk of breast cancer.

If the carrier state of a mutation is not detected in a patient with breast and/or ovarian cancer, especially with a family history, genetic testing for *BRCA1* and *BRCA2* mutations using next generation sequencing (NGS) may be performed within the Program. This test is performed in women with at least a 15% probability of detecting the mutation, including the following situations:

— the patient has been diagnosed with breast cancer or ovarian cancer and has at least two relatives of first- and/or second-degree who have been diagnosed with breast cancer and/or ovarian cancer, and at least one of these cases occurred before the age of 50;
— the patient was diagnosed with breast cancer before the age of 50 or ovarian cancer at any age and has a relative of first- and/or second-degree who has been diagnosed with breast cancer (male breast cancer) and/or ovarian cancer;
— the same patient was diagnosed with breast cancer, ovarian cancer or bilateral breast cancer, with at least one diagnosis confirmed below the age of 50;
— the patient has been diagnosed with ovarian cancer and has at least one relative whose breast cancer was detected before the age of 50 or who has been diagnosed with an ovarian cancer.

Management of patients in the categories of the highest and high risk of breast cancer

Observational studies indicate that lifestyle modification, including regular physical exercise, maintaining healthy body weight, reducing alcohol consumption, avoiding hormone replacement therapy or breastfeeding reduce the risk of breast cancer. The use of these methods is therefore particularly justified in high-risk groups. Among women with the highest and high risk of breast cancer, intensive screening programs are used, in which the MR examination plays a special role [5, 11].

The role of tamoxifen in breast cancer prevention in carriers of *BRCA1/2* genes mutation is still debatable [5–7]. Bilateral preventive breast amputation reduces the risk of breast cancer by more than 90% in this group [12], whereas bilateral adnexectomy has no effect on the risk of breast cancer in *BRCA1* mutation carriers, but reduces the risk of breast cancer in *BRCA2* mutation carriers after 5 years by about 50% [13].

The management of women in the categories of the highest and high risk of breast cancer is presented in Table 4.

Recommendations

— Individuals with aggravating family history or other factors indicating the possibility of a genetic cancer predisposition should be provided with genetic consultation (IV, A).
— Genetic testing for the carrier state of mutations associated with breast cancer is justified, among others, in patients with a strong family history, with cancer diagnosed before 40 years of age, and with triple-negative breast cancer diagnosed before 60 years of age (IV, B).
— Before performing the test, a family history analysis should be carried out, including the first- to third-degree relatives, the information about tests' limitations should be provided to the proband and written consent for the examination should be obtained (IV, A).
— The women upon evaluation should be provided with psychological care (IV, A).
— The options for management in women at high risk of breast cancer include:
  • lifestyle and diet modification (IV, B);
  • intensive screening (III, B);
  • prophylactic breast amputation (II, B);
  • prophylactic adnexectomy (in *BRCA2* mutation carriers) (II, B);
  • prophylactic use of tamoxifen (III, C).
— The choice of management method in an individual situation should be based on the estimated breast cancer risk and the patient's will (IV, A).

Pathology

Pathomorphological examination is a cornerstone for breast cancer diagnosis, allows to determine the stage of cancer and the risk of recurrence as well as to select potentially the most effective method of systemic treatment. Pathomorphological assessment of breast cancer (as well as the entire diagnosis and treatment) should take place in specialized units.

The clinician should provide the pathologist in written form with all relevant information about the patient and lesions in the breast:
— the most important data from medical history;
— results of previous biopsies;
— information on previous treatment (surgery, RTH, systemic treatment);
— the most important information from physical examination and breast imaging;
— information on the clinical status of lymph nodes;
— information on the possible presence of pregnancy or lactation.

The absolute precondition for starting the treatment is a microscopic diagnosis of cancer. Material for microscopic examination should be obtained under the control of MMG, MR or USG before the initial treatment, preferably using a core needle or vacuum-assisted breast biopsy (VABB), and if it is impossible

Table 4. Treatment of women in the categories of the highest and high risk of breast and/or ovarian cancer

Screening tests (total sensitivity of all procedures approx. 95%)
From the age of 18, breast self-exam (BSE) once a month after menstruation
From the age of 25[1] every 6–12 months:
— breast examination by a physician
— imaging examinations: MR in women under 30 years of age[2]; MMG alternating with MR in women over 30 years of age; breast ultrasonography as a supplementary examination every 12 month
From the age of 35 every 6 months:
— transvaginal ultrasonography and Ca125 measurement (between the 5th and 10th day of the cycle in premenopausal women)
Annual gynecological examination[3]
Lifestyle and diet modification
— regular physical exercise
— maintaining healthy body weight
— limitation of alcohol consumption
— breastfeeding recommendation
— avoiding the use of hormone replacement therapy
Surgical prevention
— prophylactic breast amputation (mastectomy) in carriers of *BRCA1/2* genes mutation (reducing of breast cancer risk by 90–95%)
— prophylactic mastectomy in other women in the highest risk group (reducing of breast cancer risk by 90%)
— bilateral adnexectomy in carriers of *BRCA1/2* genes mutation (reducing risk of ovarian and fallopian tube cancer by 80% lower risk, reducing breast cancer risk by 50% in *BRCA2* mutation carriers)[4]
Pharmacological prevention[5]
Tamoxifen from the age of 35 years (reducing breast cancer relative risk by 50% in the highest risk group, not established effect on risk in carriers of *BRCA1/2* genes mutation)

[1]Or since the age of 10 years below the youngest person developing cancer in the family

[2]In women under 30 years with the presence of mutations in *BRCA* genes MR is preferred because of almost double risk of breast cancer subsequent to MMG and the very low sensitivity of MMG in this population

[3]Oral contraceptives — reducing the risk of ovarian cancer by 30–60%, increasing the risk of breast cancer

[4]Premature menopause requires short-term estrogen-containing HRT

[5]Rarely used because of uncertain effect in carriers of *BRCA1/2* genes mutations and adverse effects of tamoxifen; in Poland, there is no registration and reimbursement of any of these drugs for prevention

Abbreviations developed in the text

— an aspiration fine needle biopsy (only when starting treatment from surgery). If initial systemic treatment is planned, a core needle biopsy, VABB or open biopsy with the assessment of the differentiation according to the Nottingham Histologic Grade, as well as ER, PgR, HER2 and Ki67 proliferation index status should be always performed. Biopsy without the use of imaging tests can be performed only in case of unambiguous and readily available for palpation lesions. The suspicious lesion should be marked with a metal anchor (harpoon) or other tracer, which allows its intraoperative location. The lesion marked in this way should be subjected to MMG examination after excision. In case of microcalcifications, a biopsy (core needle or VABB) should be performed under MMG control. If microcalcifications raise a great suspicion of cancer, the best method is a resection the suspect area as a whole. In the case of multifocal or multicentric lesions, a biopsy of all lesions should be performed, leaving markers in each of them.

The so-called "non-diagnostic" biopsy result (no material or material not suitable for assessment) is an indication to re-biopsy. Pregnancy or lactation is not a contraindication for a biopsy. Removal of a tumor without a prior microscopic diagnosis of cancer is only allowed if a biopsy cannot be performed or there is a contradiction between its outcome and the clinical picture. In this situation, intraoperative histopathological examination should be considered. In case of primary surgical treatment, the final microscopic diagnosis (including cancer type and grade of histologic malignancy) should include pTNM classification determined based on the examination of all the removed material. If surgery was preceded by systemic treatment, the postoperative staging should include y-characteristic (ypTNM).

Classification of breast proliferative lesions

According to WHO 2019 cancer classification system (Table 5), proliferative lesions associated with glandular (epithelial) breast structures — lobules and tubules — are divided into the following categories:
— benign epithelial proliferations and precursors;
— adenosis and benign sclerosing lesions;
— adenomas;
— epithelial — myoepithelial tumors;
— papillary neoplasms;
— noninvasive lobular neoplasia;
— ductal carcinoma *in situ* (DCIS);
— invasive breast carcinoma;
— rare and salivary gland type tumors;
— neuroendocrine neoplasms.

Non-infiltrating breast neoplasia

Non-infiltrating breast neoplasia include ductal and lobular carcinomas in situ. The diagnosis of ductal carcinoma *in situ* (DCIS) should be accompanied by its degree of differentiation (low, intermediate, high) or nuclear grade (NG1, NG2, NG3), as well as the histological subtype, size of the lesion, width of the surgical margins and the presence of foci of necrosis. Immunohistochemical tests (IHC) are helpful in differentiating diagnostically difficult intraductal lesions.

A separate category of intraductal lesions is lobular neoplasia, including usual and atypical lobular hyperplasia, as well as lobular carcinoma *in situ* (LCIS). 2019 WHO classification distinguishes 3 LCIS subtypes: (1) classic, (2) florid and (3) pleomorphic with significant atypia, characteristic for DCIS, but without the E-cadherin expression, which is typical for ductal proliferation. LCIS is a proliferation involving the terminal ductal lobular units (TDLUs). This abnormality is not a *precancerous* condition, but markedly increases the risk of invasive breast cancer. Invasive cancer can develop both in the breast where LCIS was diagnosed and in the contralateral breast; it can be both lobular carcinoma and invasive ductal carcinoma (no otherwise specified, NOS). For LCIS, the T category is not defined.

Invasive cancers

Among invasive cancers, the most common form (70–80%) is invasive ductal carcinoma (NOS), formerly referred to as infiltrative NST (no special type) cancer. The second most frequent (approximately 10%) is lobular carcinoma. Due to the distinct differences in the biological features of breast cancers, the grade of their histologic malignancy (G) is additionally given, however, this does not apply to microinvasive cancer. Currently, WHO and AJCC recommend the assessment of histologic grade only based on the Nottingham Histologic Grade classification by the Bloom-Richardson-Scarff, modified by Elston and Ellis (Table 6).

2019 WHO classification of breast cancer does not distinguish medullary cancer as a separate histological type. Cancers with this morphology are currently classified within invasive ductal cancer spectrum and belong to the so-called special morphological patterns as medullary pattern. Determination of tumor infiltrating lymphocytes (TILs) has taken over a prognostic role for cancers with this morphology; high TILs (over 60%) include cancers former belonging to the medullary type. TILs are recommended for evaluation in all cases of infiltrative cancer.

Among invasive breast cancers, the most common forms with a better prognosis are tubular, cribriform, and mucinous carcinoma. These forms are recognized when in the microscopic images structures characteristic for these tumors constitute more than 90% of the texture. There are also dozens of other forms of primary breast cancer, however, they together account for less than a few percent of all breast cancers.

Table 5. Simplified WHO classification of breast cancers [14]

Category of lesions	Disease entity with ICD-O code for selected cases (not all of the following lesions are malignancy in the biological sense — these do not have a code)	
Benign epithelial proliferations and precursors	— Usual ductal hyperplasia	
	— Columnar cell lesions including flat epithelial atypia	
	— Atypical ductal hyperplasia	
Adenosis and benign sclerosing lesions	— Sclerosing adenosis	
	— Apocrine adenoma	
	— Microglandular adenosis	
	— Radial scar/complex sclerosing lesion	
Adenomas	— Tubular adenoma NOS	
	— Lactating adenoma	
	— Duct adenoma NOS	
Epithelial-myoepithelial tumors	— Pleomorphic adenoma	
	— Adenomyoepithelioma NOS	
	— Adenomyoepithelioma with carcinoma	
	— Epithelial-myoepithelial carcinoma	
Papillary neoplasms	— Intraductal papilloma	
	— Ductal carcinoma *in situ*, papillary	
	— Encapsulated papillary carcinoma	
	— Encapsulated papillary carcinoma with invasion	
	— Solid papillary carcinoma *in situ*	
	— Solid papillary carcinoma with invasion	
	— Intraductal papillary adenocarcinoma with invasion	
Noninvasive lobular neoplasia	— Atypical lobular hyperplasia	
	— Lobular carcinoma *in situ* NOS	8520/2
	• Classic lobular carcinoma *in situ*	
	— Type A	
	— Type B	
	• Florid lobular carcinoma *in situ*	
	• Lobular carcinoma *in situ*, pleomorphic	8519/2
Ductal carcinoma *in situ* (DCIS)	— Ductal carcinoma, non-infiltrating, NOS	8500/2
	• DCIS of low nuclear grade	
	• DCIS of intermediate nuclear grade	
	• DCIS of high nuclear grade	
Invasive breast carcinoma	— Infiltrating duct carcinoma (NOS)	8500/3
	— Oncocytic carcinoma	
	— Lipid rich carcinoma	
	— Glycogen rich carcinoma	
	— Sebaceous carcinoma	
	— Lobular carcinoma NOS	8520/3
	— Tubular carcinoma	8211/3
	— Cribriform carcinoma NOS	
	— Mucinous adenocarcinoma	8480/3
	— Mucinous cystadenocarcinoma NOS	
	— Invasive micropapillary carcinoma of breast	8507/3
	— Apocrine adenocarcinoma	8401/3
	— Metaplastic carcinoma NOS	8575/3

→

Table 5 cont. Simplified WHO classification of breast cancers [14]

Category of lesions	Disease entity with ICD-O code for selected cases (not all of the following lesions are malignancy in the biological sense — these do not have a code)	
Rare and salivary gland type tumors	— Acinar cell carcinoma	
	— Adenoid cystic carcinoma	8200/3
	— Classic adenoid cystic carcinoma	
	— Solid basaloid adenoid cystic carcinoma	
	— Adenoid cystic carcinoma with high-grade transformation	
	— Secretory carcinoma	
	— Mucoepidermoid carcinoma	
	— Polymorphous adenocarcinoma (malignant tumor mixtus)	
	— Tall cell carcinoma with reversed polarity	
Neuroendocrine neoplasms	— Neuroendocrine tumor, NOS	8240/3
	— Neuroendocrine tumor, grade 1	8240/3
	— Neuroendocrine tumor, grade 2	8249/3
	— Neuroendocrine carcinoma NOS	8246/3
	— Neuroendocrine carcinoma, small cell	8041/3
	— Neuroendocrine carcinoma, large cell	8013/3
Fibroepithelial tumors and hamartomas	— Hamartoma	
	— Fibroadenoma NOS	9010/0
	— Phyllodes tumor NOS	9020/1
	— Periductal stromal tumor	
	— Phyllodes tumor, benign	9020/0
	— Phyllodes tumor, borderline	9020/1
	— Phyllodes tumor, malignant	9020/3
Tumors of the nipple	— Syringoma NOS	
	— Nipple adenoma	
	— Paget disease of the nipple	8540/3
Mesenchymal tumors	— Vascular tumors	
	— Fibroblastic and myofibroblastic tumors	
	— Peripheral nerve sheath tumors	
	— Smooth muscle tumors	
	— Adipocytic tumors	
	— Other mesenchymal tumors and tumor-like conditions	
Malignant lymphoma	— MALT lymphoma	
	— Follicular lymphoma NOS	
	— Diffuse large B cell lymphoma NOS	
	— Burkitt lymphoma NOS/Acute leukemia, Burkitt type	
	— Breast implant-associated anaplastic large cell lymphoma	
Tumors of the male breast	— Gynecomastia	
	— Florid gynecomastia	
	— Fibrous gynecomastia	
	— Intraductal, non-infiltrating cancer, NOS	
	— Duct carcinoma *in situ*	
	— Lobular carcinoma *in situ*	
	— Paget disease of the nipple	
	— Infiltrating duct carcinoma NOS	

Abbreviations developed in the text

Table 6. Assessment of the histological grade of breast cancer according to the Nottingham Histologic Grade

Characteristics	Grade
Formation of tubule and glands	
> 75%	1
10–75%	2
< 10%	3
Nuclear pleomorphism (degree of nuclear atypia)	
Small, regular, homogeneous	1
Moderately enlarged and heterogeneous	2
Clearly pleomorphic	3
The number of cancer cell division figures	
It depends on the size of view field in microscope	1–3
The final grade of malignancy including the sum of the above results	
Grade 1	3–5
Grade 2	6–7
Grade 3	8–9

Prognostic and predictive factors

The most important factors of prognostic importance include:
— tumor size;
— histological type of cancer and its malignancy;
— presence of metastases in axillary lymph nodes and the number of nodes affected by metastasis;
— ER and PgR status;
— infiltration of peritumoral lymphatic and venous vessels;
— HER2 status;
— Ki67 proliferation index;
— biological (intrinsic) subtype;
— TILs expression.

Currently, in the qualification for hormone therapy, any ER or PgR reaction in ≥ 1% of cancer cells is treated as positive (hereinafter referred to as "HR+"). Cancers without ER and PgR expression are classified as not susceptible to hormone therapy (HT), but their expression is associated with hormone sensitivity, lower sensitivity to chemotherapy (CHT) and better prognosis. Overexpression of HER2 protein or *HER2* gene amplification (hereinafter referred to as the "HER2+ feature") is an indication for the use of anti-HER2 therapy. Infiltration by tumor of peritumoral lymphatic and venous vessels is an independent unfavorable prognostic factor, irrespective of the presence of metastases in the axillary lymph nodes. In patients with triple-negative cancer, a higher percentage of TILs has a favorable prognostic value but has no predictive value.

Other predictors are only assessed as part of the qualification for molecularly targeted therapies. Programmed death-ligand 1 (PD-L1) expression on immunocompetent cells in the tumor microenvironment is a predictive factor for treatment with the PD-L1 inhibitor atezolizumab [15]. A qualifying factor for alpelysib treatment (a PI3K inhibitor) is a somatic *PIK-3CA* mutation [16]. On the other hand, the presence of hereditary *BRCA1* and *BRCA2* mutations is a predictor of benefits for the use of poly-ADP-ribose polymerase (PARP) inhibitors [17].

In selecting of postoperative systemic treatment in breast cancer patients, multigene molecular prognostic profiles (signatures) are increasingly used. These tests are performed using a variety of technologies, the best known of which are: Oncotype DX (Exact Sciences), Mamma Print (Agendia BV), Breast Cancer Index (Bio Theranostics), Genomic Grade Index (Ipsogen), Prosigna (Nanostring), and EndoPredict (Sividon Diagnostics). They are used primarily in the qualification of patients with non-advanced luminal cancer (HR+) for adiuvant CHT, in addition to HT, routinely used in this population. The only multigene predictive test included in the 8th edition of TNM classification is OncotypeDx. These tests are not reimbursed in Poland.

Components of pathological examination

The material after excision of the lesion from breast has to be prepared in a way that allows the assessment of surgical margins. For this purpose, the surgeon should carefully mark with the stitches or colored markers the extremities of the removed lesion (upper, lower, medial, lateral, superficial and deep). In addition, it is recommended to stain the lesion surface with special inks, allowing microscopic assessment of the surgical margin.

When assessing the differentiation according to the Nottingham Histologic Grade, a mitotic index should be provided based on hematoxylin and eosin (H-E) staining. Additionally, ER expression should be assessed, and in case of invasive breast cancer also PgR, HER2 and Ki67. ER and PgR expression is evaluated in tissue material fixed in buffered formalin and embedded in paraffin. These receptors can also be assessed by the immunopathological method in alcohol-fixed cytological preparations (e.g. in fine-needle aspirates), however, this test is less reliable and is only used when it is not possible to obtain tissue material. Description of the test result should include the percentage and strength of the staining of cancer cell nuclei with positive reaction. These results could be additionally presented using the Allred scale (0–8). As receptors state could change during disease progression, it is recommended to re-evaluate them within secondary lesions (relapse or metastasis).

HER2 status is determined with the use of IHC method only in the tissue specimen obtained by means of a core needle biopsy or in removed tumor fragment (diagnosis based on cytological examination is not reliable, because it is necessary to preserve cell membranes in the material). According to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) 2018 recommendations [18] HER2 assessment with IHC use implements a 4-point scale (0, 1+, 2+, 3+, Table 7). From a clinical point of view, results 0 and 1+ are defined as negative, and 3+ (strong total membrane staining in ≥ 10% of invasive cancer cells) — as positive. Expression 2+ (weak or medium total membrane staining in ≥ 10% of invasive cancer cells) indicates a borderline value (inconclusive) and requires HER2 copy number analysis by *in situ* hybridization, e.g. fluorescent *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) (Table 8).

HER2 status examination with the use of IHC is semi-quantitative. It requires appropriate equipment and trained personnel. This test should be performed only in pathomorphological laboratories with extensive experience and meeting relevant quality standards. It is also recommended to re-evaluate the postoperative material, provided that the previous assessment raises doubts (e.g. HER2 rated as 3+ in invasive ductal cancer NOS G1).

Ki67 proliferation index is determined in light microscopy based on the percentage of nuclei of cancer cells stained with the Ki67 antibody. The positive reaction criterion is not definitively established, but in general, the threshold for high proliferation in different laboratories is between 20–29%. In luminal cancers, the final report should always specify the histological subtype (A or B), including Ki67 cut-off value used in the laboratory.

Currently, the role of Ki67 evaluation is greater, as the TNM classification 8th edition takes into account histologic malignancy grade of as one of the criteria needed to determine the clinical stage of breast cancer. The proliferative component, scored on a scale of 1–3, affects the final grade of histologic malignancy (G). In some cases, there are discrepancies between the proliferation index assessed microscopically, based on the number mitotic figures and the percentage of cells positive in IHC staining for Ki67. Re-evaluation is indicated in such cases. In some patients, tissue sample from core needle biopsy may not be fully representative for entire tumor due to the small number of cancer cells or their damage. In such situations, depending on the clinical situation, the assessment of postoperative material or sample from re-biopsy is decisive.

TILs are assessed in histological tumor slides by routine H-E staining. The result is expressed as cancer stroma percentage (infiltrating component, with no necrosis) occupied by lymphocytes. High values of this parameter (60% and more) are associated with a better prognosis and a better response to systemic treatment.

Table 7. HER2 Rating Scale and ASCO Interpretation (2018) [18]

Result	Interpretation (HER2 receptor status)
0	Negative
1+	
2+	Inconclusive (border) result, requires further diagnostic procedure — ISH assessment from the same material or re-evaluation of IHC or ISH from another material from diagnosed cancer
3+	Positive

Pathological report

Reports for breast cancer depending on the type of material concern:
- diagnostic biopsy (histological or cytological);
- excision of *in situ* carcinoma;
- excision of invasive cancer;
- excision of invasive cancer after systemic treatment;
- sentinel node biopsy;
- lymphadenectomy;
- excision of a recurrent or metastatic lesion.

The report for the diagnostic biopsy should contain:
- detection of invasive and/or *in situ* cancer presence;
- for invasive cancer: the determination of histologic malignancy grade (G) with its 3 components (Table 6);
- for carcinoma in situ: determination of the degree of nuclear atypia (nuclear grade), presence of necrosis and histological type (for *in situ* cancer);
- determination of the percentage of cancer cell in material sample (for additional tests, e.g. molecular);
- histological type determination;
- IHC staining for predictive factors (ER, PgR, HER2, and Ki67).

In case of *in situ* carcinoma excision (conserving breast surgery [CBS] or mastectomy), the report should determine:
- degree of nuclear atypia, presence of necrosis and histological type;
- the largest dimension of the area with cancer in situ;
- the width of the narrowest surgical margin of the removed lesion;
- the status of lymph nodes (if they were removed): the number of lymph nodes and the possible presence of metastases (in several percent of *in situ* carcinomas metastases derive from micro-infiltration not detected in histological examination). In such

Table 8. Evaluation of HER2 receptor status by *in situ* hybridization methods

HER2 test (infiltration component) with a validated ISH probe (dual probe)											
***HER2/CEP17* ratio ≥ 2.0**					***HER2/CEP17* ratio < 2.0**						
Group 1 ***HER2* ≥ 4.0 signals/cell**	**Group 2** ***HER2* < 4.0 signals/cell**				**Group 3** ***HER2* ≥ 6.0 signals/cell**				**Group 4** ***HER2* ≥ 4.0 and < 6.0 signals/cell**			**Group 5** ***HER2* < 4.0 signals/cell**
Positive	**IHC: HER2**											Negative
	0	**1+**	**2+**	**3+**	**0**	**1+**	**2+**	**3+**	**0**	**1+**	**2+**	**3+**
	Negative, with a comment:		Second measurement 20 cells Other: ↑ Other group The same: Negative, with a comment:	Positive	Negative, with a comment:		Second measurement 20 cells Other: ↑ Other group The same: positive	Positive	Negative, with a comment:		Second measurement 20 cells Other: ↑ Other group The same: Negative, with a comment:	Positive
	The evidence for the efficacy of HER2 targeted therapy in the small subset of cases with *HER2/CEP17* ratio ≥ 2.0 and mean *HER2* copy number < 4.0 per cell is limited. In the first clinical trials in this subgroup of patients receiving trastuzumab, no improvement in DFS or OS was shown, but definitive conclusions cannot be drawn due to the small number of cases. HER2 status should be determined by IHC expression in addition to ISH. If the IHC result is not 3+, it is recommended that the sample be considered HER2-negative				There are insufficient data on the efficacy of HER2 targeted therapy in cases of *HER2/CEP17* ratio < 2.0 in the absence of protein overexpression as these patients did not participate in the first generation clinical trials with trastuzumab. With concurrent IHC negative (0–1+) results, it is recommended that the sample be considered HER2--negative				It is uncertain whether patients with a mean *HER2* copy number per cell between ≥ 4.0 and < 6.0 and a *HER2/CEP17* ratio < 2.0, expressing IHC other than 3+, benefit from anti-HER2 therapy. If the ISH result is close to the positive threshold, it is more likely that repeating the test will produce a different result by simple chance. When IHC results are other than 3+, it is therefore recommended that the sample be considered HER2-negative without additional testing on the same material		

cases, the pathological forms for invasive carcinoma should be used;
— pTNM stage.

The exemplary report in case of invasive cancer excision is presented in Table 9. Its integral part is to determine the TNM stage for assessed tumor (Table 10). If the surgery was preceded by systemic treatment, the report contains the same elements as those listed in Table 9 and additionally the response to the treatment:
— cellularity;
— the dimension of the largest metastasis, if left after treatment;
— breast changes resulting from the treatment used;

Table 9. Pathological report

1. Type of specimen	
2. Surgical procedure	
3. Macroscopic examination	
4. Microscopic examination (obligatory assessed features)	Histological type of cancer according to WHO 2019 classification
	Degree of histological malignancy (with points for particular components of evaluation)
	Size of infiltrating cancer
	Multifocal
	Organ-specific microscopic features important for pTNM assessment determined according to the 8th edition of the AJCC/UICC classification (ulceration, satellite nodules, infiltration of the pectoral muscle, chest wall infiltration, Paget's disease)
5. Surgical margins	The narrowest surgical margin width for infiltrating cancer and *in situ* component, presence or absence of fascia
6. Other organ-specific microscopic features (conditionally assessed)	Concomitant changes (e.g. cancer *in situ* extensive *vs.* non-extensive)
	Histological evaluation of nipple
	Evaluation of changes after preoperative treatment, cellularity
	pCR
	pPR
	pNR
7. Lymph nodes status	Number of lymph nodes assessed
	Number of sentinel lymph nodes assessed
	Number of lymph nodes with macrometastases, micrometastases, isolated cancer cells, diameter of the largest metastasis.
	Presence of cancerous infiltration of adipose tissue around nodes
8. pTNM and resulting stage (S), subtypes	
9. Markers of predictive and predictive factors	Estrogen receptors (ER)
	Progesterone receptors (PgR)
	HER2/neu IHC
	HER2/neu ISH
	Ki67
10. Biological subtypes of invasive breast cancer (for cancer with no special type and lobular carcinoma)	Luminal A
	Luminal B (HER2-negative)
	Luminal B (HER-positive)
	HER2-positive (non-luminal)
	Ductal triple-negative
11. Biological subtypes of invasive breast cancer (special types of breast cancer)	Hormone-dependent
	Hormone-independent (e.g. medullary, metaplastic, apocrine)

Abbreviations developed in the text

Table 10. Classification of breast cancer pTNM according to the 8th edition of the AJCC/UICC classification (2017) [21]

pT		
TX		Primary tumor cannot be assessed
T0		No evidence of primary tumor
Tis		Carcinoma *in situ*
	Tis (DCIS)	Lobular carcinoma *in situ*
	Tis (Paget)	Paget disease (not associated with invasive carcinoma and/or carcinoma *in situ* in the underlying breast parenchyma)
T1		Invasive cancer ≤ 20 mm
	T1mi	Microinvasive cancer ≤ 1 mm
	T1a	Invasive cancer > 1 mm and ≤ 5 mm
	T1b	Invasive cancer > 5 mm and ≤ 10 mm
	T1c	Invasive cancer > 10 mm and ≤ 20 mm
T2		Invasive cancer > 20 mm and ≤ 50 mm
T3		Invasive cancer > 50 mm
T4		Invasive cancer of any size with direct extension to chest wall and/or to skin (ulceration or satellite nodules but not include dermal invasion only)
	T4a	Extension to chest wall (does not include pectoralis muscle invasion only)
	T4b	Ulceration, satellite skin nodules, or skin edema, not meeting criteria of inflammatory carcinoma
	T4c	T4a + T4b
	T4d	Inflammatory carcinoma
pN		
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastases
	N0(i–)	No regional lymph node metastases in HE and IHC
	N0(i+)	Isolated cancer cells detected (HE or IHC) ≤ 0.2 mm or < 200 cells
	N0(mol–)	No regional lymph node metastases (also with use molecular biology techniques)
	N0(mol+)	Molecular metastases detected with a negative HE and IHC image
N1		Metastases in 1–3 regional lymph nodes
	N1mi	Micrometastases > 0.2 mm or > 200 cells in 1–3 lymph nodes
	N1a	Metastases in 1–3 regional lymph nodes (including at least one > 2 mm)
	N1b	Metastases (or micrometastases) in internal mammary lymph node (IMN) (sentinel lymph node biopsy, SLNB)
	N1c	N1a + N1b
N2		Metastases in 4–9 regional lymph nodes
	N2a	Metastases in 4–9 regional lymph nodes (including at least one > 2 mm)
	N2b	Metastases (or micrometastases) in internal mammary node with no metastases in axillary lymph nodes
N3		Metastases in ≥ 10 regional lymph nodes or supraclavicular lymph node or > 3 axillary and internal mammary lymph nodes
	N3a	Metastases in ≥ 10 regional lymph nodes (axillary) or subclavicular lymph node (III level of axillary fossa)
	N3b	Axillary > 3 and internal mammary lymph nodes
	N3c	Metastasis in supraclavicular lymph node
pM		
M0		No metastases
M0(i+)		Cancer cells detected microscopically or by molecular biology techniques in blood or other tissues, excluding regional lymph nodes ≤ 0.2 mm (or ≤ 200 cells), with no other symptoms of metastases
M1		Metastases to distant organs (detected clinically or pathologically)

Abbreviations developed in the text

Table 11. Definition of infiltrative breast cancer subtypes based on immunohistochemical surrogates

Feature	Luminal breast cancers				Non-luminal breast cancers	
	Luminal A	Luminal B			HER2+	Triple-negative
		HER2–		HER2+		
ER	+	+	+	+	–	–
PgR	+	Any	< 20%	Any	–	–
HER2	–	–	–	+	+	–
Ki67	< median for a center	≥ median for a center	Any	Any	Any	Any

— changes in the lymph nodes resulting from the treatment used.

In the evaluation of response to preoperative systemic therapy with CHT, it is recommended to use a residual cancer burden (RCB) system [19], available at website https://www.mdanderson.org/for-physicians/clinical-tools-resources/clinical-calculators/residual-cancer-burden.html, while for preoperative HT the PEPI index is used [20].

Subtypes of breast cancer

Based on evaluation of gene expression, there are 5 biological subtypes of breast cancer: luminal A, luminal B, HER2-enriched, triple-negative/basal-like and "normal-like", which in clinical practice are replaced by their surrogates based on routinely assessed pathological criteria (Table 11) [21]. One of the elements of a complete pathomorphological report for invasive breast cancer is the determination of these subtypes based on appropriate combination of histological type, steroid receptor status, HER2 status and proliferation index. In a small portion of breast cancers, neuroen docrine differentiation is found, but this feature is of no clinical significance. During the selection of adiuvant systemic therapy tumor polygenic molecular signature could be additionally considered.

Staging

The 8th edition of the AJCC classification distinguishes two methods for assessing the clinical stage of breast cancer. The former, referred to as anatomical, is recommended in countries where ER, PgR and HER2 cannot be assessed, but for practical reasons, it is also used in daily practice in other countries (Table 12). In other countries, the use of prognostic staging is recommended, with additional assessment of malignancy (G) grade, ER, PgR and HER2 receptors status, and possibly OncotypeDx molecular profile, which modify anatomical categories (Table 12a, available in the electronic version).

Table 12. The anatomical staging of breast cancer according to the 8th edition of the AJCC/UICC classification [20]

Stage	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T0	N1mi	M0
	T1	N1mi	M0
IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

Note: According to the 8th edition of the AJCC classification, there are two ways to assess the clinical stage of breast cancer. The first, specified above, referred to as anatomical, is recommended in countries where it is not possible to assess the so-called biomarkers or predictors (ER, PgR and HER2). In developed countries, where the assessment of predictors in breast cancer is widely available, it is recommended to use a prognostic staging, where the anatomical version is enriched with the results of the assessment of malignancy (G) and the state of ER, PgR and HER2 receptors, and possibly OncotypeDx (Table 15)

Recommendations (experts opinion)

— A precondition for starting treatment is a microscopic diagnosis of cancer (IV, A).

— The clinician should provide the pathologist in written form with all relevant information about the patient and lesion in the breast (IV, A).

— If initial systemic treatment is planned, a core needle or open biopsy should always be performed with the assessment of histological type, histologic malignancy grade, as well as ER, PgR, HER2 and Ki67 expression (IV, A).
— If metastases in axillary lymph nodes are suspect, the biopsy is necessary (IV, A).
— The pathomorphological report has to include an assessment of the most important prognostic and predictive factors (IV, A).
— The report content depends on the type of material provided for evaluation, pathomorphological diagnosis and previous treatment (IV, A).
— The biopsy report has to include (IV, A):
  • histological type;
  • histologic malignancy grade;
  • the expression level of steroid receptors;
  • HER2 expression/amplification level (only for invasive cancers).
— In postoperative report it is necessary to additionally determine tumor pTNM stage (for both primary tumor and lymph nodes) and surgical margins (IV, A).
— Diagnosis should include specific immunohistochemical subtypes classification: luminal A and B, HER2+, triple-negative (IV, A).
— In non-advanced luminal cancers (HR+, HER2–), in addition to the standard histopathological assessment, polygenic predictive tests could be included (I, B).

Diagnostics

The scope of diagnostic tests in breast cancer patients is shown in Table 13. Serum markers of breast cancer (CA15-3, Ca 27.29, CEA and others) should not be assessed in routine clinical practice; they can only be of secondary importance in monitoring the course of treatment of advanced breast cancer in case of non-measurable or difficult to measure lesions. Patients with aggravating family history or other factors indicating the possibility of a genetically driven cancer should be provided with a genetic consultation (according to the principles provided in subsection "Genetic counseling").

Diagnostic imaging: mammography (MMG), ultrasonography (USG), magnetic resonance (MR), positron emission tomography (PET)

Each report of breast imaging (MMG, USG and MR) should include information on breast structure and determination of breast lesions in BIRADS category (Table 1), and in the MR study — information on the contrast enhancement of glandular tissue in the stroma.

Table 13. Scope of preliminary diagnostic tests in patients with breast cancer

Medical history[1] and physical examination[2]
Laboratory tests:
— complete blood count with smear
— basic biochemical tests (assessing liver efficiency and calcium and alkaline phosphatase level)
Breast imaging:
— bilateral mammography
— bilateral ultrasound of breast and axillary lymph nodes
— MR mammography according to indications[3]
Microscopic examination:
— fine-needle biopsy[4]
— core-needle biopsy (preferred)[5]
— open biopsy
— US-guided fine-needle or core-needle biopsy of suspicious lymph nodes
Genetic consultation in patients with aggravating family history or other factors increasing the risk of genetic determination of cancer (e.g. young age)
Additionally in stage III and high-risk cancers:
— imaging of chest (X-ray or CT) and abdomen (US or CT)
— bone imaging[6]
— PET/CT imaging (as an alternative to all the above mentioned together)
— brain MRI[7]

[1]It refers to changes or complaints in breast (primary nature and date of occurrence), previous breast diseases, first and last menstruation, pregnancy and deliveries (dates) and data on breastfeeding, use of hormonal preparations (therapy, substitution, contraception), other diseases (concomitant or previous) and data on their treatment, the occurrence of malignant tumors in the family, current complaints

[2]It includes the assessment of performance status, weight and height measurement, palpation of both breasts (vertical and horizontal torso position), assessment of skin and nipples appearance and their symmetry, tumor location and relation to the chest wall and skin (mobility), tumor measurement in 2 dimensions, assessment of axillary and supraclavicular lymph nodes, assessment of other organs

[3]Helpful in search for primary lesions in patients with metastases in axillary lymph nodes or Paget's disease of nipples, in diagnostics of breast lesions in carriers of mutations in *BRCA* and other high-risk genes

[4]It should not be routinely used in the diagnosis of a primary breast tumor, except for very small lesions. Biopsy is performed under ultrasound or MMG control, except for lesions easily palpable; a non-diagnostic biopsy result (no material or material not suitable for assessment) is an indication for biopsy re-performance; pregnancy or lactation is not a contraindication for a biopsy (it is necessary to inform the pathologist)

[5]Necessary to reliably evaluate ER, PgR and HER2 receptors

[6]Suspected lesions in scintigraphy should be confirmed by X-ray, CT or MR

[7]In patients with symptoms suggesting CNS involvement

Abbreviations developed in the text

Mammography

MMG in two projections [craniocaudal (CC) and mediolateral oblique (MLO)] is the basic imaging diagnostic and the only screening method for breast cancer.

Radiation dose should be as low as possible (average dose for breasts with a thickness of 5.3 cm not more than 2.5 mGy for one exposure, for a smaller thickness — respectively smaller, and a larger thickness — respectively larger). Pictures must have very good quality (meet accepted standards).

All equipment elements and activities performed by MMG staff must be subject to constant quality control procedures, and the responsibility for the proper functioning of the laboratory lies with the radiologist.

MMG sensitivity in the detection of neoplastic lesions is around 85%, however, it is lower for breasts with high density, typical for younger women. Some breast cancers, even detectable during palpation are invisible in MMG. Other important reasons for the failure to detect cancer in MMG are technical errors (mainly abnormal breast position), perception error (overlook of lesion) and error of interpretation (recognition of lesion as non-malignant). The perception error is at least partly eliminated by modern artificial intelligence (AI) systems and/or evaluation by two radiologists, which allows reducing the risk of interpretation error to a greater extent than AI.

Digital MMG can be expanded by digital breast tomosynthesis (DBT) and contrast-enhanced spectral mammography (CESM). DBT allows to visualize the breast in 1 mm layers, so its structure and possible pathological changes can be examined in more thoroughly. As with all anatomical areas, layered images avoid many errors due to overlapping structures in the summation images. A 2D summation image can also be obtained from DBT layered images. According to this, DBT can be not only a supplement to MG 2D but also an independent examination. This allows the radiation dose to be reduced. CESM is a dual-energy technique involving low energy (23–32 kVp) and high energy (45–49 kVp) acquisition followed by subtraction. The test is performed after the intravenous administration of iodine contrast agent. Cancers, including breast cancer, are almost always accompanied by neoangiogenesis. The permeability of walls of cancer supplying vessels is greater than normal vessels, thanks to which the vast majority of tumors show contrast enhancement in imaging tests. CESM evaluates low-energy images, very similar to the standard MMG 2D, and subtraction images in which the contrast enhancement is much better visible. In CESM, unlike MRI, the kinetics of contrast enhancement cannot be assessed, which reduces the possibility of differentiating between benign and malignant lesions. Currently, there is no independent BIRADS lexicon for CESM. For low-energy images, it is recommended to use MMG terminology, and for subtraction images — terminology used for MRI.

Ultrasonography

Ultrasound is a supplement to MMG (especially in the differentiation of cystic and solid lesions and assessment of size and borders of focal lesions), the first or primary examination. As an independent study, this method is particularly valuable in the assessment of high-density breasts, typical for young women. This method is completely safe, which allows it to be used in young women and pregnant women. Evaluation with Doppler option and/or use of intravenously administered contrast agents increases sensitivity and specificity of ultrasound. Sonoelastography helps in the differentiation of benign and malignant lesions. Fine and core-needle biopsies as well as cyst punctures are performed under ultrasound guidance. Breast ultrasonography, alongside MMG, should be performed during baseline staging in all breast cancer patients. Ultrasound should also be routinely used to assess axillary lymph nodes; if necessary in combination with BAC of suspicious nodes.

The main disadvantage of ultrasound is a large subjectivity — the result is significantly impacted by technique and physician's experience. This researcher-dependence, as well as lack of standardized photographic documentation, limits the value of ultrasound in sequential assessment of lesions, including assessment of the effectiveness of preoperative systemic treatment. The assessment of large breasts may also be difficult. Breast ultrasound is not used in screening. Automated breast ultrasound (ABUS) is an ultrasound method that shows the breast in three planes, including the coronal plane, which cannot be obtained in a classic ultrasound scan. The examination is considered to be more reproducible than the classic ultrasound examination, but it does not allow for the assessment of the axillary cavity.

Magnetic resonance

MR examination, thanks to very good spatial and temporal tissue resolution, allows accurate imaging of breast morphology, including disturbances of their architecture, and detection of small nodules. On the other hand, this examination relatively often overestimates the extent of the tumor, which results in more frequent breast amputations [22]. Indications for MR in breast cancer are presented in Table 14 [23, 24]. This image should be carried out with the use of apparatus with a magnetic field strength of ≥ 1.5 T and an amplitude of gradients ≥ 2 mT/m, always using a surface coil making the simultaneous examination of both breasts possible. MR standard is a simultaneous examination of both breasts with a dynamic sequence, after intravenous administration with an automatic syringe paramagnetic contrast agent at a dose of 0.1 mmol Gd/kg, with a speed of 2–3 ml/s. Temporal resolution of ≤ 120 s during the dynamic study is required, preferably < 90 s. The sensitivity of the method in the detection of invasive breast cancer is over 98%, and the specificity is 90–95%. To limit a number of false-positive results the morphological features and the nature of contrast enhancement is

Table 14. Indications for MR in patients with breast cancer (according to EUSOMA [22], with modifications)

1. Before surgery:
— invasive lobular carcinoma to exclude multifocal and multiple locations in patients qualified for a breast-conserving procedure
— different tumor size in MMG and USG assessment > 1 cm in the patient < 60 years (if it affects therapeutic decisions)
— diagnosis of carcinoma occultum, i.e. the presence of metastases in axillary lymph nodes without detection of a tumor in the breast in physical examination, MMG and USG
— breast structure with predominance of glandular tissue, if there are areas of dysplastic glandular tissue
2. Screening in women with a high risk of developing breast cancer:
— *BRCA1, BRCA2, TP53* genes mutations
— the burden of 50% risk of *BRCA1, BRCA2, TP53* genes mutations (mutation in the mother or sister)
— high (> 20%) risk of breast cancer associated with a family history
— earlier RTH for the chest area in patients aged < 30 years, e.g. due to Hodgkin's lymphoma
3. Assessment of response to preoperative CHT in case of large, potentially operable cancer
4. After conserving treatment for breast cancer, if the results of classical tests are inconclusive, and biopsy cannot be performed, or the result of a biopsy is inconclusive
5. After breast enlargement/reconstruction surgery:
— in case of suspected implant rupture after aesthetic procedures (without contrast enhancement)
— the method of choice in case of clinical suspicion of recurrence in breast cancer patients after reconstructive surgery
6. Suspicion of inflammatory breast cancer: if, after the treatment of possible mastitis, it remains uncertain whether it is not an inflammatory cancer
7. In differentiation of ambiguous changes in MMG study, especially BIRADS 4A and 4B and possibly BIRADS 3

Abbreviations developed in the text

analyzed, and a study is performed between day 6 and 13 of the cycle in pre-menopausal women or at least 4 weeks after discontinuation of hormone replacement therapy. A biopsy of lesions visible only in the MR study should be carried out under the control of this method. Diffusion-weighted imaging (DWI) with the use of MR is also increasingly performed. This sequence takes 3–5 minutes, does not require the administration of a contrast agent, greatly facilitates the differentiation of benign and malignant lesions and is useful in assessing the response to CHT [25]. In some clinical situations, MR is replaced by CESM [26, 27], but in some situations (screening in women at high risk of breast cancer, assessing the response to induction CHT or the extent of neoplastic infiltration, especially chest wall infiltration) MR is still a standard imaging test.

Positron emission tomography (PET)

The indications for 18FDG PET in breast cancer are limited [28], which results from the high proportion of false-negative results in lesions < 1 cm and with a low grade of malignancy, low sensitivity in the detection of metastases in axillary lymph nodes and a high proportion of false-positive results [29]. As a result, the 18FDG PET imaging:

— is not useful in assessing the stage of primary breast cancer and in detecting of metastases in regional lymph nodes;
— can be used in diagnosing relapse after radical treatment, when the results of other imaging tests are inconclusive;
— can be used as part of initial diagnostics for spreading in high-risk and locally-advanced cancer and in case of recurrence.

Recommendations (experts opinion)

— In each patient with newly diagnosed breast cancer, MMG and ultrasonography of breasts and axillary fossa should be performed (if technically feasible) (IV, A).
— In special clinical situations and in case of diagnostic doubt, especially before conservative treatment, additional MMG with contrast enhancement or breast MR should be considered (III, B).
— During initial diagnosis of breast cancer, it is necessary to take a medical history, perform a physical examination, laboratory tests, breast imaging and chest X-ray, and in grade II (in "aggressive" phenotypes) and III — additionally a bone, abdominal and pelvic examination. Other imaging tests should be performed depending on the clinical indications (III, A).
— Serum markers of breast cancer (CA15-3, Ca 27.29, CEA and others) are not recommended for detection, diagnosis, clinical staging and follow-up after treatment (II, C).

Treatment

General principles

Diagnosis and treatment of breast cancer should be conducted by multidisciplinary teams within the so-called breast units [30], in which not less than 150 new breast cancer patients are treated every year. Such units should have a multidisciplinary team of specialists in oncological surgery, plastic surgery, oncology radiotherapy, clinical oncology, radiology, pathology, rehabilitation and psycho-oncology, as well as specialized senology nurses.

The choice of local or systemic treatment methods in particular stages is based on clinical and pathomorphological assessment, including histological type and malignancy grade, ER/PgR and Ki67 expression and HER2 status, primary tumor and axillary lymph nodes stage, presence, location and extent of metastases in distant organs, cancer-related conditions, presence of life-threatening conditions, time from primary treatment to relapse, type of previous treatment and response type, menopausal state and age of the patient, performance status, past and concomitant diseases and their treatment, as well as patient's preferences.

The individual plan before starting treatment should be developed by a team including a specialist in surgery, oncology radiotherapy, clinical oncology and radiology, and, if possible, pathologists specializing in breast cancer. All treatment decisions should be made with the patient's conscious participation, after giving her full information and presenting all options.

Recommendations

— The diagnosis and treatment of breast cancer should be conducted by multidisciplinary teams within the so-called breast units, in which every year receives treatment not less than 150 new breast cancer patients. Such units should have a multidisciplinary team of specialists (in oncological surgery, plastic surgery, oncology radiotherapy, clinical oncology, radiology, pathology, rehabilitation and psycho-oncology, senology nurse) (III, B).
— The choice of treatment methods is based on clinical and pathomorphological assessment, including the histological type and malignancy grade, ER/PgR, HER2 and Ki67 expression level, primary tumor stage, presence of metastases in axillary lymph nodes, presence, location and extent of metastases in distant organs, cancer-related conditions, presence of life-threatening conditions, menopausal state and age of the patient, performance status, past and concomitant diseases and their treatment, as well as patient's preferences (I, A).
— All treatment decisions should be made by a multidisciplinary case conference with specialists in oncology surgery, clinical oncology and radiotherapy, with the patient's informed participation after comprehensive information and presentation of all options (IV, A).
— Patients of reproductive age should be informed about the potential risk of loss of fertility and premature menopause as well as available methods of preventive treatment (III, A).

Treatment of pre-invasive cancers (stage 0)

Lobular carcinoma *in situ*

According to current TNM classification, LCIS is considered a benign lesion. In patients with LCIS diagnosis, only observation is recommended, including a clinical examination every 6–12 month for 5 years and then every 12 months, and MMG every 12 months. In patients with additional risk factors, for example, the carrier state of *BRCA1/2* genes mutation or aggravating family history, bilateral prophylactic breast amputation with reconstruction should be considered. Accidental detection of LCIS accompanying the removed benign lesions is an indication for bilateral MMG. The LCIS pleomorphic subtype and necrosis coexistence are similar to DCIS and in patients with this diagnosis lesion indicated in MMG undergoes excision (as in DCIS).

Ductal carcinoma *in situ*

The treatment of choice of patients with DCIS is excision of lesion visible in imaging tests or palpable, supplemented with radiotherapy (RTH), or breast amputation (simple or subcutaneous, possibly with simultaneous reconstruction). Conserving breast surgery is considered to be sufficient enough if the margin of tumor-free tissues is ≥ 2 mm [31]. If histopathological examinations of removed tissues reveal not only DCIS but also foci of invasive cancer (including microinfiltration within DCIS), treatment is carried out according to the principles used in invasive cancers. Half of the local recurrences after the conserving surgery are DCISs and the other half are invasive cancers. For this reason, in this group, postoperative RT is routinely applied to the entire breast at a dose of 40 Gy in 15 fractions, which reduces the relative risk of local recurrence by at least a half [32, 33]. In patients at very low risk of recurrence (at the same time: age > 50 years, tumor diameter < 10 mm, feature G1–2, surgical margin > 5 mm), the absolute benefit of using RT is small and therefore it can be abandoned [34–37].

A simple amputation with possible reconstruction is an alternative to breast-conserving treatment. It is performed when the conditions for conserving surgery or RTH are not met or at the patient's request. In patients undergoing amputation, postoperative RTH is not used.

Due to the low frequency of metastases in the lymph nodes sentinel lymph node biopsy (SLND) is not used routinely in DCIS. This examination is performed only in patients with an increased risk of microfiltration or infiltration within the DCIS due to the extent of the lesions (diameter > 4 cm in MMG), in the case of planned breast amputation without removal of the axillary lymph nodes, and when a breast tumor is clinically palpable, is located in the lymphatic drainage area (e.g. Spence's tail) or is poorly differentiated (G3) [33].

The use of tamoxifen for 5 years and additionally aromatase inhibitor in postmenopausal women reduces the risk of local recurrence in patients with HR+ breast cancer [38, 39]. For this reason, the status of ER should be determined in every patient with DCIS, while the determination of HER2 status has no influence on the management and is unfounded. Tamoxifen also reduces the risk of developing cancer in contralateral breast [38].

Recommendations

— Lobular carcinoma *in situ* is a benign lesion, in most cases not requiring therapy (I, A)
— The treatment of choice in patients with DCIS is local excision of lesion without removing lymph nodes, supplemented with RT (I, A). Postoperative RT can be dispensed only in patients with very low risk of recurrence (coexistence of very small tumor size, a wide margin of excision, features G1–2 and older age) (II, B).
— In case of local removal of the lesion, a margin of tumor-free tissue ≥ 2 mm should be preserved. If a margin of less than 2 mm is not achieved, the excision margin should be widened or breast amputation should be performed, depending on the clinical situation, (III, B). In special cases (coexistence of very small tumor size, wide margin of excision, features G1 and older age), further surgical treatment may be discontinued and adjuvant RT (III, B).
— Simple mastectomy with possible reconstruction should be performed in DCIS in case of (IV, B):
  • large extent of lesions (multicentric or single-centric encompassing more than one breast quadrant);
  • contraindications to postoperative RTH;
  • an unfavorable proportion between the size of cancerous lesions and the size of the breast, which does not allow to obtain a satisfactory aesthetic effect after conserving surgery;
  • failure to obtain patient's consent for conserving treatment.
— Sentinel node biopsy in DCIS should be performed only in the case of planned breast amputation without removal of axillary lymph nodes (III, B) and considered if the tumor is clinically detectable, poorly differentiated (G3), located in the lymphatic drainage area or its diameter in MMG exceeds 4 cm (III, B).
— In patients with HR+ feature preoperative HT should be considered: tamoxifen in pre-menopausal patients, tamoxifen or aromatase inhibitor (AI) in postmenopausal patients (I, A).

Treatment of patients with stage I and II breast cancer

The strategy of surgical treatment of patients with invasive breast cancer is shown in Figure 1.

Breast-conserving surgery (BCT)

BCT in early breast cancer is as effective as amputation and this is preferred option [40, 41]. BCT includes removal of the breast fragment, diagnostic and therapeutic procedure within axillary lymph nodes and postoperative RTH. BCT can be conducted in centers that have the possibility of cooperation between a surgeon, a specialist in oncology radiotherapy, clinical oncology, radiology and pathology. Breast-conserving surgery should be offered to each patient without contraindications to this method (Table 15).

Breast-conserving surgery requires the accurate marking of removed lesions extremities by means of suture or colored markers. This enables to accurately indicate the side with an insufficient margin and to identify the tissues that should be removed to extend the margin. The preparation must be labeled during the procedure. Surgery is considered to be complete if in the histopathological examination no cancerous cells are found in ink-stained surface of the removed lesion [42]. Otherwise, the extend of procedure (excision) should be broadened or mastectomy should be performed. The decision about further treatment should be made by a multidisciplinary team and discussed with the patient.

In order to precisely plan postoperative RTH, the borders of primary tumor bed should be marked with metal markers (one on the side walls and one on the bottom of the bed).

If there is a need to improve the aesthetic effect or obtain symmetric shape of both breasts, correction of breast or plastic surgery of contralateral breast could be performed simultaneously with tumor removal [30].

In case of primary position location behind the nipple (especially in Paget's disease of nipple with concomitant breast cancer), a conserving surgery can be performed, consisting of a conical excision the central part of breast in one central block, e.g. nipple-areola complex together with palpable primary tumor, with a microscopic margin of unchanged tissues.

Pregnancy is not a contraindication to BCT. However, it is necessary to postpone postoperative RTH until the end of pregnancy. The method of surgical treatment does not depend on the histological type of invasive cancer.

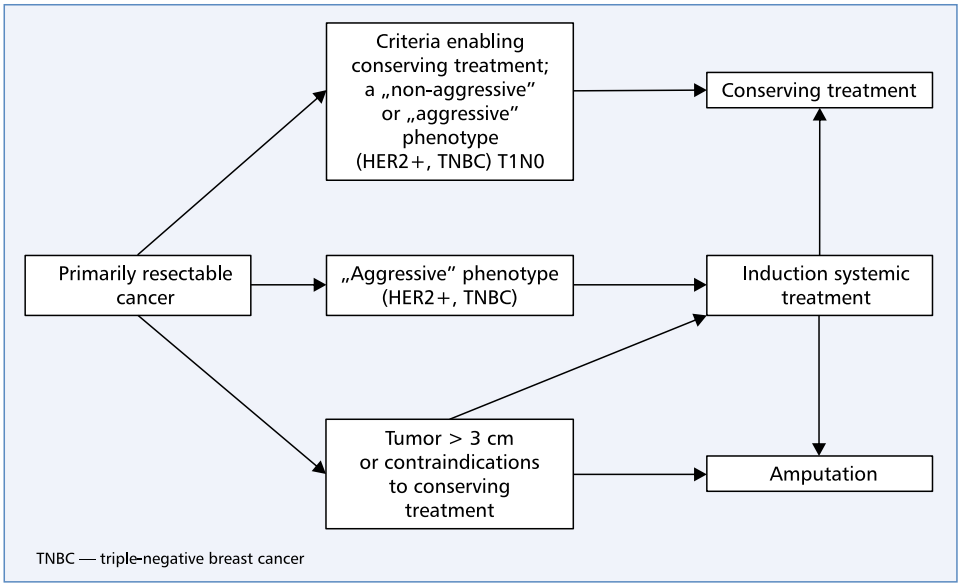

Figure 1. Strategy for the surgical treatment of invasive breast cancer

Table 15. Indications and contraindications for conserving breast cancer treatment

Indications for conserving breast cancer treatment	Contraindications for conserving breast cancer treatment
Patient consent	Carrier state of *BRCA1* or *BRCA2* gene mutations[1]
Stage T1N0M0–T2N1M0 (at baseline or after induction systemic therapy)	Multicentric cancer (including more than one breast quadrant)[1]
	Previous breast or chest RTH
Possibility of primary tumor complete excision	Extensive microcalcification visible in MMG
The possibility of obtaining a good aesthetic effect	Dermal forms of collagenases
No contraindications	

[1]Relative contraindication
Abbreviations developed in the text

The carrier state of *BRCA1* and *BRCA2* genes mutations is considered a relative contraindication to BCT because the risk of developing of another cancer in the breast undergoing BCT or contralateral breast is significantly increased in this group. Such patients should rather be proposed to undergo amputation of breast with tumor with a possibly prophylactic mastectomy on the other side.

If BCT is impossible to perform due to the size of the primary tumor, the conserving surgery can be performed after induction systemic therapy, provided that:

— preoperative systemic treatment resulted in cancer remission, confirmed in imaging tests, preserving oncological safety and enabling satisfactory aesthetic effect (lack of remission means the need for breast amputation);
— the location of primary tumor was marked before initiation of systemic treatment with the use of a tattoo or implantation of a metal marker; however, the tumor should be marked in the same position of patient in which the surgery will be performed.

Pre- and postoperative breast MRI is helpful in assessing the response to preoperative systemic treatment and the possibility of safe BCT.

Currently, preoperative systemic treatment is also increasingly used in cancers initially qualified for BCT, especially in subtypes with higher aggressiveness (triple-negative carcinomas, HER2+).

Breast amputation

Breast amputation consists of removing the entire breast with the skin covering the gland (this does not apply only to the subcutaneous amputation). The following types of amputation are distinguished:

— simple;
— subcutaneous (skin sparing);
— subcutaneous, nipple sparing;
— modified radical according to Madden method;
— radical according to Halsted method.

Simple amputation is a procedure in which axillary nodes are not removed.

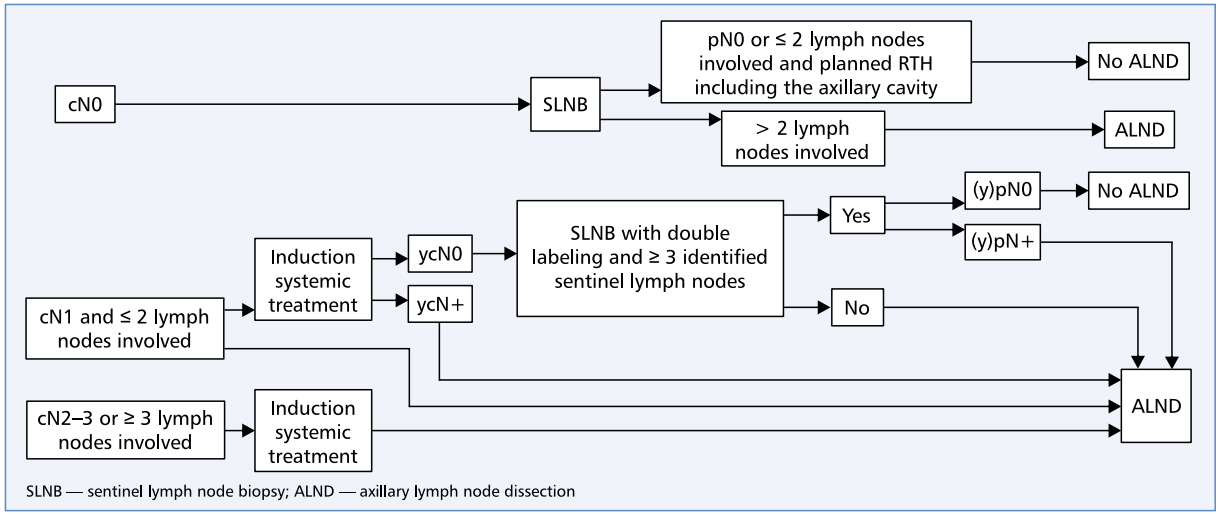

Figure 2. Strategy of management in axillary region

Subcutaneous amputation is performed (as a preparation for reconstructive surgery) in case of:
— extensive dysplastic changes occurring with severe pain, not responding to conserving treatment;
— the presence of extensive pre-cancerous lesions of non-invasive lobular neoplasms (atypical lobular hyperplasia and LCIS) or benign epithelial proliferation and precursors (usual ductal hyperplasia, columnar cell lesions including flat epithelial atypia, atypical ductal hyperplasia) type, especially in women with familial cancer;
— carrier state of *BRCA1* and *BRCA2* genes mutations (preventive subcutaneous amputation to reduce the risk of breast cancer);
— selected breast cancer patients (T1–T3, location > 2 cm from the nipple, with no nipple infiltration in imaging studies and bloody nipple exudate), in whom breast reconstruction is planned (removal of gland and possibly nipple-areola complex).

The indication for amputation according to Madden method is cancer in stage I, II or III, which is not eligible for conserving surgery or skin sparing amputation with simultaneous reconstruction. The radical breast amputation according to Patey method is currently performed in case of presence of metastases in Rotter's lymph nodes with infiltration of pectoralis minor muscle).

The only indication for a breast amputation according to Halsted method (currently rarely performed) is the infiltration of pectoralis major muscle: in this situation, tumor should be removed even if preoperative CHT or HT resulted in its shrinkage and mobility.

In patients with no high risk of contralateral breast cancer associated with the carrier of hereditary mutations or with a family history, there is no indication for breast amputation.

Recommendations
— The treatment of choice in patients with early breast cancer is excision of local lesion supplemented with RTH (I, A).
— A careful assessment of surgical margins is required. Breast-conserving treatment is considered complete if no cancerous cells are found in the ink-stained surface of the cut lesion in histopathological examination (II, B).
— If the first surgery was considered not complete it should be widened or amputation should be performed, depending on the clinical situation (II, A).
— If there is a need to improve the aesthetic effect or obtain symmetric shape of both breasts, correction of breast or plastic surgery of contralateral breast could be performed simultaneously with tumor removal (IV, B).
— Breast amputation should be performed in case of contraindications to breast-conserving treatment or at the patient's request (II, B).
— Surgical treatment after induction systemic therapy should be performed in accordance with the general principles of breast cancer treatment (II, A).
— In patients with conserving treatment planned after induction systemic therapy, the tumor localization has to be labeled (IV, A).

Procedures within regional lymph nodes

General principles of management in the axillary cavity are presented in Figure 2.

Sentinel lymph node biopsy

Sentinel lymph node biopsy (SLNB) is a standard diagnostic procedure in patients with early breast cancer who have no metastases in the axillary lymph nodes in the clinical and ultrasound examination [43].

SLNB allows a reliable assessment of axillary lymph nodes. If there are no metastases in the sentinel lymph node, its removal can be safely aborted. In patients with T1–T2 feature and metastases in 1–2 sentinel nodes, RTH from tangential fields including the lower part of axilla is as efficient as lymphadenectomy [44]. Axilla irradiation is also equivalent to lymphadenectomy (with a lower risk of arm edema) in patients with metastases in 1–2 sentinel nodes who have undergone amputation or conserving surgery [45]. There is also no need to perform a lymphadenectomy if micrometastases (< 2 mm) are detected in sentinel nodes [46]. In contrast, axillary lymphadenectomy is a routine procedure in with ≥ 3 sentinel nodes involved [47]. Axillary lymphadenectomy is always performed in the case of failure to identify and collect the sentinel node for examination during the operation.

In patients receiving preoperative systemic treatment, SLNB can be performed before or after its completion [47]. SLNB can be also performed if no more than 2 axillary lymph nodes were involved at baseline, and their complete clinical remission was achieved after preoperative systemic treatment. In patients with N2 feature or baseline involvement of at least 3 lymph nodes, SLNB is not performed regardless of the response to induction therapy. Proper evaluation of SLNB after systemic treatment in patients with baseline lymph node involvement requires the use of double labeling (dye + isotope) and the identification of at least 3 sentinel lymph nodes [47].

SLNB can only be performed as part of a close cooperation of a nuclear medicine, surgery and pathology specialists. Appropriate experience in performing these procedures is also necessary (in specialized centers this should be at least 30 successful biopsies). Any breast cancer patient who meets the SLNB qualification criteria should be informed of this possibility and, if necessary, be referred to the center where the treatment is performed.

Axillary lymphadenectomy

Axillary lymphadenectomy is associated with risk of irreversible functional complications and should be performed only in patients with clinical presence of metastases in lymph nodes (cN+ feature), with contraindications for SLNB or in absence of sentinel lymph nodes. Lymphadenectomy involves the removal of lymph nodes of I and II levels. Removal of lymph nodes of III level is justified only in case of clinical features of metastases in lymph nodes of II or III levels of axilla [30].

Recommendations

— SLNB should be performed in patients with early breast cancer with no metastases in axillary lymph nodes before surgery (I, A).
— If there are no metastases in sentinel lymph nodes or micrometastases are present in 1–2 sentinel nodes, there is no indication for further local treatment of nodal areas (I, B).
— In patients with T1 or T2 cN0 breast cancer and metastases in 1–2 sentinel nodes with no previous systemic treatment, lymphadenectomy could be abandoned after conserving surgery with whole breast radiation therapy (WBRT) (I, B).
— In patients with metastases in 1–2 sentinel lymph nodes, RTH of axillary fossa could be an alternative to axillary lymphadenectomy (I, A).
— In patients with baseline cN0 feature receiving preoperative systemic treatment, SLNB could be performed before or after its completion (II, B).
— SLNB could be performed in patients with baseline pN+ feature (assessed on the basis of a FINE or thick-needle biopsy), with complete clinical remission of metastatic lymph nodes after preoperative systemic treatment, however, in this case it is necessary to assess at least 3 lymph nodes and to use double marking of sentinel nodes (III, B).
— If metastases in sentinel lymph nodes in patients with baseline axillary lymph node involvement are not found after preoperative systemic treatment, there is no need for lymphadenectomy; however, it should be performed in all cases of persistent lymph node metastases (II, B).
— Axillary lymphadenectomy should be performed in patients with clinical presence of metastases in lymph nodes (cN+ feature), with contraindications for SLNB or if sentinel lymph nodes could not be identified (II, B).

Breast reconstruction procedures

Surgical reconstructive treatment is an immanent part of modern breast cancer management and this option should be provided to every patient. Reconstructive procedures concern patients undergoing amputation, but also conserving treatments if their aesthetic effect is unsatisfactory.

Reconstruction can be performed (in immediate or deferred mode) using the patient's own tissues (usually skin-muscle flaps), implants or a combination of these methods, in immediate or delayed setting. The choice of reconstruction time and reconstructive material depends on the individual determinants and patient's preferences. Immediate reconstruction can be carried out if there are no anatomical or medical contraindications for this procedure and patient undergoing amputation expresses a strong will to do reconstruction. Immediate reconstruction is not performed in patients with inflammatory breast cancer.

Delayed reconstruction is performed after at least 6 months from the end of adjuvant treatment (RTH

and/or CHT), while in the remaining patients, including patients undergoing adjuvant HT, the procedure can be performed earlier.

Delayed reconstruction is performed in patients with a strong desire to carry it out, without the features of tumor recurrence and general contraindications to surgery. The presence of unfavorable prognostic factors is not an absolute contraindication for breast reconstruction.

Recommendations

— Each patient should be given the opportunity to perform breast reconstruction (III, A).
— The time of reconstruction and its type is determined by the individual clinical situation and patient's will (III, A).
— Immediate breast reconstruction does not change indications for adjuvant systemic treatment and RTH (II, A).

Postoperative radiotherapy

In patients after breast-conserving surgery postoperative RTH significantly reduces the risk of local recurrence and death [48]. The most commonly used are photons with energy 4–6 MeV at a dose of 40 Gy in 15 fractions in 3 weeks, which is as effective as a dose of previously used 50 Gy in 25 fractions [49]. Similar results can also be obtained by administering irradiation dose 26 Gy in 5 fractions over 1 week [50]. Increased RTH dose with the use of photon beam, electron beam or brachytherapy (boost) on tumor bed in patients after breast-conserving treatment reduces the risk of local recurrence [51]. However, in some groups of patients (e.g. completely resected T1, G1–G2 tumor, without extensive DCIS component in patients over 50 years of age) this benefit is small, which justifies the omission of the additional dose. Depending on individual situation, the boosting dose on tumor bed can be administered simultaneously with WBRT (simultaneous integrated boost) or after its completion. In patients at low risk of recurrence (tumor < 2 cm, no lymph node metastases, surgical margin ≥ 2 mm, age > 50 years and ductal cancer or other forms with a good prognosis), accelerated partial breast irradiation (APBI) is an alternative to WBRT [52–54]. In elderly patients (> 80 years) without high-risk factors of recurrence, RT after conserving surgery may be aborted. In patients undergoing conserving surgery with lymph nodes metastases or at high risk of recurrence, irradiation of the nodal areas (retrosternal, supraclavicular and axillary nodes) in addition to irradiation of the breast reduces the risk of recurrence, but not the risk of death [54].

The clinical benefit from supplemental RTH after breast amputation applies to all patients with T4 or N+ feature, regardless of number of metastatic lymph nodes [55]. Indications for RTH after amputation also include the presence of narrow (< 1 mm) surgical margins. The role of adjuvant RTH in patients with T3N0 is debatable and its use depends on other risk factors. The most commonly used dose during RT after amputation is 40 Gy in 15 fractions [56].

In patients with the N+ feature irradiation of the nodal areas reduces the risk of recurrence and death from breast cancer, although it has not been clearly determined which areas should be irradiated. Irradiation of retrosternal lymph nodes is particularly controversial due to cardiotoxic effects [57]. RTH increases risk of upper limb edema whilst the risk of relapse in operated axilla is small. Irradiation of this area after lymphadenectomy is indicated only in patients at high risk of relapse (e.g. massive passage of infiltration through lymph nodes capsule or the presence of infiltration in the surgical incision line in this area).

The indications for postoperative RT in patients after breast amputation with simultaneous reconstruction are the same as in patients without reconstruction, but patients should be informed about the higher risk of complications and the possibility of a worse aesthetic effect.

In postoperative RTH of breast cancer, three-dimensional planning based on CT images is used. The best protection of the lungs and heart is achieved using the tangential field technique. To improve the homogeneity of the dose, it can be supplemented with arc beams with geometry similar to tangential fields and with low load. The use of classic IMRT/VMAT techniques is inadvisable due to the inevitable increase in the dose on heart and lungs.

If there is a risk of administering a high dose to the heart (primarily left-sided carcinomas, but also RTH of the right-sided retrosternal lymph nodes), deep inspiration breath hold (DIBH) technique radiation is indicated. In some situations, heart and lung dose reduction may be achieved by RTH in the prone position.

Recommendations

— Postoperative RT is an indispensable component of treatment for all patients undergoing conserving breast surgery (I, A).
— In postoperative RT of breast cancer, the recommended dose is 40 Gy in 15 fractions over 3 weeks (I, A) or (in patients after conserving surgery) 26 Gy in 5 fractions over 1 week (only in patients without irradiation of nodal areas, provided obtaining a high homogeneity of the dose) (I, B).
— In patients at increased recurrence risk, an additional dose (boost) of 10–15 Gy (photon or electron beam in 4–8 fractions or brachytherapy) should be administered to the tumor bed (I, A). This procedure is

negligible in patients at low risk of recurrence (completely resected T1, G1–G2 tumor without extensive DCIS component and age over 50 years) (II, B).
— In patients at very low recurrence risk, irradiation of only part of the breast can be an alternative to whole breast RTH (II, B).
— RTH after breast amputation should be used:
  • in all patients with T4 breast cancer or metastases in at least 4 axillary lymph nodes (I, A);
  • in patients with metastases in 1–3 axillary lymph nodes, with other poor prognosis factors (age up to 40 years, HR–, G3 or lymphatic vessels infiltration) (II, B);
  • in the case of narrow (< 1 mm) surgical margins (III, B);
  • in patients with T3N0 feature and additional risk factors (III, B).
— The irradiated area should always include the chest wall, and in patients with metastases in the axillary lymph nodes — also the regional lymph nodes (II, A).
— In patients after lymphadenectomy, a routine irradiation of axillary fossa is contraindicated (II, B).
— Retrosternal lymph nodes should be irradiated in patients with medial or central tumor localization and metastases in axillary lymph nodes (II, B).
— Indications for RTH of nodal areas in patients undergoing breast-conserving treatment are the same as in patients after breast amputation (I, A).
— In adjuvant RTH three-dimensional planning based on CT images taken in therapeutic position is recommended and in left-sided location of the lesions — with use of deep inspiration breath-hold technique or in the prone position (II, B).
— Adjuvant RTH should be carried out after completion of adjuvant CHT (II, B).
— Adjuvant RT can be used simultaneously with adjuvant HT and treatment with trastuzumab (II, B).
— RTH is strictly contraindicated during pregnancy (IV, A).

Perioperative systemic treatment

General principles

Systemic treatment in combination with surgery can be used before or after surgery. The effectiveness of both strategies in primary operable tumors is similar [58]. In recent years there is a tendency to increasingly frequent use of preoperative (inductive, neoadjuvant) systemic treatment because it allows to limit the extent of surgery in breast and axillary fossa and to assess the individualized effectiveness of therapy. The longer time before surgery also allows to perform genetic tests for hereditary mutations associated with breast cancer in selected groups of patients and, if necessary, to modify the surgery. Assessment of the response to CHT in the postoperative samples enables the individualization of further local and systemic treatment. Potential risks associated with preoperative systemic treatment include the possibility of both overestimating the initial stage of the tumor and thus applying excessive treatment, as well as underestimating the extent of the tumor, which in the case of subsequent BCT may increase the risk of local recurrence [59]. Contraindications to preoperative systemic treatment include extensive DCIS lesions that make it impossible to accurately determine the extent of the infiltrating component and difficulties in the clinical assessment of the extent of the lesion in breast.

Preoperative systemic therapy includes CHT, HT and molecular targeted drugs. The condition for starting preoperative systemic treatment is obtaining a full histopathological diagnosis and to determine the expression of ER, PgR, HER2 and Ki67 by means of core-needle or open biopsy, as well as the assessment of stage and grade of histopathologic malignancy grade. Before starting initial systemic therapy, the primary tumor should be marked with markers; this is particularly important if a breast-onserving surgery is envisaged in the second step.

Preoperative systemic treatment is routinely used in stage IIB and III breast cancer, as well as - more and more often — also in stage IIA (≥ T2 or N1 feature), in triple-negative and HER2+ subtypes, as well as in situations which provide the opportunity to limit extend of local treatment. In initially inoperable tumors the use of preoperative CHT allows performing a radical surgery in many patients.

The decision to use systemic perioperative treatment in breast cancer patients is made based on recurrence risk assessment (estimated on basis of known prognostic factors), potential sensitivity to specific strategies of systemic treatment and benefits resulting from their use. Additionally, the anticipated adverse effects of particular methods, performance status, concomitant diseases and individual patient's preferences should be taken into account. The efficacy of postoperative CHT decreases with the time between surgery and systemic treatment [60].

The type of perioperative systemic treatment depends on the biological subtype of cancer determined with the use of IHC assay (Table 12). The general principles on which the choice of treatment for each of subtypes is based are shown in Table 16. Useful tools for estimating the benefit of systemic adjuvant treatment in specific clinical situations are computed models of relapse risk, e.g. PREDICT (www.predict.nhs.uk). In HR+/HER2– phenotypes an adjuvant treatment of choice is HT, and the indication for additional use of CHT is based on individual risk of relapse and patient's preference (Table 17). Simultaneous use of adjuvant HT and CHT is less effective than the sequential use of both methods (this does not apply to LHRH analogues

Table 16. Choice of adjuvant systemic treatment including biological subtypes of cancer, determined with use of IHC assay (based on St. Gallen Conference 2013, 2015 and 2017 recommendations)

Breast cancer subtype	Treatment	Comments
Luminal A	HT	CHT in case of involvement of > 4 lymph nodes or other risk factors
Luminal B, HER2–	HT ± CHT (majority of patients)	The use of CHT and its type depending on the intensity of hormone receptors expression, degree of risk and patient's preferences
Luminal B, HER2+	CHT + trastuzumab + HT	No data on treatment without CHT
Non-luminal HER2+	CHT + trastuzumab	Trastuzumab recommended from stage T1b (diameter > 5 mm)[1] and in patients with pN+
Triple-negative, with no special type (formerly ductal)	CHT	
Special histological type (Table 5)		
ER+	HT	
ER–	CHT	In early (T1–2N0) apocrine cancer and adenoid cystic carcinoma (ACC) CHT could be abandoned

[1]In Poland reimbursed from stage T1c (diameter > 10 mm) and in patients with pN+
Abbreviations developed in the text

Table 17. Factors determining the use of adjuvant chemotherapy (in addition to hormone therapy) in patients with hormone-dependent breast cancer (based on St. Gallen 2009 conference recommendations)

Clinical and pathological features	For chemotherapy	Factors not influencing the choice	Against chemotherapy
HR expression	Low		High
Malignancy grade	3	2	1
Proliferation	High	Moderate	Low
Lymph nodes	≥ 4N+	1–3N+	N–
pT	> 5 cm	2.1–5 cm	≤ 2 cm
Patient's choice	Use of all available treatment methods		Avoidance of CHT side effects
Risk based on molecular profile	High	Moderate	Low

Abbreviations developed in the text

used to reduce the risk of premature menopause and loss of fertility) [61].

In doubtful situations, molecular tests based on the expression of selected genes were used to assess the individual risk of recurrence, e.g. OncotypeDX, MammaPrint, Prosigna, EndoPredict or Breast Cancer Index, which are not reimbursed. An alternative method is Magee Equation prognostic calculator (http://path.upmc.edu/onlineTools/mageeequations.html), which is validated in relation to OncotypeDX and include the classic histopathological parameters — tumor diameter, malignancy grade (the sum of Nottingham Histologic Grade scores), ER and PgR expression (according to H-score on 0–300 scale), HER2 and Ki67 expression level. Prospective validation of the OncotypeDX test indicates that postoperative CHT in addition to HT can be safely withdrawn in postmenopausal HR+/HER2– patients without nodal metastases and with a recurrence score (RS) ≤ 25, while the analysis of partial data from this study shows that patients < 50 years of age with a RS of 16–25 may gain some benefit from CHT [62]. CHT in addition to HT is also not beneficial in HR+/HER2– patients without metastases in the axillary lymph nodes and with metastases in ≤ 3 lymph nodes, with a low risk of recurrence in the MammaPrint test [63]. CHT can also be abandoned in patients with luminal A lobular carcinoma without nodal metastases or with metastases in ≤ 3 lymph nodes [47].

General principles on adjuvant systemic treatment for breast cancer patients are presented in Figure 3 and in Table 16.

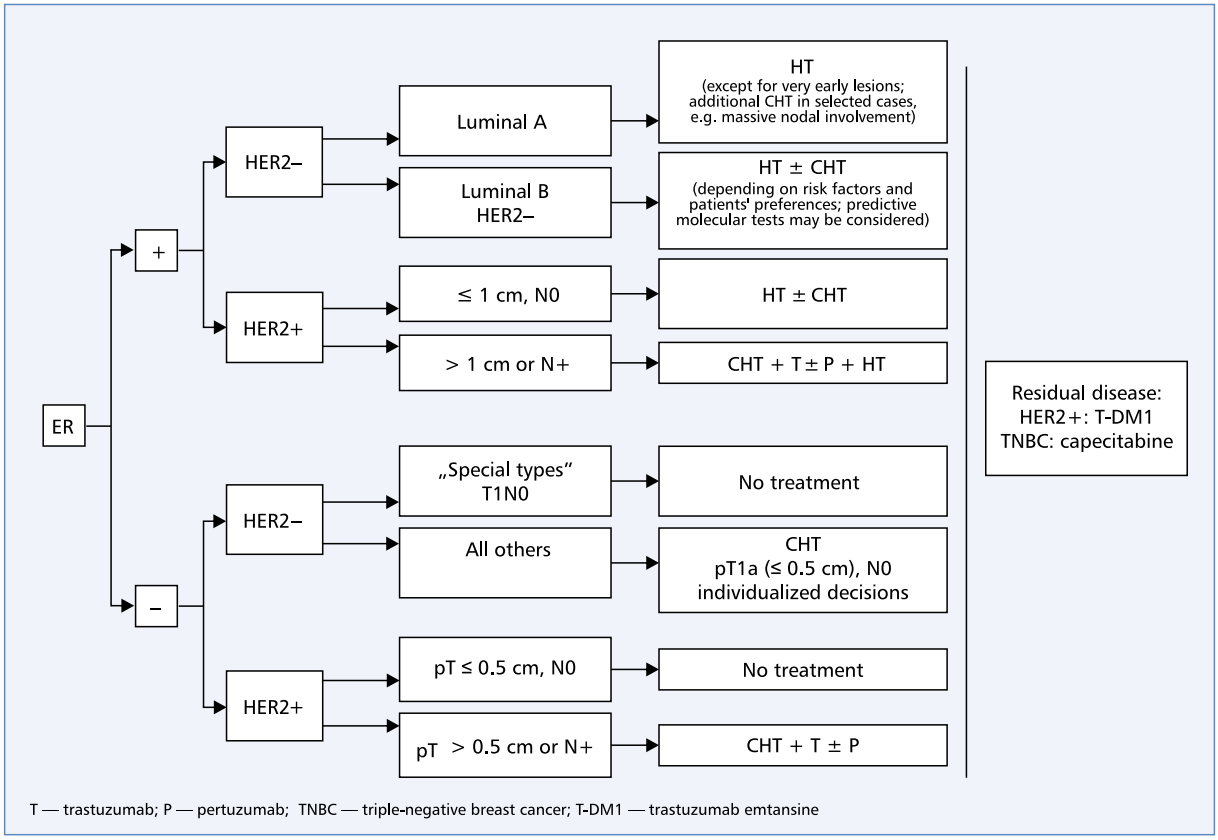

Figure 3. General principles of perioperative systemic treatment of early breast cancer

Recommendations

— Systemic treatment in combination with surgery can be used before or after operation, depending on the individual clinical situation (I, A).
— Before initiating preoperative systemic therapy, it is necessary to obtain a full histopathological diagnosis, to determine ER, PgR, HER2 and Ki67 expression as well as to assess the clinical stage (IV, A).
— Preoperative systemic treatment should be routinely used in stage IIB and III breast cancer and considered in stage II with ≥ T2 or N1 feature, particularly in triple-negative and HER2+ subtypes, as well as in situations which provide the opportunity to limit the extent of local treatment (II, B).
— Adjuvant (postoperative) systemic therapy should be started, where possible, within 4–6 weeks of surgery (especially in the case of triple-negative cancer) (III, C).
— The type of perioperative systemic treatment depends on biological cancer subtype (I, A).
— In HR+/HER2– phenotypes, an adjuvant treatment of choice is HT (I, A), and the indication for additional use of CHT is based on individual risk of relapse and patient's preference (II, B).
— In case of doubts about the indications for CHT in patients with HR+ breast cancer, individual risk of recurrence can be determined with the use of molecular tests (e.g. Oncotype DX, MammaPrint, Prosigna, EndoPredict or Breast Cancer Index) or Magee Equation (I, B).
— The majority of patients with HER2+ cancers should be treated with CHT in combination with anti-HER2 therapy (I, A), and in the case of the simultaneous HR+ feature — additionally with the later HT (II, A).
— In the majority of patients with triple-negative cancers, CHT should be used (I, A).
— Adjuvant HT and CHT should not be used simultaneously (I, A), except for LHRH analogues administered to reduce the risk of premature menopause and loss of fertility) (I, A).

Hormone therapy

HT is used in patients with breast cancer with ER expression in > 1% of cells. However, breast cancers with low ER expression (1–9%) usually have a clinical course typical for hormone-dependent cancers, which is generally an indication for use of CHT in addition to HT [64]. The drugs used in adjuvant HT include tamoxifen,

nonsteroidal aromatase inhibitors (AIs) — anastrozole and letrozole, steroid AI — exemestane, and gonadotropin-releasing hormone (GnRH) analogs inhibiting hormonal ovarian activity — goserelin, leuprolein and triptorelin and, in selected cases, bilateral adnexectomy (surgical castration).

HT before menopause

The fundamental hormonal drug in premenopausal patients is tamoxifen 20 mg/d, used for 5–10 years. In patients who achieved biochemically confirmed menopause during tamoxifen treatment, HT with AI may be continued. The role of prolonged HT is ambiguous; available data indicate that this method allows increasing the time to disease-free survival (DFS) and overall survival (OS) in patients with lymph node metastases [65, 66]. However, prolonging the use of tamoxifen is associated with increased risk of endometrial cancer and thromboembolic complications.

Data regarding the efficacy of preoperative HT in pre-menopausal patients are insufficient to consider this method as a standard procedure. Preoperative HT can be used in selected patients with luminal A cancer, in whom CHT is not indicated and the tumor size makes it impossible to perform optimal surgical treatment. In such cases, it is recommended to combine the GnRH analog (currently goserelin is the only reimbursed drug in this indication) and AI [67].

In pre-menopausal patients, AI could not be used without simultaneous suppression of ovaries activity. GnRH analogue combined with tamoxifen or exemestane prolongs DFS and OS in HR+ patients in whom previous adjuvant CHT did not induce menopause [68, 69]. Administration of GnRH analogues at monthly intervals allows obtaining better hormonal suppression. There are no data on the optimal duration of use of GnRH analogues, but five years of treatment is generally recommended [47]. Ovarian suppression, combined with oral hormonal drugs, is associated with lower libido and higher risk of osteopenia and osteoporosis.

In pre-menopausal patients with contraindications to tamoxifen, a combination of AI and GnRH analogs is used. In case of poor tolerance of both tamoxifen and AI, treatment with GnRH analogue administered alone may be continued for up to 5 years.

The use of GnRH analogues during perioperative CHT reduces the risk of loss of fertility and premature menopause [70].

Recommendations

— The fundamental hormonal drug in premenopausal patients is tamoxifen used for 5–10 years (I, A).
— In patients who during have experienced biochemical menopause during treatment with tamoxifen, HT with AI use may be continued (I, B).
— Extension of HT > 5 years may be considered in patients with metastases in lymph nodes (II, B).
— In pre-menopausal patients, AI should not be used without simultaneous suppression of ovaries activity (III, A).
— In patients with menopause caused by systemic therapy, the concentration of sex hormones should be regularly monitored (III, B).
— The use of a GnRH analogue in combination with tamoxifen or exemestane is justified in patients younger than 35 years of age, as well as in patients in whom previous adjuvant CHT did not induce menopause (I, B).
— In patients of reproductive age, the use of GnRH analogues during perioperative CHT reduces the risk of premature menopause and loss of fertility (I, B).

HT after menopause

Menopause is defined as one of the following criteria: prior bilateral adnexectomy, age ≥ 60 years, age < 60 years, and amenorrhea for at least 12 months without CHT, tamoxifen, or GnRH, or FSH and estradiol levels within the range typical of menopause. Preoperative HT is used in postmenopausal patients diagnosed with luminal carcinoma A (especially in the case of lobular carcinoma less sensitive to CHT) and in selected cases of HER2– luminal carcinoma B. AIs, especially in patients with G3 feature, high Ki67 index and cancer lobular, are more effective than tamoxifen [71–73]. Preoperative HT is used for 4–8 months or until the maximum response is achieved, and then continued after surgery for a total of 5–10 years. In patients who do not respond to preoperative HT, preoperative CHT can be used.

In perioperative HT in post-menopausal breast cancer patients, both tamoxifen and AIs are used. The duration of adjuvant treatment with tamoxifen is 5–10 years. The indication for the use of prolonged HT is stage II or III with pN+ feature.

As compared to tamoxifen, the use of AIs is associated with a slightly lower risk of recurrence and death [74] and it is recommended that in patients at increased risk, drugs of this group should constitute at least part of postoperative HT [47].

Continuation of HT with the use of AIs after 5 years of treatment with tamoxifen, as well as the use of AIs over 5 years after sequential therapy (tamoxifen 2–3 years + AIs 2–3 years or TAM + AIs 4.5–6 years) slightly, prolongs the time to recurrence but has no significant effect on the risk of death [75–77].

In lobular cancer, the efficacy of AI appears to be significantly higher compared to tamoxifen, both in terms of risk of recurrence and death [78].

Typical side effects of AI are joint and muscle pain, a decrease in bone mineral density (osteopenia, osteoporosis) and the associated increased risk of bone fractures, as well as disorders in lipid metabolism [71–73]. Osteoporosis is a relative contraindication

to AIs administration. Increased physical activity, in addition to its preventive effect on the development of osteoporosis, significantly reduces the risk of relapse [79]. Typical side effects of tamoxifen are endometrial hyperplasia, increased risk of endometrial cancer, cataracts and venous thromboembolism, and in pre-menopausal patients additionally forming of ovarian cysts. Concomitant use of strong or moderate CYP2D6 inhibitors decreases tamoxifen efficacy, especially antidepressants from the group of selective serotonin reuptake inhibitors — fluoxetine, paroxetine or fluvoxamine and sertraline [80]. On the other hand, venlafaxine has little effect on tamoxifen metabolism. Thanks to intrinsic agonist activity against ER tamoxifen prevents bone demineralization in postmenopausal women and reduces the risk of death from heart attack by protecting blood vessels endothelium [81].

Recommendations

- In postmenopausal patients with luminal cancer A or luminal B HER2– breast cancer HT is the treatment of choice (I, A).
- Adjuvant HT in post-menopausal breast cancer patients includes tamoxifen for 5–10 years, AIs or sequential use of both drugs (I, A).
- Relative contraindications to tamoxifen are venous thromboembolism in medical history and varicose veins of lower extremities (II, B).
- In patients treated with tamoxifen the concomitant use of strong or moderate CYP2D6 inhibitors and antidepressants from the group of selective serotonin reuptake inhibitors — fluoxetine, paroxetine or fluvoxamine and sertraline — should be avoided (III, A). If there are indications for use of these drugs, the safest of these is venlafaxine (III, B).
- Except for patients at low recurrence risk, postoperative HT should at least in some part include AI (II, B).
- Osteoporosis is a relative contraindication to AI administration (II, B).
- As part of postoperative HT the AIs could be used from the beginning and 2–3 or 5 years after treatment with tamoxifen (I, A).
- Prolonging of HT use over 5 years should be considered in patients with metastases in lymph nodes (II, B).
- Routinely prolonged treatment with AIs (> 5 years) is not sufficiently justified (I, B).
- Baseline bone mineral density (BMD) with use of densitometry should be evaluated at the beginning of AI treatment; calcium and vitamin D3 administration (1,000 IU daily) during the treatment is indicated together with regular BMD monitoring (III, B).

Chemotherapy

Almost all patients with triple-negative or HER2+ cancer are eligible for perioperative CHT (lasting 3–6 months) (the exception is a subgroup with a very low risk of recurrence and some special types of triple-negative cancer), as well as some patients with luminal tumors with the HR+/HER2– phenotype).

The effectiveness of peri-operative CHT is associated with maintaining its assumed intensity (appropriate doses of medication, intervals between cycles, and use of bone marrow stimulating factors) [82]. The intensity of perioperative CHT should be adjusted to the risk of recurrence.

In preoperative and postoperative CHT alkylating agents, anthracyclines and taxoids are used, most often as multi-drug regimens (Table 18). Anthracycline-based regimens (AC and EC) are generally administered in 4 cycles. The addition of fluorouracil to regimens containing anthracyclines and taxoids increases the toxicity and has no clinical benefits [83]. In patients with early HER2– breast cancer (HR– or N+), regimens based on the sequential use of anthracyclines and taxoids are slightly more effective than 6 × TAC regimen (docetaxel, doxorubicin and cyclophosphamide) [84]. After 4 cycles of AC, paclitaxel is administered weekly at a dose of 80 mg/m^2 (12 times) and docetaxel at a dose of 75–100 mg/m^2 (4 times) every 3 weeks [85]. Of the two mentioned protocols, a regimen containing paclitaxel is better tolerated [85]. The reverse sequence of anthracyclines and taxoids can also be used.

In patients at intermediate recurrence risk, the administration of 4 cycles of a two-drug regimen based on anthracyclines or taxoids may be sufficient. In low-risk HER2+ cancer (pT1N0), paclitaxel monotherapy in combination with trastuzumab can be used. Shortening the intervals between CHT cycles (mainly AC or EC) — generally from 3 to 2 weeks — in combination with granulocyte colony-stimulating factors (the so-called dose-dense chemotherapy) allows to prolong DFS compared to conventional regimens, especially in patients with HR– feature and in high-risk B luminal cancer patients [86]. The use of platinum derivatives in carriers of *BRCA1* or *BRCA2* gene mutations does not significantly improve the results of perioperative CHT [87, 88]. In patients with triple-negative cancer who are not carriers of the *BRCA1/2* mutation, the use of platinum derivatives in preoperative CHT increases pathological complete remission (pCR) rate although the impact on the long-term results is unclear [89, 90].

In patients with triple-negative and luminal B cancer, in whom pCR was not achieved after preoperative CHT (infiltrating cancer foci greater than 1 cm or N+ feature), administration of 6–8 cycles of capecitabine

Table 18. Perioperative chemotherapy regimens used in breast cancer patients

Recommended regimens in patients with HER2-negative breast cancer	Recommended regimens in patients with HER2-positive breast cancer
AC/EC	**AC/EC → T + H (± P)**
DOX 60 mg/m^2 *i.v*. day 1.	DOX 60 mg/m^2 *i.v*. day 1.
or	or
Epi 75–90 mg/m^2 *i.v.* day 1.	Epi 75–90 mg/m^2 *i.v*. day 1.
CTX 600 mg/m^2 *i.v*. day 1., 4 cycles, every 21 days	CTX 600 mg/m^2 *i.v.* day 1., 4 cycles, every 21 days, and then
AC/EC → P*	DXL 75–100 mg/m^2 *i.v.* day 1.
DOX 60 mg/m^2 *i.v.* day 1.	T: 8 mg/kg *i.v.* (loading dose) day 1. → 6 mg/kg *i.v*. (maintenance dose) day 1., every 21 days
or	or
Epi 75–90 mg/m^2 *i.v.* day 1.	T: 600 mg *s.c.* day 1., every 21 days
CTX 600 mg/m^2 *i.v.* day 1., 4 cycles, every 21 days, and then PXL 80 mg/m^{2,} weekly for 12 weeks	± P: 840 mg (loading dose) day 1. → 420 mg (maintenance dose) *i.v*., day 1. 4 cycles, every 21 days (T in total for 1 year, P 3–6 cycles in preoperative treatment)
AC/EC → T*	**AC/EC → P + H (± P)**
DOX 60 mg/m^2 *i.v.* day 1.	DOX 60 mg/m^2 *i.v*. day 1.
or	or
Epi 75–90 mg/m^2 *i.v.* day 1.	Epi 75–90 mg/m^2 *i.v.* day 1.
CTX 600 mg/m^2 *i.v.* day 1., 4 cycles, every 21 days, and then DXL 75–100 mg/m^2 *i.v.* day 1., 4 cycles, every 21 days	CTX 600 mg/m^2 *i.v*. day 1., 4 cycles, every 21 days, and then PXL 80 mg/m^2 weekly for 12 weeks
ddAC/EC → P*	T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (maintenance dose) day 1., every 21 days
DOX 60 mg/m^2 *i.v.* day 1.	or
or	T: 600 mg *s.c.* day 1., every 21 days
Epi 75–90 mg/m^2 *i.v*. day 1	± P: 840 mg (loading dose) day 1. → 420 mg (maintenance dose) *i.v.,* day 1. every 3 weeks (T in total for 1 year, P 3–6 cycles in preoperative treatment)
CTX 600 mg/m^2 *i.v*. day 1., 4 cycles, every 14 days (after each cycle G-CSF support), and then PXL 80 mg/m^2, weekly for 12 weeks	**TCH ± P**
ddAC/EC → T*	DXL 75 mg/m^2 *i.v.* day 1.
DOX 60 mg/m^2 *i.v.* day 1.	CBPL AUC6 *i.v.* day 1.
or	T: 8 mg/kg *i.v.* (loading dose) day 1. → 6 mg/kg *i.v.* (maintenance dose) day 1, every 21 days
Epi 75–90 mg/m^2 *i.v.* day 1	or
CTX 600 mg/m^2 *i.v.* day 1., 4 cycles, every 14 days (after each cycle G-CSF support), and then DXL 75–100 mg/m^2 *i.v*. day 1.,	T: 600 mg *s.c.* day 1., every 21 days
4 cycles, every 21 days	± P: 840 mg (loading dose) day 1. → 420 mg (maintenance dose) *i.v.,* day 1. every 3 weeks (T in total for 1 year, P 3–6 cycles in preoperative treatment)
TC	**PCH ± P**
DXL 75 mg/m^2 *i.v.* day 1.	PXL 80 mg/m^2 *i.v*. day 1., 8., 15.
CTX 600 mg/m^2 *i.v*. day 1., 4 cycles, every 21 days	CBPL AUC2 *i.v.* day 1., 8., 15.
CMF	6 cycles, every 21 days
CTX 100 mg/m^2 orally day 1.–14.	T: 8 mg/kg *i.v.* (loading dose) day 1. → 6 mg/kg *i.v*. (maintenance dose) day 1., every 21 days
MTX 40 mg/m^2 *i.v.* day 1. and 8.	or
FU 600 mg/m^2 *i.v*. day 1. and 8., 6 cycles, every 28 days	T: 600 mg *s.c.* day 1., every 21 days
Post-induction treatment in patients with residual tumor	± P: 840 mg (loading dose) day 1. → 420 mg (maintenance dose) *i.v.,* day 1. every 3 weeks (T in total for 1 year, P 3–6 cycles in preoperative treatment)
Capecitabine 1250 mg/m^2 twice daily on days 1.–14., 6–8 cycles, every 21 days	**PH****
	PXL 80 mg/m^2 weekly for 12 weeks simultaneously with T: 8 mg/kg *i.v.* (loading dose) day 1. → 6 mg/kg *i.v.* (maintenance dose) day 1. (T in total for 1 year)
	or
	T: 600 mg *s.c.* day 1., every 21 days
	Post-induction treatment in patients with residual tumor
	T-DM1: 3.6 mg/kg day 1., every 21 days (14 cycles)

*Reverse sequence can also be used

**Regimen used in patients with I stage, in Poland reimbursed in stage IC

CTX — cyclophosphamide; DOX — doxorubicin; DXL — docetaxel; GCSF — granulocyte-colony stimulating factor; CBPL — carboplatin; MTX — methotrexate; T — trastuzumab; P — pertuzumab; PXL — paclitaxel

after surgery prolongs OS [91]. This benefit is greatest in triple-negative cancers, especially with the pN+ feature.

In some premenopausal women, chemotherapy could result in loss of fertility, with the risk increasing with age. For this reason, each patient of childbearing age should be informed about the possibility of using methods that increase the chance of preserving fertility (cryopreservation, e.g. freezing the egg, part of the ovary or embryo). The risk of premature menopause and loss of fertility is also reduced by the administration of GnRH during CHT, but this management is not equivalent to the above-mentioned treatment. methods and should not replace them.

Recommendations

— Perioperative CHT should be used for 3–6 months (4–8 cycles) (I, A).
— In the majority of patients sequential use of multidrug anthracycline-based and taxane-based regimens in preoperative and postoperative CHT is recommended (I, A).
— In patients from intermediate-risk groups (HR+, N0) administration of 4 CHT cycles (AC, EC or TC) could be sufficient (I, B).
— The simultaneous use of anthracyclines and taxanes (e.g. AT, TAC) is not recommended (III, B).
— Fluorouracil containing regimens (e.g. FAC or FEC) are not justified in perioperative treatment (I, B).
— In patients with HR– and luminal B breast cancer, especially at a young age, it is advisable to shorten the intervals between the cycles of CHT containing anthracyclines (the so-called "dose-dense" schemes) (I, B).
— In patients with triple-negative cancer, preoperative CHT with the use of platinum derivatives could be used (I, B).
— In the perioperative treatment of *BRCA1/2* genes mutation carriers, the routine use of platinum derivatives is not recommended, whereas these patients, in addition to anthracyclines and taxanes, should receive cyclophosphamide (II, C).
— Regardless of tumor regression level scheduled preoperative CHT should be entirely given before surgery, i.e. it should not be divided into periods before and after surgery (III, B).
— In case of progression during preoperative CHT a surgery, radical RTH or different regimen potentially not cross-reacting with the previous scheme should be considered (III, C).
— In patients with triple-negative or luminal B cancers, in whom pCR was not achieved after preoperative CHT, 6–8 cycles of capecitabine should be considered after surgery (I, B).
— All pre-menopausal patients who are expected to receive chemotherapy should be informed about its potential effects on fertility and the possibilities to reduce this risk (III, A).

Anti-HER2 treatment

The combination of pre- and postoperative CHT with trastuzumab in patients with HER2+ breast cancer significantly reduces the risk of tumor recurrence and death compared to CHT alone [92–94]. In routine perioperative treatment, trastuzumab is administered for 12 months, but shortened schedules (6 months) have very similar efficacy and can be considered in individual situations [95–98]. Biosimilar equivalents are an alternative to trastuzumab, but they should be used in accordance with international recommendations [99]. Adding pertuzumab to the regimen containing taxoid and trastuzumab in preoperative treatment allows achieving higher complete remission rate in histopathological examination but with no proven effect on DFS [100].

Due to cardiotoxic effects trastuzumab is not administered simultaneously with anthracyclines. In patients receiving sequential CHT with an initial anthracycline application (e.g. 4 × AC → 4 × DXL or 4 × AC → 12 × PXL), starting trastuzumab at the same time as taxoids is more effective than postponing it until the end of CHT [101]. Anti-HER2 treatment is also combined with non-anthracycline-containing CHT schemes, e.g. regimens with docetaxel and carboplatin (TC), which have similar activity to 4 × AC → 4 × DXL regimen with a lower risk of cardiotoxicity [93].

Administration of trastuzumab concomitantly with HT and/or RTH is safe and does not increase the toxicity [92–94].

Anti-HER2 treatment is associated with an increased risk of functional cardiac disorders and is contraindicated in patients with documented heart failure or left ventricle ejection fraction (LVEF) < 50%. Heart functions are regularly assessed throughout the treatment. In case of symptomatic heart failure, trastuzumab is discontinued and standard pharmacological treatment is used, which in most patients enables resumption of drug administration. There is no evidence to justify the use of trastuzumab as the single agent or in combination with exclusive HT (without CHT). In HER2+ breast cancer patients from the lowest risk groups (T1N0), adjuvant treatment has been very successful when using only paclitaxel (80 mg/m^2 weekly for 12 weeks) in combination with trastuzumab for 12 months [102].

In patients with HER2+ breast cancer who did not achieve pCR after preoperative treatment, the use of T-DM1 in postoperative treatment reduces the relative risk of recurrence by half (not reimbursed therapy) [103].

In postoperative treatment, adding lapatinib to trastuzumab and CHT does not increase the effectiveness of treatment [104], while adding pertuzumab slightly reduces the risk of relapse in patients with N+ feature [105]. The use of neratinib in adjuvant therapy for one year after completion of trastuzumab treatment slightly prolongs DFS in patients with N+ feature, but

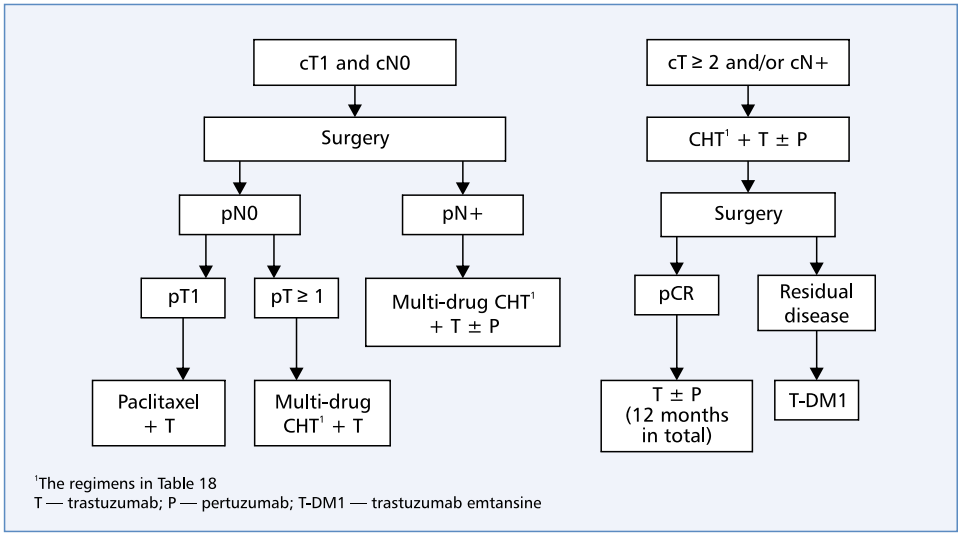

Figure 4. Strategy for the perioperative treatment of HER2+ breast cancer

this applies only to the HR+ group, and the treatment is associated with significant gastrointestinal toxicity [106]. Pertuzumab and neratinib are not reimbursed in this indication.

The perioperative treatment strategy for HER2+ patients is presented in Figure 4, and the treatment regimens in Table 18.

Recommendations

— In perioperative treatment of patients with HER2+ breast cancer it is recommended to administer CHT combined with anti-HER2 treatment for a total of 1 year if there has not been any progression or undesirable clinically significant effects before (I, A). In justified cases, a reduction of anti-HER2 treatment to 6 months may be considered (I, B).
— Trastuzumab should be administered intravenously every 3 weeks (at a loading dose of 8 mg/kg, then at a dose of 6 mg/kg) or subcutaneously (at a fixed dose of 600 mg every 3 weeks) (I, A).
— To reduce the risk of cardiotoxicity, sequential use of anthracyclines and trastuzumab is recommended, and in the case of concurrent treatment, the use of anthracycline-free regimens (TC, PC) (I, A).
— In patients receiving sequential treatment with anthracycline-containing regimens followed by taxoids, anti-HER2 treatment should be initiated at the same time as the administration of taxoids (I, B).
— Anti-HER2 treatment could be used simultaneously with non-anthracycline CHT regimens, e.g. combining docetaxel and carboplatin (I, B).
— In patients in high-risk group the so-called "dual blockade" involving trastuzumab and pertuzumab in combination with CHT could be considered in preoperative treatment (I, B).
— In patients who did not achieve pCR after preoperative treatment containing CHT with trastuzumab or trastuzumab and pertuzumab, T-DM1 should be used after surgery (I, A).
— In patients with the N+ feature, the so-called "dual blockade" involving trastuzumab and pertuzumab could be considered in postoperative treatment (I, C).
— In postoperative treatment of patients with T1N0 tumor, it is sufficient to administer only paclitaxel (80 mg/m^2 weekly for 12 weeks) in combination with trastuzumab for 12 months (II, B).
— In HR+ patients receiving CHT and anti-HER2 treatment, HT should be started immediately after CHT completion (II, B).
— There are no contraindications for combination of anti-HER2 treatment with postoperative RTH or HT (II, B).
— Treatment with trastuzumab is contraindicated in patients with documented heart failure or left ventricle ejection fraction (LVEF) < 50% (II, A).
— Heart functions should be assessed every 3 months during anti-HER2 treatment (III, A). In case of symptomatic heart failure, anti-HER2 treatment should be discontinued and standard pharmacological treatment should be used (III, B).

Bisphosphonates

Osteoporosis related to the degradation of bone structures by osteoclasts and the release of a number of molecular signaling factors in this process creates favorable conditions for the survival and proliferation of cancer cells, which justifies the use of drugs modulating bone turnover in breast cancer patients. The use of bisphosphonates in the supportive treatment of

premenopausal patients does not bring clinical benefits, whereas in patients after menopause (natural or pharmacologically induced) significantly reduces the risk of bone metastases and death from breast cancer [107]. These benefits are independent of type of bisphosphonate and its dosage regimen, cancer stage, HR expression and prior systemic treatment. In addition to improving the prognosis, administration of bisphosphonates prevents complications associated with iatrogenic bone loss (especially in patients receiving AIs). Most commonly used drug in this supportive care is zoledronic acid, administered at a dose of 4 mg *i.v.* every 6 months for 3–5 years or 4 mg *i.v.* every 3 months for 2 years. Replacing bisphosphonates with denosumab is not justified. The use of bisphosphonates in supportive care is not reimbursed in Poland. During administration bisphosphonates, vitamin D3 and calcium should be also taken.

Recommendations

— In addition to other methods of supportive care in patients after natural or pharmacologically induced menopause, it is justified to use bisphosphonates, especially in the case of an increased risk of relapse (I, A).
— In supportive care zoledronic acid is recommended at a dose of 4 mg *i.v.* every 6 months or for 3–5 years or 4 mg *i.v.* every 3 months for 2 years (I, B).

Adjuvant treatment of pregnant patients

Diagnosis of breast cancer in the second and third trimester of pregnancy is not an indication for its premature termination, and the only factor affecting the condition of the child is the age of pregnancy at the time of its completion. In patients with breast cancer diagnosed during pregnancy, CHT based on anthracyclines and taxoids seems to be safe (especially in the second and third trimesters). Use of RTH, anti-metabolites, HT and anti-HER2 treatment is associated with a significant risk of fetal harm.

Recommendations

— Anthracyclines, alkylating agents and taxoids could be relatively safely used in perioperative treatment during the second and third trimester of pregnancy (III, B).
— RTH, anti-metabolites, HT and anti-HER2 treatment are contraindicated throughout the pregnancy (III, A).

Adjuvant treatment of elderly patients

In the "biologically old" patients with the HR+ breast cancer, it is usually recommended to use HT, while in patients with the HR– cancer CHT should be considered with considering of general condition and "biological" age as well as previous and concomitant diseases. In patients qualifying for standard adjuvant CHT, monotherapy with docetaxel or capecitabine is less effective than classical regimens (AC, CMF) [108, 109]. In other patients, less toxic RT regimens may be considered.

Recommendations

— Chronological age should not be a criterion for the choice of treatment. Treatment type and intensity should be adjusted to cancer type and stage, biological age, concomitant disorders and patients preferences (II, A).
— In elderly patients with good general condition with indications to CHT, it is recommended to use standard multidrug regimens at full dose rates (II, A).

Adjuvant treatment in breast cancer with so-called a special type of histology

The majority of cribriform, tubular and mucinous cancers are hormone-dependent, which in case of proven HR expression justifies the use of adjuvant HT (in the absence of HR expression it is advisable to verify the diagnosis). In HR– cancers with a high risk of recurrence (e.g. metaplastic cancer) CHT should be used. The treatment of breast cancer patients with medullary pattern is the same as for invasive ductal cancer NOS. In case of rare subtypes, such as "secretory juvenile", apocrine cancer or adenoid-*cystic* carcinoma with N0 feature withdrawal from chemotherapy is possible.

Recommendations

— Treatment of cancers with so-called a special type of histology should be based on the same principles as for other breast cancers (III, B).
— In some rare subtypes, CHT may be abandoned (III, B).

Adjuvant treatment of breast cancer in men

Breast cancer in men accounts for about 1% of the total number of all cases. The majority of breast cancers in men indicates HR expression. Standard postoperative management in this group is the administration of tamoxifen for 5–10 years. Due to insufficient inhibition of estradiol production, the exclusive use of AIs is not recommended [110]. In patients with contraindications to treatment with tamoxifen, AIs are used in combination with GnRH analogues.

Recommendations

— In adjuvant HT in men tamoxifen should be used (III, A).
— Other principles of adjuvant treatment are the same as for breast cancer in women (III, A).

Occult primary breast cancer

The term of occult primary breast cancer (OPBC) is defined as the presence of metastases in regional lymph nodes (usually axillary), the most commonly with breast cancer morphology, but without the possibility of identifying the primary tumor in breast. They are very rare

situations (< 0.5% of cases). In addition to standard tests, diagnostics includes breast MR and/or PET/CT. Local treatment within axillary fossa usually involves the removal of axillary lymph nodes. Within the breast, similar results are obtained after breast amputation or radical RTH; both methods are much more effective than lymphadenectomy alone [111].

Recommendations

— The treatment of choice of occult primary breast cancer (the presence of metastases in the axillary lymph nodes, without clinical features of the presence of the primary focus in the breast) is the removal of axillary lymph nodes and amputation or irradiation of the breast (III, B).

Treatment of patients with stage T3N0 and III breast cancer

Locally advanced breast cancer is defined as stage IIB (T3N0) and IIIA–IIIC. In most patients in this group induction, systemic treatment is used, followed by surgery combined with RTH or radical RTH.

Pre- and postoperative systemic treatment in this group of patients with locally advanced breast cancer include the same methods as in patients with early breast cancer. The choice of the local treatment method (surgery or RTH) depends on response to induction systemic treatment (Figure 5). In patients with initial stage T4, eligible for surgery after systemic treatment, breast amputation is generally performed. In other cases, amputation or conserving surgery is performed, depending on the treatment response, the anatomical situation and patient's preferences. In all patients with T4 and/or ≥ N2 features postoperative RTH is used.

Recommendations

— Before starting the treatment of patients with locally advanced breast cancer, in addition to routine tests performed in early breast cancer, imaging tests of the lungs, abdomen and bones, as well as assessment of bone marrow, liver and kidney function should be performed, and in patients with symptoms suggesting CNS involvement additional brain imaging (Table 14) (III, A).
— Most patients with locally advanced breast cancer require initial systemic treatment prior to surgery and RTH (Figure 4) (I, A).
— Systemic treatment of locally advanced breast cancer is based on the same eligibility rules and methods as in primary operable breast cancer (II, A).
— The choice of the local treatment method (surgery or RTH) depends primarily on baseline cancer stage and response to induction systemic treatment (II, B).
— In eligible patients the type of surgery (amputation or conserving treatment) depends on baseline cancer stage, treatment response, anatomical situation and patient's preferences (II, B).
— In all patients with locally advanced breast cancer adjuvant RTH should be used, which after conserving treatment includes the breast, and after amputation the chest wall; in both situations, the lymph nodes in supraclavicular area are also covered (I, A).
— The decision regarding RTH of axillary and retrosternal area depends on type of surgical treatment within axillary fossa and recurrence risk, determined individually based on the extent of the cancer before induction treatment and in postoperative histopathological examination (II, B).
— In patients with inflammatory cancer eligible for surgery after induction systemic therapy, the procedure of choice is breast amputation without simultaneous reconstruction (III, A).
— In breast cancer patients illegible for surgery after induction systemic treatment, the treatment of choice is radical RTH (III, A).

Supportive care in patients receiving systemic therapy

The emetogenic effect of CHT is reduced by antiemetics used in accordance with the ESMO/MASCC recommendations (Table 19) [112]. The risk of hair loss due to CHT is reduced by cooling caps [113], and the risk of neuropathy by systems causing temporary ischemia of the hands and feet (tight surgical gloves or cooling systems) [114]. In patients receiving HT leading to a decrease in estrogen levels (surgical castration, LHRH analogues, AIs), administration of calcium and vitamin D3, and in patients with low bone mineral density — early treatment with bisphosphonates or denosumab reduces the risk of bone complications [115].

Recommendations

— In patients receiving CHT with intermediate and high emetogenic potential, the use of antiemetic drugs is indicated (I, A).
— In patients receiving CHT associated with the risk of hair loss, cooling caps can be used (II, B).
— In patients treated with taxoids and platinum derivatives, temporary ischemia of the hands and feet (pressure, low temperature) can be used (II, B).
— In patients receiving treatment lowering estrogen levels, administration of calcium and vitamin D3 is indicated, and in patients with low bone mineral density early initiation of treatment with bisphosphonates or denosumab (I, A).

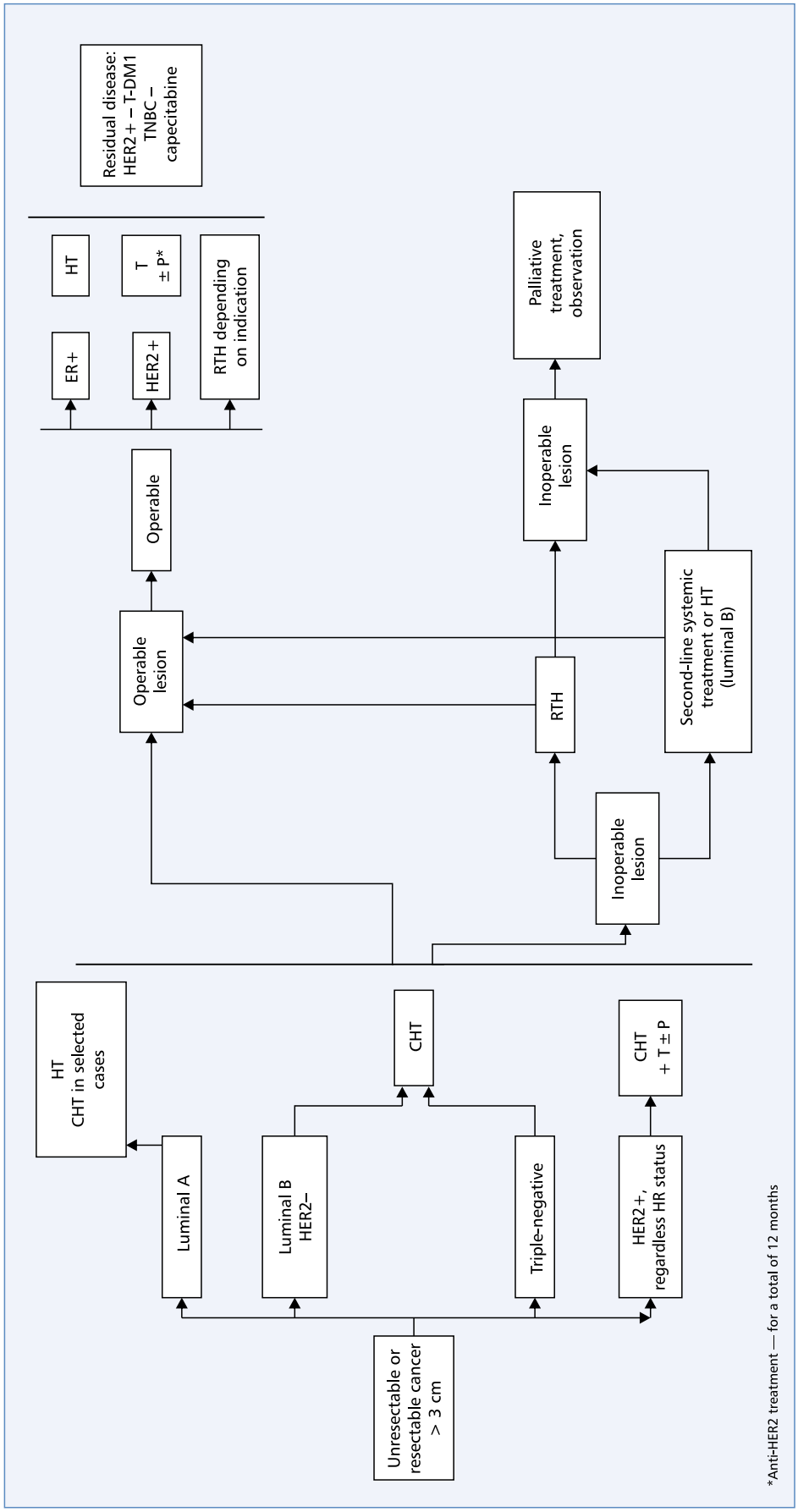

Figure 5. Strategy for treatment of locally advanced breast cancer

Table 19. Antiemetic treatment of patients receiving CHT with intermediate or high emetogenic effects (based on ESMO/MASCC recommendations [112])

On CHT day
— aprepitant 125 mg (orally 1 hour before CHT)
— ondansetron 8 mg every 8 hours
— dexamethasone 4 mg (in the afternoon)
— olanzapine 5 mg once daily
On day 1. and 2. after CHT
— aprepitant 80 mg (orally)
— dexamethasone 2 × 4 mg (in the morning and in the afternoon)
— olanzapine 5 mg once daily
On day 3. after CHT
— dexamethasone 2 × 4 mg (in the morning and in the afternoon)
— olanzapine 5 mg once daily
On demand
— ondansetron 8 mg every 8 hours
— thiethylperazine 6.5 mg (tablet or suppository) every 8 hours
Delayed vomiting:
— metoclopramide 3 × 10 mg

Treatment of local or regional recurrences

Treatment of patients with only local or regional relapse depends on type of primary treatment, tumor phenotype, time to relapse, as well as tumor volume and location (Figures 6 and 7).

In most patients with a recurrence in the breast after primary conserving treatment, amputation with axillary lymphadenectomy is preferred unless performed previously. In selected patients renewed conserving procedure could be an option.

In patients with recurrence after breast amputation, which iseligible for surgical treatment, excision of the recurrent lesion is recommended followed by RTH covering entire chest wall, with possible boosting dose on tumor bed and surrounding lymph nodes. In patients who have previously underwent adjuvant RTH to the chest wall area, reirradiation may be applied to a limited area. Depending on clinical situation in patients who are not eligible for surgical treatment it is possible to use radical RTH (≥ 50 Gy) with a possible boost to recurrence area or palliative RTH.

As in early breast cancer, systemic treatment in local and regional recurrences depends on the tumor phenotype. In patients with HR– feature an adjuvant CHT improves long-term treatment outcomes [116], and in patients with HR+ feature local treatment should be supplemented with HT [117].

Recommendations

— Before starting the treatment In patients with local or regional recurrence, imaging tests of the lungs, abdomen and bones, as well as assessment of bone marrow, liver and kidney function should be performed, and in patients with symptoms suggesting CNS involvement additional brain imaging (Figure 8) (II, A).
— In patients with local and regional recurrences, the possibility of treatment with a radical intention should always be considered (Figures 6, 7) (II, A).
— In most patients with a recurrence in the breast after primary conserving treatment, amputation with axillary lymphadenectomy should be performed (II, A). Renewed conserving procedure could be considered in selected cases, depending on the size and location of the recurrent lesion and patient's preferences (III, C).
— In patients with recurrence limited to breast, who have not undergone a sentinel lymph node biopsy (SLNB) or lymphadenectomy as part of the initial treatment, SLNB could be performed. In the case of previous surgery within axillary fossa, no sentinel node identified or metastases in the axillary lymph nodes, a lymphadenectomy should be performed (III, C).
— In patients with recurrence in chest wall eligible for surgical treatment, excision of recurrent lesion, nodal surgery according to the criteria described above and (unless performed previously) RTH covering entire chest wall and surrounding lymph nodes are recommended (III, B).
— In patients who have previously underwent adjuvant RTH to the chest wall, reirradiation may be applied to a limited area (III, C).
— In case of recurrence in axillary fossa, lymphadenectomy supplemented with RT is recommended (III, A).
— In case of recurrence in supraclavicular region or the area of internal thoracic lymph nodes, radiotherapy is recommended (III, C).
— In patients ineligible for surgical treatment, radical or palliative RTH should be considered (III, B).
— In patients with HR– breast cancer with local or regional relapse adjuvant CHT should be used after local treatment (II, B).
— In patients with HR+ breast cancer, local treatment should be supplemented with adjuvant HT (II, B).
— In patients with HER2+ feature, trastuzumab is indicated, especially if it has not been used before or more than 12 months have elapsed since the completion of adjuvant treatment with its use (II, B).

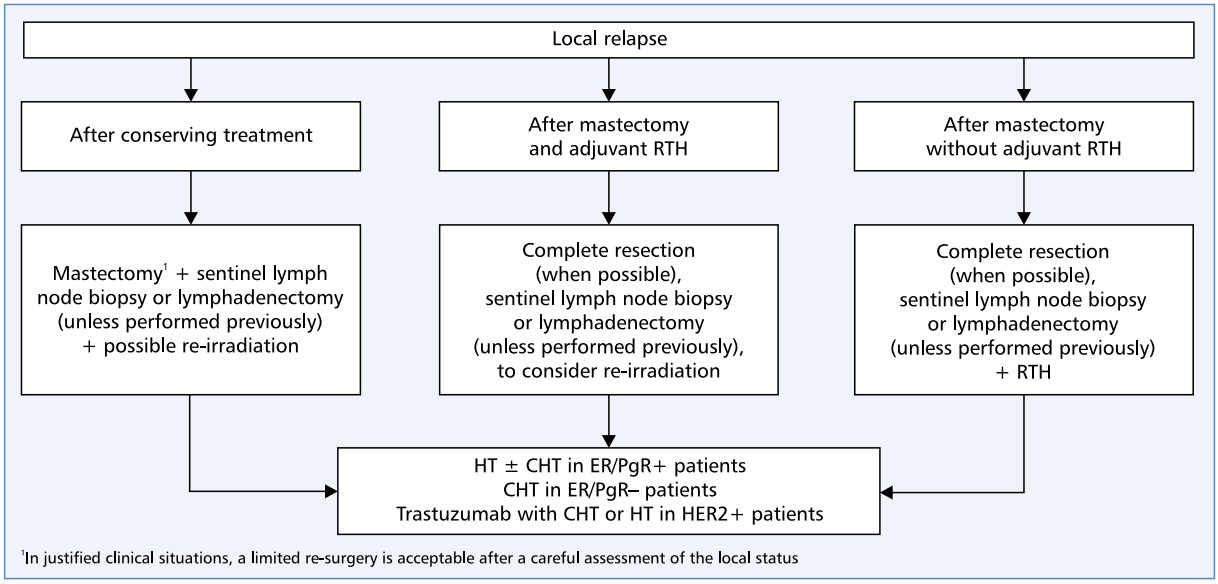

Figure 6. Treatment strategy for patients with local recurrence

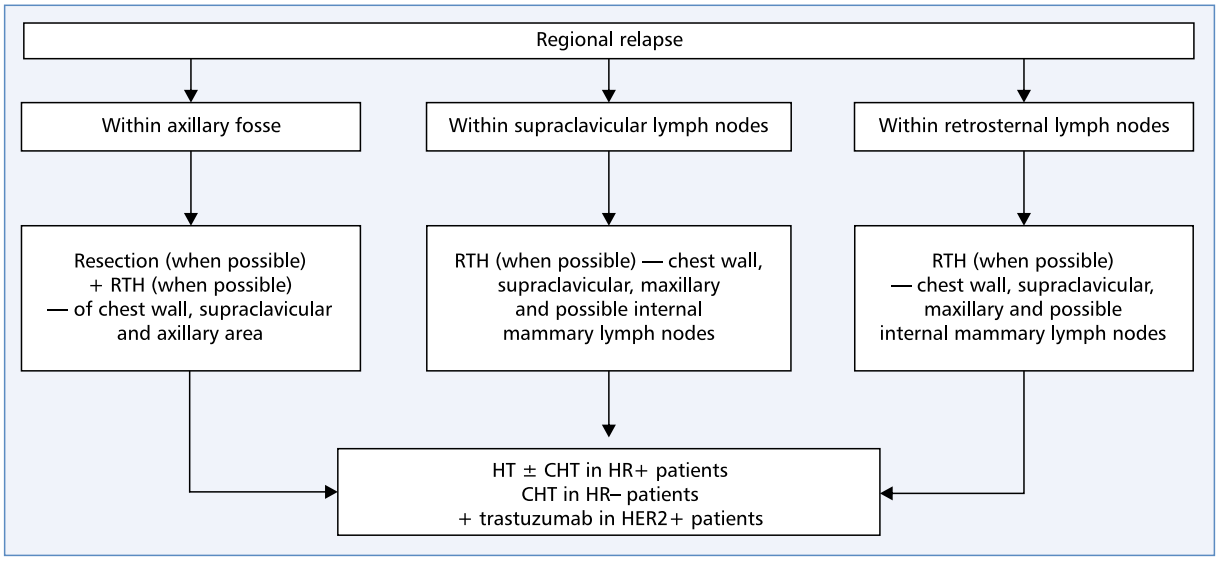

Figure 7. Treatment strategy for patients with regional relapse

— Exclusive systemic treatment of patients with local or regional breast cancer recurrence is permissible only when local methods are not available (III, C).

Treatment of patients with stage IV breast cancer

Zasady ogólne

In patients with generalized breast cancer systemic and local therapies are used. In most patients the treatment is palliative, but in some of them, a long-term survival could be achieved. The main goal of treatment is to extend life and improve its quality. The factors that determine the choice of treatment for patients in stage IV include:

— number, volume and location of neoplastic lesions;
— presence and severity of cancer-related complaints;
— HR expression and HER2 status at the primary (and/or metastatic) lesions;
— relapse-free survival after primary treatment;
— dynamics of cancer development;
— previously used systemic treatment and response;
— menopausal status;
— previous and concomitant diseases and their treatment;

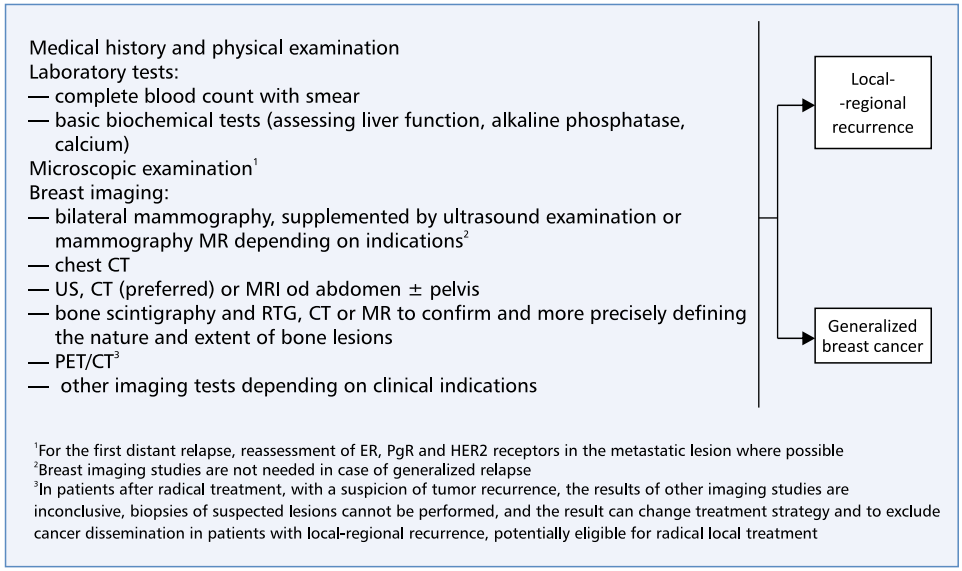

Figure 8. The scope of diagnostic tests in patients with recurrence or dissemination of breast cancer

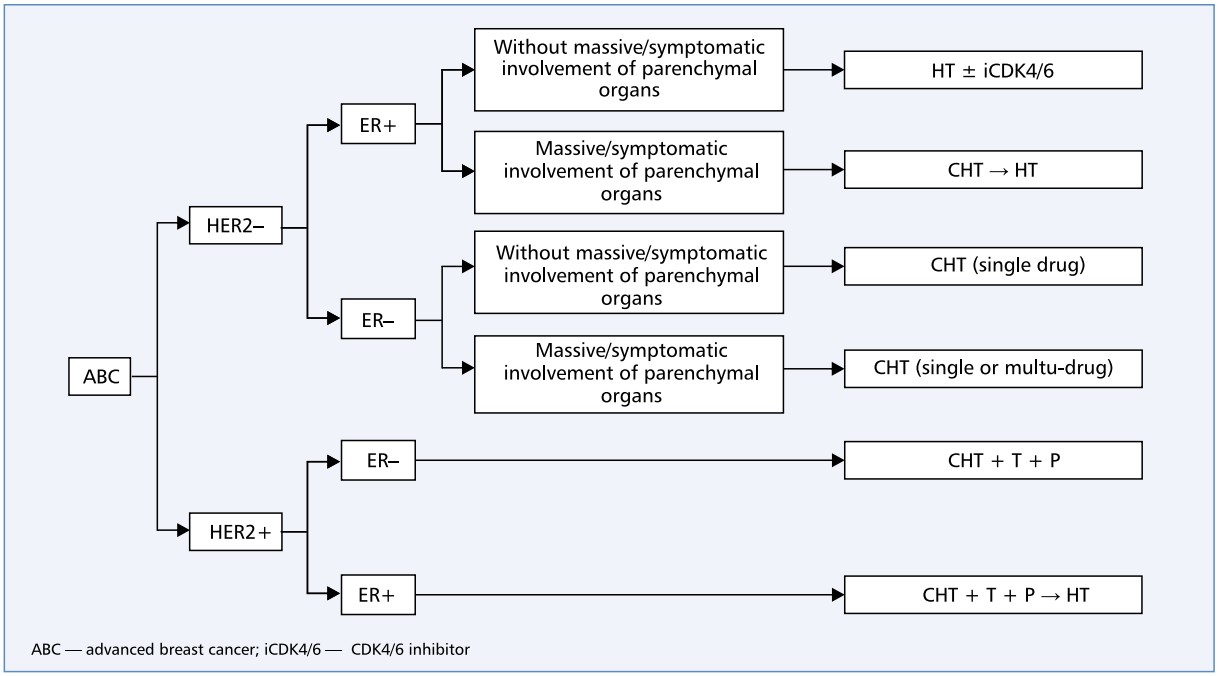

Figure 9. Frontline systemic treatment of advanced breast cancer

— performance status;
— biological age;
— patient's preferences and psychosocial determinants;
— pharmacoeconomic indicators and financing options.

In patients with HR+ breast cancer HT is most commonly used, possibly in combination with CDK4/6 inhibitors. In this group of patients CHT is used only in case of rapid progression and life-threatening, massive involvement of parenchymal organs (so-called visceral crisis), e.g. lymphangiosis carcinomatosa. In turn, CHT is treatment of choice in HR– patients and in HR+ patients with exhausted possibilities of HT (Figures 9 and 10).

The type of HT depends on adjuvant HT scheme and time since its completion. In case of obtaining an objective response or long-term stabilization after first--line HT, further HT lines should be used. Combining several HT methods is pointless, except for surgical or pharmacological castration combined with other forms

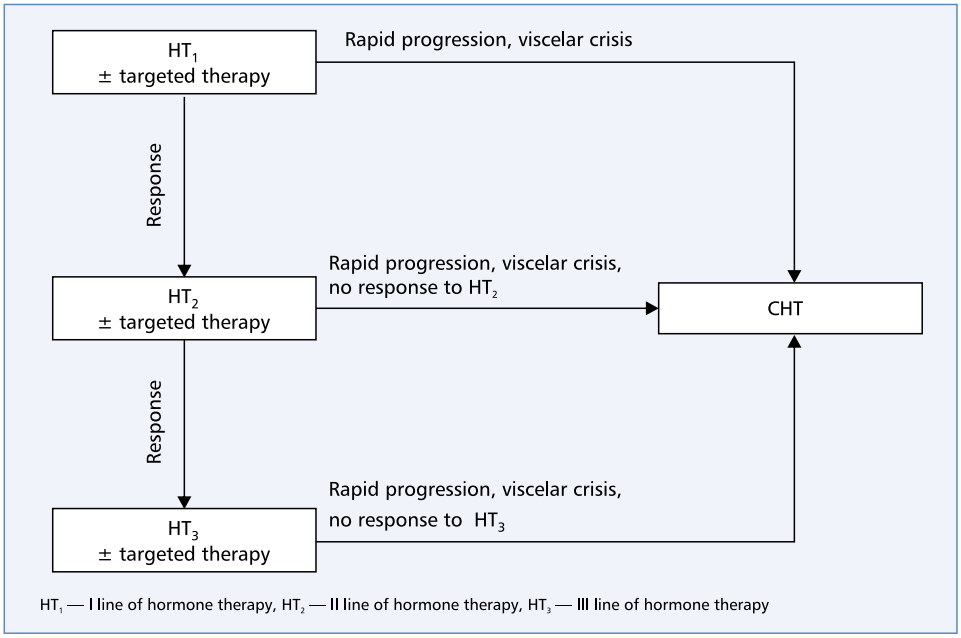

Figure 10. General principles of advanced ER+ breast cancer treatment

of HT in pre-menopausal patients, which is more effective than the use of single method [118].

Sequentially use of single-drug CHT regimens has similar effectiveness to multi-drug regimens but lower toxicity [119].

In patients with HER2+ cancer adding anti-HER2 treatment to non-anthracycline-containing CHT regimens prolongs OS and progression-free survival (PFS) [120].

Recommendations

— Patients with generalized breast cancer should be cared for by the multidisciplinary team, consisting of clinical oncologist, radiotherapy specialist, surgical oncologist, specialist in palliative medicine, a specialist in rehabilitation/physiotherapy, specialist nurse and clinical psychologist (IV, A).
— The choice of treatment of advanced breast cancer should take into account the type of cancer, its extent and location of lesions, dynamics of growth, previously used treatment and response achieved as well as patient's general condition, concomitant diseases and patient's preferences (Figure 9) (I, A).
— If possible, at the time of disease generalization it is recommended to take a tissue sample from the metastatic lesion to confirm the diagnosis and determine cancer phenotype (III, B).
— In patients with HR+ breast cancer HT should be used first (II, A).
— It is recommended to use similar HT in pre- and postmenopausal women, however in patients before menopause, effective ovaries suppression is necessary (II, B).
— In patients with HR+ breast cancer CHT is used only in case of rapid progression and massive, life-threatening involvement of parenchymal organs (so-called visceral crisis) (III, B).
— CHT and HT should not be used simultaneously (III, B).
— CHT is the treatment of choice in patients with HR– feature and in patients who have not responded to previous HT (II, A).
— It is recommended to sequentially use of single-drug CHT regimens (I, B). Multi-drug CHT should only be used when a prompt response is needed (III, B).
— In patients with HR+ feature, starting treatment with CHT a following HT after CHT completion should be considered (IV, C).
— In patients with HER2+ breast cancer, it is advisable to combine non-anthracycline containing CHT with anti-HER2 therapy (I, A).
— In selected cases, HT could be combined with anti-HER2 treatment (II, C).
— Response during HT and CHT should be evaluated every 2–4 months, considering possible treatment interruptions, with consistent use of the same imaging methods (IV, A).
— HT and anti-HER2 treatment should be conducted to progression or intolerable toxicity (II, B), and CHT to achieve an expected therapeutic effect, progression and/or intolerable toxicity (I, B).
— Systemic treatment requires monitoring of side effects, patient's performance status and therapeutic response. Complete blood count should be performed before each CHT cycle, and biochemical tests every 4–8 weeks (III, A).

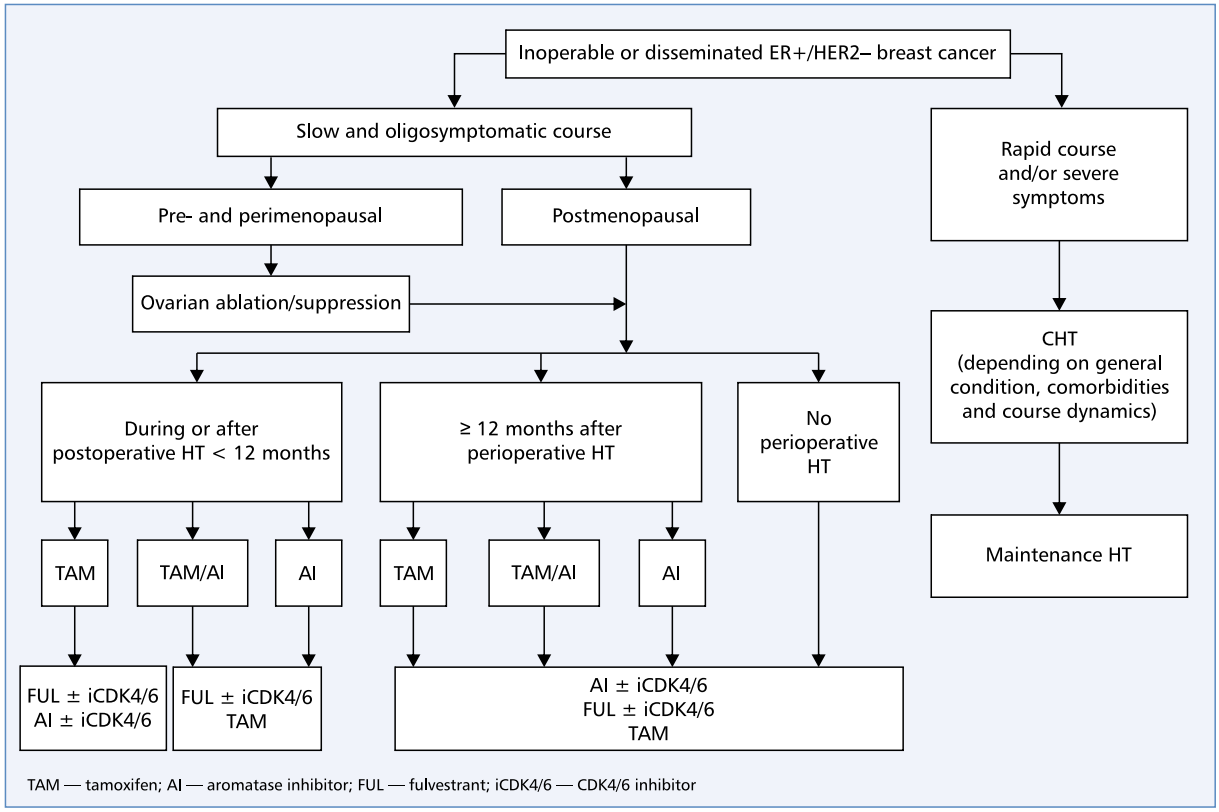

Figure 11. First-line systemic treatment strategy in advanced ER+/HER2– breast cancer

— If imaging-based response is not evaluable or ambiguous, monitoring of serum breast cancer biomarkers might be helpful (II, C).
— Polygenic prediction tests are not recommended in routine practice (II, B).

HR-positive, HER2-negative breast cancer

The HT sequence in patients with HR+ breast cancer is shown in Figure 11. Surgical or pharmacological castration (GnRH analogue) in pre-menopausal patients inhibits the mitogenic effect of estrogen and allows to extend HT with drugs used in post-menopausal patients; for this reason, castration should be an integral part of the management of all patients in this group.

Other medications used in HT for advanced breast cancer include tamoxifen, AIs and fulvestrant, and megestrol acetate, medroxyprogesterone acetate or estrogens in subsequent lines. The type of HT depends on the individual clinical situation and possibly contraindications (similarly to HT in perioperative treatment). The overall survival (OS) of patients receiving AI in first-line treatment is slightly longer compared to those treated with tamoxifen [121]. The use of fulvestrant prolongs time to progression (PFS) compared to letrozole treatment [122]. Significant PFS (and in some studies also OS) prolongation during first and second line HT is also possible after adding to AI or fulvestrant one of the cyclin-dependent 4/6 (CDK4/6) kinases inhibitors — palbociclib, ribociclib or abemaciclib [123–130]. In patients with progression during non-steroidal AI therapy, the combination of exemestane with the mTOR serine-threonine kinase inhibitor — everolimus (non-reimbursed drug) — allows for longer PFS compared to exemestane monotherapy, at the cost of greater toxicity and without a significant effect on OS [131]. In patients with the *PIK3CA* mutation who progressed after prior HT with AI, the addition of alpelysib (non-reimbursed drug) to fulvestrant prolongs PFS, at the cost of significant toxicity of treatment [132].

HT regimens and targeted therapies used in generalized hormone-dependent breast cancer are presented in Table 20.

Recommendation

— In pre-menopausal patients with advanced HR+ breast cancer surgical or pharmacological castration is indicated (I, B).
— In first-line treatment of advanced HR+/HER2– breast cancer tamoxifen (I, B) can be used, and in patients with confirmed menopausal status — fulvestrant (I, B), AIs (I, B) and the combination of AI or fulvestrant with a CDK4/6 inhibitor (I, A).

Table 20. Hormone and molecularly targeted drugs used in advanced hormone-dependent breast cancer

Agent	Dosage
Tamoxifen	20 mg daily *p.o.*
Anastrozole[1, 2]	1 mg daily *p.o.*
Letrozole[2, 3]	2.5 mg daily *p.o.*
Exemestane[2, 4]	25 mg daily *p.o.*
Goserelin	3.6 mg every 28 days *s.c.*
Fulvestrant[4]	500 mg day 1., 14. and 28., and then every 28 days *i.m.*
Megestrol acetate	160 mg daily *p.o.*
Combination treatment*	
Palbociclib 125 mg/day 1–21. days of cycle every 28 days *p.o.* with letrozole 2.5 mg/day *p.o.*[2] or anastrozole 1 mg/day *p.o.*[2] or fulvestrant 500 mg *i.m*. day 1, 15, 28. → day 1. every 28 days[2]	
Ribociclib 600 mg/day 1–21. days of cycle every 28 days with letrozole 2.5 mg/day *p.o.*[2] or anastrozole 1 mg/day *p.o.*[2] or fulvestrant 500 mg *i.m.* day 1, 15, 28. → day 1. every 28 days[2]	
Abemaciclib 2 × 150 mg mg/day *p.o.* with letrozole 2.5 mg/day *p.o.*[2] or anastrozole 1 mg/day *p.o.*[2] or fulvestrant 500 mg *i.m*. day 1, 15, 28. → day 1. every 28 days[2]	
Exemestane 25 mg and everolimus 10 mg daily *p.o.*[2*]	
Alpelysib 300 mg/day *p.o.* with fulvestrant 500 mg *i.m.* day 1, 15, 28. → day 1. every 28 days[2, 5]	

*Check current reimbursement regulations in Poland

[1]In patients with HER2+ breast cancer it could be combined with trastuzumab

[2]In pre-menopausal patients with the GnRH analogue (goserelin 3.6 mg every 28 days)

[3]In patients with HER2+ breast cancer it could be combined with lapatinib (check current reimbursement regulations in Poland)

[4]In patients with resistance to a non-steroidal aromatase inhibitor (cancer relapse during or within 12 months of completing adjuvant treatment and disease progression within one month after completing of palliative treatment)

[5]In patients with the *PIK3CA* mutation

— In postmenopausal patients with progression during postoperative HT with tamoxifen or within 12 months of its completion, fulvestrant (I, B) or AIs (II, B) in combination with a CDK4/6 inhibitor (I, A) can be used in the second line.
— In patients with progression during postoperative AI treatment or less than 12 months after its completion, fulvestrant in combination with a CDK4/6 inhibitor (I, B), tamoxifen (III, C), exemestane in combination with everolimus (I, B) or (in patients with *PIK3CA* mutation) alpelysib with fulvestrant (I, B) can be used.
— In subsequent treatment lines, depending on previously used therapy, non-steroidal or steroidal AIs, tamoxifen fulvestrant, megestrol acetate, medroxyprogesterone acetate or estrogens can be used (II, B).

HER2-positive breast cancer

In patients with advanced HER2+ breast cancer, first-time treatment with trastuzumab in combination with CHT significantly increases PFS and OS [133]. Original trastuzumab can be replaced with its biosimilar substitutes, provided that they are used in accordance with international recommendations [99]. Adding pertuzumab to the combination of docetaxel and trastuzumab significantly prolongs PFS and OS [134]. The duration of CHT in combination with anti-HER2 antibodies depends on response achieved and treatment tolerance; in general, it is 4–6 months [135]. Treatment with anti-HER2 antibodies is continued until tumor progression or treatment intolerance. In postmenopausal patients with HR+/HER2+ phenotype, the combination of trastuzumab with anastrozole or lapatinib with letrozole extends PFS compared with the HT alone [136, 137]. Adding pertuzumab or lapatinib to the combination of trastuzumab and HT prolongs PFS [138, 139] (non-refundable regimens). However, the combination of anti-HER2 antibodies with HT in first-line treatment is not an alternative to CHT combined with anti-HER2 antibodies and should not be used routinely. However, this approach may be considered in the case of worsening CHT tolerance, with satisfactory tumor control.

In patients with progression after treatment with taxoids in combination with trastuzumab, the use of T-DM1 results in prolonged OS and PFS compared to the combination of lapatinib with capecitabine, with lower toxicity [140]. In the next treatment line (after the combination of lapatinib with capecitabine), the use of T-DM1 allows for longer PFS and OS compared to physician's choice treatment, with better tolerance [141].

The combination of lapatinib with capecitabine significantly prolongs PFS compared to capecitabine monotherapy [142]. There are no data on the efficacy of this regimen in patients who previously received CHT in combination with trastuzumab and pertuzumab.

Combination of trastuzumab with lapatinib (regimen currently not reimbursed) in patients with progression after adjuvant treatment with trastuzumab significantly increases OS and PFS compared to lapatinib alone [143]. Lapatinib is contraindicated in patients with impaired left ventricle ejection fraction, malabsorption or receiving drugs that interact with lapatinib (affecting CYP3A4 enzyme activity).

In patients after failure of trastuzumab, pertuzumab and T-DM1 treatment, the addition of a selective HER2 tyrosine kinase inhibitor, tucatinib (not reimbursed drug) to the combination of trastuzumab with capecitabine, prolongs PFS and OS with acceptable toxicity [144]. In turn, the use of trastuzumab-derukstecan (a conjugate of trastuzumab with a cytotoxic drug from the group of topoisomerase I inhibitors, not reimbursed drug) in patients with previous treatment including T-DM1 results in 61% response rate [145]. Replacing lapatinib with neratinib (not reimbursed drug) in combination with neratinib (not reimbursed drug) in combination with capecitabine in patients after 2 or more anti-HER2 treatment lines can prolong PFS and reduce the risk of symptomatic brain metastases [146].

Chemotherapy regimens used in patients with disseminated HER2+ breast cancer is shown in Table 21, while the principles of anti-HER2 treatment in the first and subsequent lines in Figures 12 and 13.

Recommendations

— Anti-HER2 therapy in patients with HER2+ breast cancer should be started at diagnosis of generalized disease (I, A).
— The combination of docetaxel, trastuzumab and pertuzumab is most effective in first-line treatment (I, A).
— In case of progression during first-time treatment with trastuzumab, it is advisable to continue anti-HER2 treatment in subsequent treatment lines (II, B).
— In the second and subsequent treatment lines T-DM1 should be used (I, A).
— In further treatment lines the combination of lapatinib with capecitabine (I, B) or trastuzumab (II, B), trastuzumab with capecitabine and tucatinib (I, B) or trastuzumab-derukstecan (II, B) could be used.
— Due to the risk of cardiovascular complications, trastuzumab should not be combined with anthracyclines (II, B).
— Heart function should be monitored during treatment with trastuzumab (III, A).
— In patients with HR+/HER2+ feature after completion of CHT in combination with anti-HER2, anti-HER2 treatment should be continued in combination with HT (III, B).

HER2-negative, ER/PgR-negative or ER/PgR-positive hormone-resistant breast cancer

HT resistance can be primary and secondary. Primary resistance means tumor recurrence in the first 2 years of postoperative HT, and in advanced cancer — progression during the first 6 months of first HT. Secondary resistance is defined as tumor recurrence after more than 2 years of postoperative HT or 12 months after its completion, and in advanced breast cancer, progression later than in the first 6 months of HT. Similarly to HR– cancer, CHT is used in patients resistant to HT. The decision to choose single or multi-drug CHT depends on the individual clinical situation. The decision to select single or multi-drug CHT depends on the individual clinical situation. Sequential use of single drugs allows to obtain similar efficacy with less toxicity as compared to multi-drug regimens and is preferred option [147]. Multi-drug CHT is used only if rapid remission is necessary due to discomfort, large metastases volume or rapid progression. In generalized breast cancer, anthracyclines, taxoids, capecitabine, vinorelbine, eribulin (not reimbursed in Poland), platinum derivatives, gemcitabine, cyclophosphamide and methotrexate are used (Table 22).

The maximum cumulative lifetime doses of doxorubicin and epirubicin are 450 mg/m^2 and 900 mg/m^2, respectively, and in patients after heart covering radiotherapy and in high-risk group (hypertension since more than 5 years, coronary heart disease, valvular defect or history of myocardial injury, age > 70 years) — 400 mg/m^2 and 800 mg/m^2, respectively. During anthracycline treatment, heart function should be monitored.

The relatively good palliative effect can be achieved by metronomic CHT with low doses of cyclophosphamide and methotrexate, capecitabine or vinorelbine [148]. This treatment option seems to be particularly justified in patients who do not require rapid tumor mass reduction.

In patients with triple-negative breast cancer after the previous adiuvant CHT with anthracyclines and possibly taxoids, carboplatin and docetaxel show similar efficacy with better carboplatin tolerance, while in the subgroup of patients with inherited *BRCA1/2* gene mutation carboplatin use allows achieving higher response rate [149]. In patients with HER2– (triple-negative or HR+, HT-resistant) cancer with a hereditary *BRCA1/2* mutation, the use of PARP inhibitors (olaparib or talazoparib; not reimbursed drugs) in the first or subsequent treatment lines allows, compared to standard single-agent CHT, to obtain a longer PFS,

Table 21. Treatment regimens used in relapsed or advanced HER2-positive breast cancer*

First line
P/T/DXL P: 840 mg (loading dose) day 1.→ 420 mg (maintenance dose) *i.v.* day 1. T1: 8 mg/kg *i.v.* (loading dose) day 1. → 6 mg/kg *i.v*. (maintenance dose) day 1. DXL: 75–100 mg/m^2 *i.v.* day 1., every 21 days
P/T/PXL* P: 840 mg (loading dose) day 1.→ 420 mg (maintenance dose) *i.v*. day 1. T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (maintenance dose) day 1., every 21 days PXL: 80 mg/m^2 *i.v*., every 7 days
Subsequent lines
T-DM1: 3.6 mg/kg day 1., every 21 days
PXL/T PXL: 80 mg/m^2 *i.v*. day 1., every 7 days T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v.* (dose maintenance) day 1., every 21 days or T: 600 mg *s.c*. day 1., every 21 days DXL/T DXL: 80–100 mg/m^2 *i.v.* day 1., every 21 days T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (dose maintenance) day 1., every 21 days or T: 600 mg *s.c.* day 1., every 21 days
VRB/T VRB: 25 mg/m^2 *i.v.* day 1. every 7 days or VRB: 30–35 mg/m^2 *i.v.* day 1. i 8., every 21 days or VRB: 60–80 mg/m^2 orally day 1. every 7 days T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (dose maintenance) day 1., every 21 days or T: 600 mg *s.c*. day 1., every 21 days
CAP/T CAP: 1000–1250 mg/m^2 *p.o.* twice daily, day 1–14, every 21 days T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (dose maintenance) day 1., every 21 days or T: 600 mg *s.c.* day 1., every 21 days
LAP/CAP* LAP: 1250 mg *p.o.* daily day 1–21 CAP: 1000 mg/m^2 *p.o.* twice daily, day 1–14., every 21 days
LAP/T* LAP: 1000 mg *p.o.* daily day 1–21. T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (dose maintenance) day 1., every 21 days or T: 600 mg *s.c*. day 1., every 21 days
Neratinib + CAP* Neratinib: 240 mg *p.o*. daily, day 1–21. CAP: 750 mg/m^2 *p.o.* twice daily, day 1–14., every 21 days
Tucatinib + T + CAP* Tucatinib: 300 mg *p.o*. twice daily, day 1–21. T: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (dose maintenance) day 1., every 21 days or CAP: 1000 mg/m^2 *p.o*. twice daily, day 1–14., every 21 days
Trastuzumab deruxtecan*: 5.5 mg/kg day 1., every 21 days

*Check current reimbursement regulations in Poland

P — pertuzumab; T — trastuzumab; T1 — regimen: 8 mg/kg *i.v*. (loading dose) day 1. → 6 mg/kg *i.v*. (maintenance dose) day 1., cycle every 3 weeks; DXL — docetaxel; PXL — paclitaxel; CAP — capecitabine; T-DM1 — trastuzumab emtansine; VRB — vinorelbine

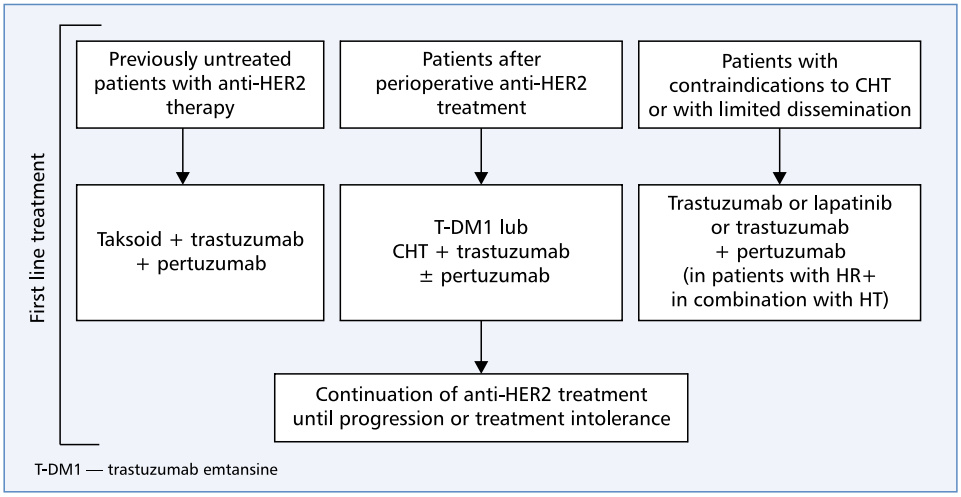

Figure 12. First line treatment regimen for patients with advanced HER2+ breast cancer

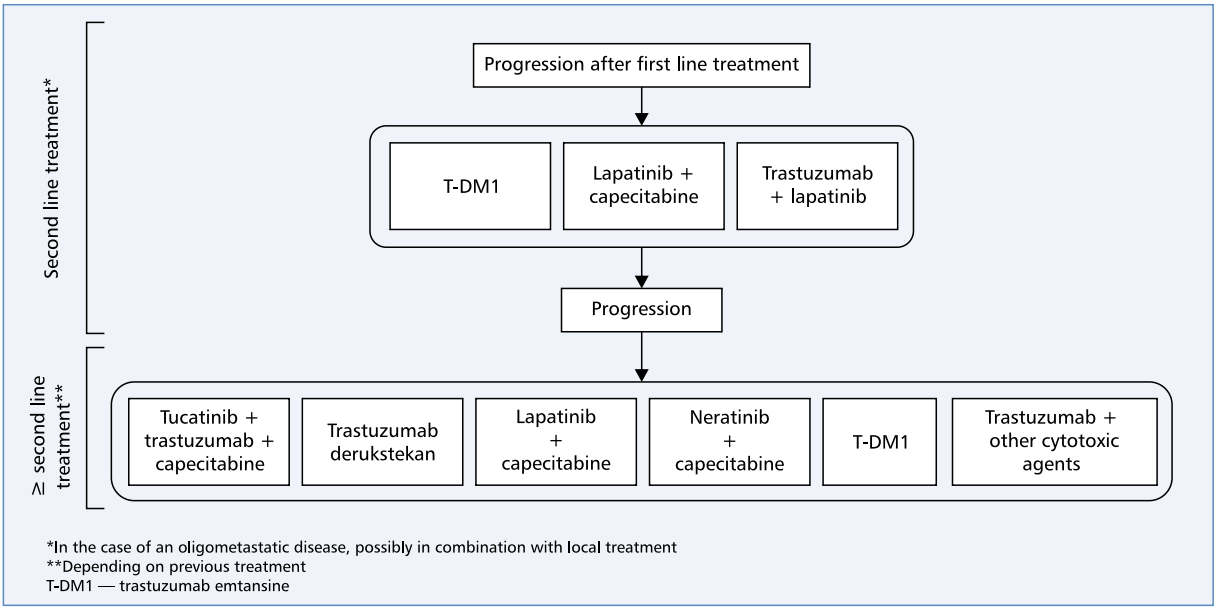

Figure 13. Second and subsequent lines treatment regimen for patients with HER2+ breast cancer

with less toxicity, the better quality of life, and a similar OS [17, 150] (Table 23). In the first-line treatment of patients with triple-negative cancer, the addition of the anti-PD-L1 antibody atezolizumab (not reimbursed drug) to nab-paclitaxel allows obtaining a longer PFS, especially in the subgroup of patients with PD-L1 expression [15]. In patients with triple-negative cancer, after treatment multiple lines, about 30% of the response is achieved by the drug sacituzumab-gowitecan-hziy, which is a conjugate of an antibody against human trophoblast surface antigen-2 and SN-38 (the active metabolite of irinotecan) [151]. This drug is not reimbursed.

Recommendations

— In majority of patients receiving CHT the sequential use of single drugs is preferred (I, A).
— Use of multi-drug CHT could be considered only if rapid response is necessary due to discomfort, large metastases volume or rapid progression (III, B).
— In patients with recurrence after previous postoperative CHT containing anthracyclines or taxoids, the use of capecitabine, vinorelbine or eribulin (not reimbursed drug) could be considered. Anthracyclines or taxoids could be reused at least 12 months after their first administration and (in the case of anthracyclines) if the maximum lifetime cumulative dose has not been reached (III, B).

Table 22. Chemotherapy regimens used in advanced HER2-negative breast cancer*

Monotherapy	Multi-drugs regimens (to be used only in selected cases)
DOX	**AC**
20 mg/m^2 *i.v.* day 1., every 7 days or	DOX 60 mg/m^2 *i.v.* day 1.
60–75 mg/m^2 *i.v.*, every 21 days	CTX 600 mg/m^2 *i.v.* day 1, every 21 days
or pegylated liposomal DOX 50 mg/m^2	**EC**
i.v. every 28 days	EPI 75 mg/m^2 *i.v.* day 1.
EPI	CTX 600 mg/m^2 *i.v.* day 1., every 21 days
60–90 mg/m^2 *i.v.* day 1.,	**CMF**
every 21 days or	CTX 100 mg/m^2 *p.o.* day 1–14.
30 mg/m^2 *i.v.* day 1., every 7 days	MTX 40 mg/m^2 *i.v.* day 1.and 8.
PXL	FU 600 mg/m^2 *i.v.* day 1. and 8, every 28 days
80 mg/m^2 *i.v.* day 1., every 7 days	**NA**
DXL	VRB 25 mg/m^2 *i.v.* day 1.and 8.
60–100 mg/m^2 *i.v.* day 1.,	DOX 50 mg/m^2 *i.v.* day 1. or 25–30 mg/m^2, day 1. and 8., every 21 days
every 21 days	**MC**[1]
CAP	Liposomal DOX 60–75 mg/m^2 *i.v.* day 1.
800–1250 mg/m^2 *p.o.* twice daily	CTX 600 mg/m^2 *i.v.* day 1., every 21 days
day 1–14., every 21 days	**NK**
GCB	VRB 80 mg *p.o.* day 1. and 8. (in 1. cycle 60 mg/m^2 *p.o.*)
800–1200 mg/m^2 *i.v.* day 1., 8., and 15.,	CAP 1000 mg/m^2 twice daily, day 1–14., every 21 days
every 28 days	**DXL/CAP**
VRB	DXL: 75 mg/m^2 *i.v.* day 1.
25 mg/m^2 *i.v.* day 1., every 7 days or	CAP: 950 mg/m^2 *p.o.* twice daily, day 1–14., every 21 days
60–80 mg/m^2 *p.o.* day 1.,	**Nab-PXL/ATEZO*** (in TNBC with PDL1 expression[2])
every 7 days	NAB-PXL: 100 mg/m^2 *i.v.* day 1., 8. and 15.
Eribulin*	ATEZO: 840 mg *i.v.* day 1. and 15., every 28 days
1,4 mg/m^2 *i.v.* day 1. and 8.,	**Metronomic regimens**
every 21 days	**CTX**
CBDCA	50 mg *p.o.* daily, treatment without interruptions
AUC 6 *i.v.* day 1., every 21–28 days or	**CTX + MTX**
AUC 2 *i.v.* day 1., every 7 days	CTX 50 mg *p.o.* daily and MTX 5 mg twice weekly, treatment without interruptions
Cisplatin	**CAP**
75 mg/m^2 *i.v.* day 1., every 21 days	3 × 500 mg/m^2 *p.o.* 3 times a day after meals, treatment without interruptions
or 25 mg/m^2 *i.v.* day 1., every 7 days	**VRB**
Nab-PXL*	50 mg *p.o.* (Monday, Wednesday, Friday)
100–125 mg/m^2 *i.v.* day 1., 8.,	30 mg *p.o.* every other day (in elderly patients)
and 15., every 28 days	**VK**
Sacituzumab govitekan-hziy	VRB 40 mg *p.o.* (Monday, Wednesday, Friday) and
(in TNBC) 10 mg/kg *i.v.* day 1.	CAP 500 mg *p.o.* 3 times a day after meals, treatment without interruptions
and 8., every 21 days	**VEK**
	VRB 40 mg *p.o.* (Monday, Wednesday, Friday)
	CTX 50 mg/day *p.o.*, treatment without interruptions and
	CAP 500 mg *p.o.* 3 times a day after meals, treatment without interruptions

*Check current reimbursement regulations in Poland

[1]Treatment with liposomal anthracyclines should be considered in patients with cardiological burdens

[2]Assessment of PD-L1 expression on immune cells in tumor microenvironment; VENTANA SP142 IHC test, cut-off point ≥ 1%

CTX — cyclophosphamide; DOX — doxorubicin; EPI — epirubicin; DXL — docetaxel; PXL — paclitaxel; FU — fluorouracil; CAP — capecitabine; MTX — methotrexate; VRB — vinorelbine; CBDCA — carboplatin; GEM — gemcitabine; Nab-PXL — paclitaxel in form of a nanoparticle complex with albumin; M — liposomal doxorubicin; GCS-F — granulocyte colony-stimulating factor

— In subsequent treatment lines, single-drug or multi-drug metronomic CHT with low doses of cyclophosphamide and methotrexate, capecitabine or vinorelbine may be used (II, B).

— In patients with triple-negative cancer with PD-L1 expression, the combination of nab-paclitaxel and atezolizumab can be used in frontline treatment (I, B).

Table 23. Molecularly targeted drugs (PARP inhibitors) used in patients with advanced breast cancer with germline BRCA1/2 gene mutations

Agent	Dosing
Olaparib*	300 mg *p.o.* twice daily, every 28 days
Talazoparib*	1 mg p.o. daily, every 28 days

*Check current reimbursement regulations in Poland

— In patients with triple-negative breast cancer on *BRCA1/2* genes mutations basis platinum derivatives could be used in first line treatment (II, B).
— In patients with HER2– breast cancer on *BRCA1/2* genes mutations PARP inhibitors may be used in the first or subsequent treatment lines (I, B).

Special clinical situations

In HER2+ breast cancer patients with brain metastases and with no progression in extracranial foci, local therapy is usually applied (neurosurgery or RTH), and then anti-HER2 therapy is continued [152] (procedure currently not reimbursed in Poland).

In men with HR+ breast cancer, tamoxifen is the drug with the best-known efficacy [153, 154]. In patients with contraindications to tamoxifen or with progression after tamoxifen treatment, clinical benefit can be obtained using AIs, but always in combination with a GnRH analogue or orchidectomy [155].

Recommendations

— In patients with a limited number of metastases (so-called oligometastatic disease), local ablative treatment (surgery, stereotactic RTH, thermoablation, intra-arterial CHT) could be considered in addition to systemic treatment (III, C).
— In HER2+ breast cancer patients with brain metastases and with no progression in extracranial foci after local therapy, a continuation of anti-HER2 treatment should be considered (III, C).
— If brain metastases develop with concurrent extracranial progression, another systemic treatment line should be considered in addition to local treatment (III, C).
— In men with breast cancer with HR, expression tamoxifen should be used in first-line treatment (III, A) and in case of contraindications to tamoxifen or progression after treatment with tamoxifen — AIs in combination with GnRH analogue or surgical castration (III, C).

Supportive treatment in bone metastases

Bisphosphonates used in patients with bone metastases reduce the risk and delay subsequent bone metastases and related complications, reduce pain severity and improve the quality of life [156–158]. In addition to bone metastases, the indication for bisphosphonates (intravenous) is acute hypercalcemia (serum calcium > 3 mmol/L) with accompanying multiorgan clinical symptoms. If there are no contraindications, bisphosphonates should be used in combination with calcium supplements (1200–1500 mg daily) and vitamin D3 (400–800 IU) daily (II, A). The dose and duration of bisphosphonates administration should be based on renal function The main contraindication for bisphosphonates administration is renal failure (creatinine > 3.0 mg/dL), as well as an active inflammatory processes and planned invasive procedures within the maxilla or mandible. The most important side effects of bisphosphonate therapy are kidney damage and osteonecrosis of the jaw (ONJ). Bisphosphonates are used in the following doses: zoledronic acid 4 mg *i.v.*, 15-minute infusion every 3–12 weeks [159], clodronate 1600 mg *p.o.* (in 1–2 doses) daily. The optimal duration of bisphosphonate therapy has not been established.

Denosumab, fully human monoclonal antibody directed against receptor activator of nuclear factor-κB ligand (RANKL) (not reimbursed drug) reduces the risk and delays the occurrence of bone complications compared to zoledronate, but has no significant effect on PFS and OS [160]. Hypocalcemia and ONJ are more frequent during the use of denosumab than in case of zoledronate, however, there is no need to modify the dose in case of renal failure. Denosumab is administered at a dose of 120 mg every 4 weeks by subcutaneous injection, in combination with calcium supplements and vitamin D3. The optimal duration of treatment is not established.

Recommendations

— In patients with bone metastases, bone-modifying agents (bisphosphonates, denosumab) should be used since diagnosis (I, A).
— Qualification for bone-modifying agents is based on bone metastases diagnosed in radiological examination (X-ray, CT, MRI). These agents should not be used only based on lesions in scintigraphy (III, B).
— Bone-modifying agents can be combined with anticancer treatment (III, A).
— Bone-modifying agents should be used in combination with calcium supplements and vitamin D3 (II, A).
— Before starting the treatment with the bone-modifying agent, sanitation of the oral cavity should be performed (III, A).
— During the treatment with bone-modifying agents, dental procedures that violate the continuity of the mucous membrane should be avoided as much as possible. If this procedure is necessary, bisphosphonates should be discontinued at least 4 weeks before and 4 weeks after the procedure. Renal function

(creatinine concentration) should be assessed every 2–3 months, if the osteonecrosis of the jaw and/or maxilla is suspected, oral examination and a panoramic X-ray should be performed (III, A).

Supportive care in patients receiving systemic therapy

Supportive care is described in the section on treatment of patients with stage I–III breast cancer.

The role of other treatment methods

In patients with a limited number of brain metastases, in good general condition and without non-cerebral tumor foci, the treatment of choice is surgical resection or radiosurgery. In case of multiple metastases, whole-brain RTH or symptomatic treatment is used. RTH allows achieving a good palliative effect in inoperable local and regional recurrences, compartment syndromes and painful or fracture-threatening bone metastases. In bone metastases, single high doses of RTH are as effective as fractionated diagrams but less burdensome [161].

In multiple painful metastases, especially ineligible for RTH, treatment with radioisotopes is used. However, this procedure is rarely used due to significant hematological toxicity that may hinder the administration of subsequent lines of systemic treatment [162].

In patients with neoplastic symptomatic pleural effusion, pleural cavity drainage with possible intrapleural infusion of talc is used in addition to systemic therapy. Drainage, possibly with bleomycin administration, or pericardiocentesis is used in patients with malignant pericardial effusion (MPE).

Palliative surgical treatment includes mitigation treatments, for example, excision of ulcerative lesions, anastomosis of bone fractures, or formation of nutritional stomies.

The role of breast amputation in patients with cancer generalization is not clearly defined and there is no justification for its routine use [163, 164]. Palliative amputation (so-called toilet amputation) is performed to remove bleeding or ulcerated primary tumor in patients after failure of systemic treatment and RTH.

Recommendations

— In patients with a limited number of brain metastases, in good performance status and without extra-cranial tumors, the treatment of choice is surgical resection or stereotactic RTH (II, B).
— For multiple brain metastases, whole-brain RTH or symptomatic treatment could be used (II, B).
— Palliative RTH is therapy of choice in inoperable local and regional recurrences, compartment syndromes and painful or fracture-threatening bone metastases (II, A).
— In RTH of limited bone metastases the use of single high doses is recommended (I, B).
— In multiple painful metastases, especially ineligible for RTH, treatment with radioisotopes could be used (II, B).
— In limited metastases in lungs, liver and bones, stereotactic RTH could be considered (II, B).
— In patients with primary generalized cancer, routine breast amputation is unjustified (II, C).
— Palliative surgical treatment could be used for the relief of tumor symptoms if it cannot be achieved by other methods (III, B).

Rehabilitation

Psychophysical rehabilitation is an integral part of the treatment of patients with breast cancer. It is aimed at reducing the physical, mental, professional and social maladjustment that results from cancer or its treatment.

The goals of psychological rehabilitation are to improve mental state of patients and help in the acceptance of cancer. Psychological help should apply to both patient and her relatives. The methods of psychoeducation, short- and long-term individual therapy of patients and their families as well as social activities in support groups are used.

Physiotherapy (physical rehabilitation) is aimed at improving patients during various treatment periods (hospital, outpatient, sanatorium). For specific purposes, physiotherapy includes:

— achieving full mobility within the shoulder girdle after surgical treatment of breast cancer;
— prevention of secondary lymphoedema of limb on operated side;
— conducting comprehensive conserving edema therapy in case of its occurrence;
— prevention and conserving treatment of postural defects resulting from breast amputation.

As part of rehabilitation, the selection of external breast implants (one reimbursed for 2 years), wigs during CHT (one reimbursed per year) and compression sleeves after lymphatic edema treatment of upper limbs (one reimbursed per year) should be ensured.

Recommendations

— All breast cancer patients should have full access to physical and psychological rehabilitation (III, A).
— Psychological assistance in the form of psychoeducation, short- and long-term, individual therapy and social activities in support groups should apply to both the patient and her relatives (III, A).
— Physical rehabilitation should include prevention of secondary lymph edema of the operated side limb, achieving full mobility within the shoulder girdle

after surgical treatment, complex non-surgical treatment of edema and prevention and conservative treatment of postural defects resulting from breast amputation (III, A).
— As part of rehabilitation, the selection of external breast implants (reimbursement every 2 years), wigs during CHT (refund every year) and compression sleeves after lymphatic edema treatment of upper limbs (reimbursement every year) (IV, A).
— During the follow-up period after primary treatment, it is necessary to ensure the possibility of improvement due to possible co-morbidities; a breast cancer diagnosis is not a contraindication to physiotherapy (III, A).

Modification of lifestyle after a breast cancer diagnosis

Moderate-intensive physical activity, both before and after cancer diagnosis, reduces the risk of death and cancer-related death in patients diagnosed with breast cancer [165]. Increasing physical activity in breast cancer patients also improves the quality of life, emotional state and social activity, and reduces anxiety [166]. Weight gain translates into poorer prognosis. Compared to patients maintaining baseline body weight (± 5%), weight gain after diagnosis of breast cancer by ≥ 10% was associated with a significant increase in the relative risk of death by 23% and increase in the risk of breast cancer-related death by 17% (non-significant difference) [167].

The use of hormone replacement therapy in women after treatment of breast cancer significantly increases the risk of cancer recurrence [168].

Consumption of alcohol, even in small amounts, is a well-known factor that increases the risk of breast cancer. The results of research on the relationship between alcohol consumption after the diagnosis of breast cancer and treatment outcomes are contradictory.

In smokers, stopping smoking after a diagnosis of breast cancer significantly improves the prognosis. Study involving over 20,000 patients indicated that smoking cessation was associated with a 33% reduction in the relative risk of death from breast cancer [169].

There are no fundamental contraindications to preventive vaccinations in cancer patients, except of periods of profound immunosuppression associated with anticancer treatment.

Recommendations
— Patients diagnosed with breast cancer should be advised to have regular physical activity (at least 150 minutes/week) (II, B).
— Patients should be advised to prevent weight gain and keep BMI within the range of 20–25 (II, B).
— The use of hormone replacement therapy in patients after breast cancer treatment is contraindicated (I, A).
— Patients should be advised to limit alcohol consumption (III A).
— Smoking patients should be advised to stop smoking and supported in order to overcome addiction (II, A).
— There are no contraindications to preventive vaccinations in patients with breast cancer (II, B).

Observation after treatment

The goals of follow-up after primary breast cancer treatment include early detection of local and regional recurrence and secondary cancers, observation toward late-onset complications, psychological and social counseling (including encouraging to physical activity, tobacco abstinence and maintaining proper body weight), motivating patients receiving long-term adjuvant treatment for its continuation, as well as evaluation of late treatment results. Majority of breast cancer recurrences are detected based on medical history, physical examination and MMG [170–172], and these three components are an indispensable part of follow-up evaluations [173, 174]. Performing an extended range of imaging or laboratory tests to earlier detect asymptomatic distant metastases does not have a significant impact on patients survival and quality of life [174]. Detailed rules regarding observation of patients after treatment are presented in Table 24.

Recommendations
— In breast cancer patients who have received treatment with a radical intention, it is recommended to perform a monthly breast self-examination, periodic medical examination including medical history and physical examination, and yearly MMG, supplemented with breast ultrasound or MRI, if necessary (II, B).
— Performing an extended range of imaging or laboratory tests to actively search for asymptomatic distant metastases is not recommended (I, B). However, it is justified in case of clinical features suggestive of tumor recurrence (IV, B).

Conflicts of interest

JJ — advisory roles: AstraZeneca, BMS, Pfizer, MSD, Takeda; travel support: Roche, Pfizer. MK — advisory roles: Pierre Fabre, AstraZeneca, Roche, Novartis, Lilly. RD — travel grants: Roche, Teva, GSK, Pfizer, AstraZeneca; Science Advisory Boards: AstraZeneca, Novartis, Teva, Roche, Lilly. ESK — honoraria: Amgen, AstraZeneca, Clinigen, Egis, Eli Lilly, Genomic

Table 24. Recommendations for follow-up after radical treatment for breast cancer

Recommended tests	**Frequency**
Self-examination	Monthly
Medical history and physical examination, education of patients about typical symptoms of tumor recurrence	Every 3–4 months for first 2 years[1]
	Every 6–8 months for 3–5 years
	Every 12 months > 5 years
MMG[2] (if necessary supplemented with breast ultrasound)	Every 12 months
	In patients after BCT the first examination after 6 months
Imaging and laboratory tests	Only according to clinical indications
Evaluation of bone mineralization status (densitometry)[3]	Every 12–24 months
Body weight measurement	BMI maintenance within the range of 20–25
Not recommended tests	
Complete blood count	
Blood biochemistry	
Circulating tumor markers	
Chest X-ray	
Other imaging studies (CT, MRI, PET)	
Transvaginal US[4]	

[1]In ductal cancer *in situ* follow-up examinations every 6 months for the first 2 years; then every 12 months
[2]MRI to consider in carriers of *BRCA* genes mutations
[3]Applies to patients at high risk of osteoporosis associated with the treatment of aromatase inhibitors or with ovarian suppression — *see* supportive treatment
[4]In patients without symptoms from the reproductive system there is no indications for transvaginal US and endometrial biopsy
Abbreviations developed in the text

Health, Novartis, Pfizer, Pierre Fabre, Roche, Sandoz, TLC Biopharmaceuticals; travel support: Amgen, AstraZeneca, Egis, Novartis, Pfizer, Roche; clinical research: Amgen, AstraZeneca, Eli Lilly, Novartis, Pfizer, Roche, Samsung; stock: Eli Lilly. PW — consultant, speaker: Roche, Pfizer, Novartis, Eli Lilly, AstraZeneca. BBB, AJ, WO, HTK — none declared.

Piśmiennictwo

1. Didkowska J, Wojciechowska U, Czaderny K, et al. Nowotwory złośliwe w Polsce w 2017 roku. Ministerdstwo Zdrowia, Warszawa 2019.
2. Duffy SW, Vulkan D, Cuckle H, et al. Effect of mammographic screening from age 40 years on breast cancer mortality (UK Age trial): final results of a randomised, controlled trial. Lancet Oncol. 2020; 21(9): 1165–1172, doi: 10.1016/S1470-2045(20)30398-3, indexed in Pubmed: 32800099.
3. Perry N, Broeders M, de Wolf C. European guidelines for quality assurance in breast cancer screening and diagnosis European Commission. Ed. 4. European guidelines for quality assurance in breast cancer screening and diagnosis European Commission. Ed. 4. European Commission, Office for Official Publications of the European Communities, Luksemburg 2006.
4. Spak DA, Plaxco JS, Santiago L, et al. BI-RADS fifth edition: A summary of changes. Diagn Interv Imaging 2017; 98(3): 179–190, doi: 10.1016/j.diii.2017.01.001, indexed in Pubmed: 28131457.
5. Brożek I, Limon J. Poradnictwo genetyczne w dziedzicznym raku piersi. In: Jassem J, Krzakowski M (ed.). Rak piersi. Praktyczny przewodnik dla lekarzy. Via Medica, Gdańsk. 2009: 20–31.
6. NCCN Clinical practice guidelines in oncology: genetic/familial high risk assessment: breast & ovarian. Version 2.2016 ed2016. www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf (24.10.2017).
7. Paluch-Shimon S, Cardoso F, Sessa C, et al. ESMO Guidelines Committee. Prevention and screening in BRCA mutation carriers and other breast/ovarian hereditary cancer syndromes: ESMO Clinical Practice Guidelines for cancer prevention and screening. Ann Oncol. 2016; 27(suppl 5): v103–v110, doi: 10.1093/annonc/mdw327, indexed in Pubmed: 27664246.
8. Kurian AW, Hare EE, Mills MA, et al. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol. 2014; 32(19): 2001–2009, doi: 10.1200/JCO.2013.53.6607, indexed in Pubmed: 24733792.
9. Antoniou AC, Hardy R, Walker L, et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. J Med Genet. 2008; 45(7): 425–431, doi: 10.1136/jmg.2007.056556, indexed in Pubmed: 18413374.
10. Levy-Lahad E, Friedman E. Cancer risks among BRCA1 and BRCA2 mutation carriers. Br J Cancer. 2007; 96(1): 11–15, doi: 10.1038/sj.bjc.6603535, indexed in Pubmed: 17213823.
11. Kuhl C, Schrading S, Leutner C, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. J Clin Oncol. 2005; 23(33): 8469–8476, doi: 10.1200/jco.2004.00.4960.
12. De Felice F, Marchetti C, Musella A, et al. Bilateral risk-reduction mastectomy in BRCA1 and BRCA2 mutation carriers: a meta-analysis. Ann Surg Oncol. 2015; 22(9): 2876–2880, doi: 10.1245/s10434-015-4532-1, indexed in Pubmed: 25808098.
13. Mavaddat N, Antoniou AC, Mooij TM, et al. Risk-reducing salpingo-oophorectomy, natural menopause, and breast cancer risk: an international prospective cohort of BRCA1 and BRCA2 mutation carriers. Breast Cancer Res. 2020; 22(1): 8, doi: 10.1186/s13058-020-1247-4, indexed in Pubmed: 31948486.
14. Tan PH, Ellis I, Allison K, et al. The 2019 WHO classification of tumours of the breast. Histopathology 2020; 77: 181–185.
15. Schmid P, Adams S, Rugo HS, et al. IMpassion130 Trial Investigators. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018; 379(22): 2108–2121, doi: 10.1056/NEJMoa1809615, indexed in Pubmed: 30345906.
16. Wang M, Li J, Huang J, et al. The Predictive Role of Mutation Status on Inhibitors in Breast Cancer Therapy: A Systematic Review and Meta-Analysis. Biomed Res Int. 2020; 2020: 1598037, doi: 10.1155/2020/1598037, indexed in Pubmed: 32461963.

17. Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med. 2017; 377(6): 523–533, doi: 10.1056/NEJMoa1706450, indexed in Pubmed: 28578601.
18. Wolff A, Hammond M, Allison K, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med. 2018; 142(11): 1364–1382, doi: 10.5858/arpa.2018-0902-sa.
19. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol. 2007; 25(28): 4414–4422, doi: 10.1200/JCO.2007.10.6823, indexed in Pubmed: 17785706.
20. Ellis MJ, Tao Yu, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst. 2008; 100(19): 1380–1388, doi: 10.1093/jnci/djn309, indexed in Pubmed: 18812550.
21. Amin BA, Edge SB. AJCC cancer staging manual. 8th ed. Springer, New York 2017.
22. Miller BT, Abbott AM, Tuttle TM. The influence of preoperative MRI on breast cancer treatment. Ann Surg Oncol. 2012; 19(2): 536–540, doi: 10.1245/s10434-011-1932-8, indexed in Pubmed: 21751044.
23. Sardanelli F, Boetes C, Borisch B, et al. Magnetic resonance imaging of the breast: recommendations from the EUSOMA working group. Eur J Cancer. 2010; 46(8): 1296–1316, doi: 10.1016/j.ejca.2010.02.015, indexed in Pubmed: 20304629.
24. Mann RM, Balleyguier C, Baltzer PA, et al. European Society of Breast Imaging (EUSOBI), with language review by Europa Donna — The European Breast Cancer Coalition. Breast MRI: EUSOBI recommendations for women's information. Eur Radiol. 2015; 25(12): 3669–3678, doi: 10.1007/s00330-015-3807-z, indexed in Pubmed: 26002130.
25. Iacconi C. Diffusion and perfusion of the breast. Eur J Radiol. 2010; 76(3): 386–390, doi: 10.1016/j.ejrad.2010.03.009, indexed in Pubmed: 20413239.
26. Sardanelli F, Fallenberg EM, Clauser P, et al. European Society of Breast Imaging (EUSOBI), with language review by Europa Donna — The European Breast Cancer Coalition. Mammography: an update of the EUSOBI recommendations on information for women. Insights Imaging. 2017; 8(1): 11–18, doi: 10.1007/s13244-016-0531-4, indexed in Pubmed: 27854006.
27. Bhimani C, Matta D, Roth RG, et al. Contrast-enhanced Spectral Mammography: Technique, Indications, and Clinical Applications. Acad Radiol. 2017; 24(1): 84–88, doi: 10.1016/j.acra.2016.08.019, indexed in Pubmed: 27773458.
28. Krzakowski M, Rutkowski P, Jassem J, et al. Recommendations on the application of positron emission tomography in oncology. Oncol Clin Pract. 2015; 11: 155–171.
29. Kumar R, Chauhan A, Zhuang H, et al. Clinicopathologic factors associated with false negative FDG-PET in primary breast cancer. Breast Cancer Res Treat. 2006; 98(3): 267–274, doi: 10.1007/s10549-006-9159-2, indexed in Pubmed: 16555126.
30. Biganzoli L, Cardoso F, Beishon M, et al. The requirements of a specialist breast centre. Breast. 2020; 51: 65–84, doi: 10.1016/j.breast.2020.02.003, indexed in Pubmed: 32217457.
31. Morrow M, Van Zee KJ, Solin LJ, et al. Society of Surgical Oncology-American Society for Radiation Oncology-American Society of Clinical Oncology Consensus Guideline on Margins for Breast-Conserving Surgery With Whole-Breast Irradiation in Ductal Carcinoma In Situ. J Clin Oncol. 2016; 34(33): 4040–4046, doi: 10.1200/JCO.2016.68.3573, indexed in Pubmed: 27528719.
32. Goodwin A, Parker S, Ghersi D, et al. Post-operative radiotherapy for ductal carcinoma *in situ* of the breast. Cochrane Database Syst Rev. 2013; 11: CD000563.
33. Sagara Y, Freedman RA, Vaz-Luis I, et al. Patient Prognostic Score and Associations With Survival Improvement Offered by Radiotherapy After Breast-Conserving Surgery for Ductal Carcinoma In Situ: A Population-Based Longitudinal Cohort Study. J Clin Oncol. 2016; 34(11): 1190–1196, doi: 10.1200/JCO.2015.65.1869, indexed in Pubmed: 26834064.
34. Stuart KE, Houssami N, Taylor R, et al. Long-term outcomes of ductal carcinoma *in situ* of the breast: a systematic review, meta-analysis and meta-regression analysis. BMC Cancer. 2015; 15: 890, doi: 10.1186/s12885-015-1904-7, indexed in Pubmed: 26555555.
35. Mitchell KB, Kuerer H. Ductal carcinoma in situ: treatment update and current trends. Curr Oncol Rep. 2015; 17(11): 48, doi: 10.1007/s11912-015-0473-x, indexed in Pubmed: 26373411.
36. Wapnir IL, Dignam JJ, Fisher B, et al. Long-term outcomes of invasive ipsilateral breast tumor recurrences after lumpectomy in NSABP B-17 and B-24 randomized clinical trials for DCIS. J Natl Cancer Inst. 2011; 103(6): 478–488, doi: 10.1093/jnci/djr027, indexed in Pubmed: 21398619.
37. Narod SA, Iqbal J, Giannakeas V, et al. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. JAMA Oncol. 2015; 1(7): 888–896, doi: 10.1001/jamaoncol.2015.2510, indexed in Pubmed: 26291673.
38. Cuzick J, Sestak I, Pinder S, et al. Effect of tamoxifen and radiotherapy in women with locally excised ductal carcinoma in situ: long-term results from the UK/ANZ DCIS trial. Lancet Oncol. 2011; 12(1): 21–29, doi: 10.1016/s1470-2045(10)70266-7.
39. Margolese R, Cecchini R, Julian T, et al. Anastrozole versus tamoxifen in postmenopausal women with ductal carcinoma *in situ* undergoing lumpectomy plus radiotherapy (NSABP B-35): a randomised, double-blind, phase 3 clinical trial. The Lancet. 2016; 387(10021): 849–856, doi: 10.1016/s0140-6736(15)01168-x.
40. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. N Engl J Med. 2002; 347(16): 1233–1241, doi: 10.1056/NEJMoa022152, indexed in Pubmed: 12393820.
41. Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med. 2002; 347(16): 1227–1232, doi: 10.1056/NEJMoa020989, indexed in Pubmed: 12393819.
42. Moran MS, Schnitt SJ, Giuliano AE, et al. Society of Surgical Oncology, American Society for Radiation Oncology. Society of Surgical Oncology-American Society for Radiation Oncology consensus guideline on margins for breast-conserving surgery with whole-breast irradiation in stages I and II invasive breast cancer. J Clin Oncol. 2014; 32(14): 1507–1515, doi: 10.1200/JCO.2013.53.3935, indexed in Pubmed: 24516019.
43. Lyman GH, Temin S, Edge SB, et al. American Society of Clinical Oncology Clinical Practice. Sentinel lymph node biopsy for patients with early-stage breast cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2014; 32(13): 1365–1383, doi: 10.1200/JCO.2013.54.1177, indexed in Pubmed: 24663048.
44. Giuliano AE, Ballman KV, McCall L, et al. Effect of Axillary Dissection vs No Axillary Dissection on 10-Year Overall Survival Among Women With Invasive Breast Cancer and Sentinel Node Metastasis: The ACOSOG Z0011 (Alliance) Randomized Clinical Trial. JAMA. 2017; 318(10): 918–926, doi: 10.1001/jama.2017.11470, indexed in Pubmed: 28898379.
45. Donker M, Tienhoven Gv, Straver M, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol. 2014; 15(12): 1303–1310, doi: 10.1016/s1470-2045(14)70460-7.
46. Galimberti V, Cole BF, Zurrida S, et al. International Breast Cancer Study Group Trial 23-01 investigators. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. Lancet Oncol. 2013; 14(4): 297–305, doi: 10.1016/S1470-2045(13)70035-4, indexed in Pubmed: 23491275.
47. Burstein HJ, Curigliano G, Loibl S, et al. Members of the St. Gallen International Consensus Panel on the Primary Therapy of Early Breast Cancer 2019. Estimating the benefits of therapy for early-stage breast cancer: the St. Gallen International Consensus Guidelines for the primary therapy of early breast cancer. Ann Oncol. 2019; 30(10): 1541–1557, doi: 10.1093/annonc/mdz235, indexed in Pubmed: 31373601.
48. Darby S, McGale P, Correa C, et al. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10, 801 women in 17 randomised trials. Lancet. 2011; 378: 1707–1716, doi: 10.1016/S0140-6736(11)61629-2, indexed in Pubmed: 22019144.
49. Smith BD, Bentzen SM, Correa CR, et al. Fractionation for whole breast irradiation: an American Society for Radiation Oncology (ASTRO) evidence-based guideline. Int J Radiat Oncol Biol Phys. 2011; 81(1): 59–68, doi: 10.1016/j.ijrobp.2010.04.042, indexed in Pubmed: 20638191.
50. Brunt AM, Haviland JS, Wheatley DA, et al. Hypofractionated breast radiotherapy for 1 week versus 3 weeks (FAST-Forward): 5-year efficacy and late normal tissue effects results from a multicentre, non-inferiority, randomised, phase 3 trial. Lancet. 2020; 395: 1613–1626, doi: 10.1016/S0140-6736(20)30932-6.
51. Bartelink H, Maingon P, Poortmans P, et al. Whole-breast irradiation with or without a boost for patients treated with breast-conserving surgery for early breast cancer: 20-year follow-up of a randomised phase 3 trial. The Lancet Oncology. 2015; 16(1): 47–56, doi: 10.1016/s1470-2045(14)71156-8.

52. Vicini FA, Cecchini RS, White JR, et al. Long-term primary results of accelerated partial breast irradiation after breast-conserving surgery for early-stage breast cancer: a randomised, phase 3, equivalence trial. Lancet. 2019; 394(10215): 2155–2164, doi: 10.1016/S0140-6736(19)32514-0, indexed in Pubmed: 31813636.
53. Whelan TJ, Julian JA, Berrang TS, et al. RAPID Trial Investigators. External beam accelerated partial breast irradiation versus whole breast irradiation after breast conserving surgery in women with ductal carcinoma *in situ* and node-negative breast cancer (RAPID): a randomised controlled trial. Lancet. 2019; 394(10215): 2165–2172, doi: 10.1016/S0140-6736(19)32515-2, indexed in Pubmed: 31813635.
54. Whelan TJ, Olivotto IA, Levine MN, et al. MA.20 Study Investigators. Regional Nodal Irradiation in Early-Stage Breast Cancer. N Engl J Med. 2015; 373(4): 307–316, doi: 10.1056/NEJMoa1415340, indexed in Pubmed: 26200977.
55. McGale P, Taylor C, Correa C, et al. EBCTCG (Early Breast Cancer Trialists' Collaborative Group). Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta-analysis of individual patient data for 8135 women in 22 randomised trials. 2014; 383:. Lancet. 2014; 383: 2127–2135, doi: 10.1016/S0140-6736(14)60488-8, indexed in Pubmed: 24656685.
56. Wang SL, Fang H, Song YW, et al. Hypofractionated versus conventional fractionated postmastectomy radiotherapy for patients with high-risk breast cancer: a randomised, non-inferiority, open-label, phase 3 trial. Lancet Oncol. 2019; 20(3): 352–360, doi: 10.1016/S1470-2045(18)30813-1, indexed in Pubmed: 30711522.
57. Poortmans PM, Collette S, Kirkove C, et al. EORTC Radiation Oncology and Breast Cancer Groups. Internal Mammary and Medial Supraclavicular Irradiation in Breast Cancer. N Engl J Med. 2015; 373(4): 317–327, doi: 10.1056/NEJMoa1415369, indexed in Pubmed: 26200978.
58. Mauri D, Pavlidis N, Ioannidis JPA. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. J Natl Cancer Inst. 2005; 97(3): 188–194, doi: 10.1093/jnci/dji021, indexed in Pubmed: 15687361.
59. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. Lancet Oncol. 2018; 19: 27–39.
60. Gagliato Dd, Gonzalez-Angulo AM, Lei X, et al. Clinical impact of delaying initiation of adjuvant chemotherapy in patients with breast cancer. J Clin Oncol. 2014; 32(8): 735–744, doi: 10.1200/JCO.2013.49.7693, indexed in Pubmed: 24470007.
61. Albain K, Barlow W, Ravdin P, et al. Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. Lancet. 2009; 374(9707): 2055–2063, doi: 10.1016/s0140-6736(09)61523-3.
62. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. N Engl J Med. 2018; 379(2): 111–121, doi: 10.1056/NEJMoa1804710, indexed in Pubmed: 29860917.
63. Cardoso F, van't Veer LJ, Bogaerts J, et al. MINDACT Investigators. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med. 2016; 375(8): 717–729, doi: 10.1056/NEJMoa1602253, indexed in Pubmed: 27557300.
64. Yi M, Huo L, Koenig KB, et al. Which threshold for ER positivity? a retrospective study based on 9639 patients. Ann Oncol. 2014; 25(5): 1004–1011, doi: 10.1093/annonc/mdu053, indexed in Pubmed: 24562447.
65. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet. 2013; 381(9869): 805–816, doi: 10.1016/s0140-6736(12)61963-1.
66. Al-Mubarak M, Tibau A, Templeton AJ, et al. Extended adjuvant tamoxifen for early breast cancer: a meta-analysis. Miller TW (ed.). PloS ONE. 2014; 9: e88238.
67. Masuda N, Sagara Y, Kinoshita T, et al. Neoadjuvant anastrozole versus tamoxifen in patients receiving goserelin for premenopausal breast cancer (STAGE): a double-blind, randomised phase 3 trial. Lancet Oncol. 2012; 13(4): 345–352, doi: 10.1016/S1470-2045(11)70373-4, indexed in Pubmed: 22265697.
68. Pagani O, Regan MM, Walley BA, et al. TEXT and SOFT Investigators, International Breast Cancer Study Group. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med. 2014; 371(2): 107–118, doi: 10.1056/NEJMoa1404037, indexed in Pubmed: 24881463.
69. Francis PA, Regan MM, Fleming GF, et al. SOFT Investigators, International Breast Cancer Study Group, TEXT and SOFT Investigators, International Breast Cancer Study Group. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med. 2014; 371: 107–118, doi: 10.1056/NEJMoa1404037, indexed in Pubmed: 24881463.
70. Lambertini M, Moore HCF, Leonard RCF, et al. Gonadotropin-Releasing Hormone Agonists During Chemotherapy for Preservation of Ovarian Function and Fertility in Premenopausal Patients With Early Breast Cancer: A Systematic Review and Meta-Analysis of Individual Patient-Level Data. J Clin Oncol. 2018; 36(19): 1981–1990, doi: 10.1200/JCO.2018.78.0858, indexed in Pubmed: 29718793.
71. Cataliotti L, Buzdar AU, Noguchi S, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. Cancer. 2006; 106(10): 2095–2103, doi: 10.1002/cncr.21872, indexed in Pubmed: 16598749.
72. Smith IE, Dowsett M, Ebbs SR, et al. IMPACT Trialists Group. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol. 2005; 23(22): 5108–5116, doi: 10.1200/JCO.2005.04.005, indexed in Pubmed: 15998903.
73. Eiermann W, Paepke S, Appfelstaedt J, et al. Letrozole Neo-Adjuvant Breast Cancer Study Group. Preoperative treatment of postmenopausal breast cancer patients with letrozole: A randomized double-blind multicenter study. Ann Oncol. 2001; 12: 1527–1532, doi: 10.1023/a:1013128213451, indexed in Pubmed: 11822750.
74. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015; 386: 1341–1352, doi: 10.1016/S0140-6736(15)61074-1, indexed in Pubmed: 26211827.
75. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. J Natl Cancer Inst. 2005; 97(17): 1262–1271, doi: 10.1093/jnci/dji250, indexed in Pubmed: 16145047.
76. Jakesz R, Greil R, Gnant M, et al. Austrian Breast and Colorectal Cancer Study Group. Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a. J Natl Cancer Inst. 2007; 99(24): 1845–1853, doi: 10.1093/jnci/djm246, indexed in Pubmed: 18073378.
77. Goss PE, Ingle JN, Pritchard KI, et al. Extending aromatase-inhibitor adjuvant therapy to 10 years. N Engl J Med. 2016; 375(3): 209–219, doi: 10.1056/NEJMoa1604700, indexed in Pubmed: 27264120.
78. Metzger Filho O, Giobbie-Hurder A, Mallon E, et al. Relative Effectiveness of Letrozole Compared With Tamoxifen for Patients With Lobular Carcinoma in the BIG 1-98 Trial. J Clin Oncol. 2015; 33(25): 2772–2779, doi: 10.1200/JCO.2015.60.8133, indexed in Pubmed: 26215945.
79. Ibrahim EM, Al-Homaidh A. Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies. Med Oncol. 2011; 28(3): 753–765, doi: 10.1007/s12032-010-9536-x, indexed in Pubmed: 20411366.
80. Desmarais JE, Looper KJ. Interactions between tamoxifen and antidepressants via cytochrome P450 2D6. J Clin Psychiatry. 2009; 70(12): 1688–1697, doi: 10.4088/JCP.08r04856blu, indexed in Pubmed: 20141708.
81. Braithwaite RS, Chlebowski RT, Lau J, et al. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J Gen Intern Med. 2003; 18(11): 937–947, doi: 10.1046/j.1525-1497.2003.20724.x, indexed in Pubmed: 14687281.
82. Bonadonna G, Valagussa P, Moliterni A, et al. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer: the results of 20 years of follow-up. N Engl J Med. 1995; 332(14): 901–906, doi: 10.1056/NEJM199504063321401, indexed in Pubmed: 7877646.
83. Del Ma, De Pl, Bruzzi P, et al. Gruppo Italiano Mammella (GIM) investigators. Fluorouracil and dose-dense chemotherapy in adjuvant treatment of patients with early-stage breast cancer: an open-label, 2 × 2 factorial, randomised phase 3 trial. Lancet. 2015; 385: 1863–1872, doi: 10.1016/S0140-6736(14)62048-1, indexed in Pubmed: 25740286.
84. Blum JL, Flynn PJ, Yothers G, et al. Anthracyclines in Early Breast Cancer: The ABC Trials-USOR 06-090, NSABP B-46-I/USOR 07132, and NSABP B-49 (NRG Oncology). J Clin Oncol. 2017; 35(23): 2647–2655, doi: 10.1200/JCO.2016.71.4147, indexed in Pubmed: 28398846.
85. Sparano JA, Zhao F, Martino S, et al. Long-Term Follow-Up of the E1199 Phase III Trial Evaluating the Role of Taxane and Schedule in Operable Breast Cancer. J Clin Oncol. 2015; 33(21): 2353–2360, doi: 10.1200/JCO.2015.60.9271, indexed in Pubmed: 26077235.
86. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomised trials. Lancet.

2019; 393(10179): 1440–1452, doi: 10.1016/S0140-6736(18)33137-4, indexed in Pubmed: 30739743.
87. Golshan M, Cirrincione CT, Sikov WM, et al. Alliance for Clinical Trials in Oncology. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol. 2015; 33(1): 13–21, doi: 10.1200/JCO.2014.57.0572, indexed in Pubmed: 25092775.
88. Tung N, Arun B, Hacker MR, et al. TBCRC 031: Randomized Phase II Study of Neoadjuvant Cisplatin Versus Doxorubicin-Cyclophosphamide in Germline Carriers With HER2-Negative Breast Cancer (the INFORM trial). J Clin Oncol. 2020; 38(14): 1539–1548, doi: 10.1200/JCO.19.03292, indexed in Pubmed: 32097092.
89. Loibl S, O'Shaughnessy J, Untch M, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. Lancet Oncol. 2018; 19(4): 497–509, doi: 10.1016/S1470-2045(18)30111-6, indexed in Pubmed: 29501363.
90. Loibl S, Weber KE, Timms KM, et al. Survival analysis of carboplatin added to an anthracycline/taxane-based neoadjuvant chemotherapy and HRD score as predictor of response-final results from GeparSixto. Ann Oncol. 2018; 29(12): 2341–2347, doi: 10.1093/annonc/mdy460, indexed in Pubmed: 30335131.
91. Masuda N, Lee SJ, Ohtani S, et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. N Engl J Med. 2017; 376(22): 2147–2159, doi: 10.1056/NEJMoa1612645, indexed in Pubmed: 28564564.
92. Goldhirsch A, Gelber RD, Piccart-Gebhart MJ, et al. Herceptin Adjuvant (HERA) Trial Study Team. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. Lancet. 2013; 382: 1021–1028, doi: 10.1016/S0140-6736(13)61094-6, indexed in Pubmed: 23871490.
93. Slamon D, Eiermann W, Robert N, et al. Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011; 365(14): 1273–1283, doi: 10.1056/NEJMoa0910383, indexed in Pubmed: 21991949.
94. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a andomized controlled superiority trial with a parallel HER2-negative cohort. Lancet Oncol. 2014; 15: 640–647, doi: 10.1016/s1470-2045(14)70080-4.
95. Joensuu H, Fraser J, Wildiers H, et al. Effect of Adjuvant Trastuzumab for a Duration of 9 Weeks vs 1 Year With Concomitant Chemotherapy for Early Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: The SOLD Randomized Clinical Trial. JAMA Oncol. 2018; 4(9): 1199–1206, doi: 10.1001/jamaoncol.2018.1380, indexed in Pubmed: 29852043.
96. Pivot X, Romieu G, Debled M, et al. PHARE trial investigators, PHARE trial investigators. 6 months versus 12 months of adjuvant trastuzumab for patients with HER2-positive early breast cancer (PHARE): a randomised phase 3 trial. Lancet Oncol. 2013; 14(8): 741–748, doi: 10.1016/S1470-2045(13)70225-0, indexed in Pubmed: 23764181.
97. Dieci MV, Conte P, Bisagni G, et al. Association of tumor-infiltrating lymphocytes with distant disease-free survival in the ShortHER randomized adjuvant trial for patients with early HER2+ breast cancer. Ann Oncol. 2019; 30(3): 418–423, doi: 10.1093/annonc/mdz007, indexed in Pubmed: 30657852.
98. Earl HM, Hiller L, et al. Vallier A-L PERSEPHONE: 6 versus 12 months (m) of adjuvant trastuzumab in patients (pts) with HER2 positive (+) early breast cancer (EBC): Randomised phase 3 non-inferiority trial with definitive 4-year (yr) disease-free survival (DFS) results. J Clin Oncol. 2018; 36: 506.
99. Tabernero J, Vyas M, Giuliani R, et al. Biosimilars: a position paper of the European Society for Medical Oncology, with particular reference to oncology prescribers. ESMO Open. 2016; 1(6): e000142, doi: 10.1136/esmoopen-2016-000142, indexed in Pubmed: 28848668.
100. Gianni L, Pienkowski T, Im YH, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. Lancet Oncol. 2016; 17: 791–800, doi: 10.1016/S1470-2045(16)00163-7, indexed in Pubmed: 27179402.
101. Perez EA, Suman VJ, Davidson NE, et al. Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. J Clin Oncol. 2011; 29(34): 4491–4497, doi: 10.1200/JCO.2011.36.7045, indexed in Pubmed: 22042958.
102. Tolaney SM, Barry WT, Dang CT, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. N Engl J Med. 2015; 372(2): 134–141, doi: 10.1056/NEJMoa1406281, indexed in Pubmed: 25564897.
103. von Minckwitz G, Huang CS, Mano MS, et al. KATHERINE Investigators. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. N Engl J Med. 2019; 380(7): 617–628, doi: 10.1056/NEJMoa1814017, indexed in Pubmed: 30516102.
104. Piccart-Gebhart M, Holmes E, Baselga J, et al. Adjuvant Lapatinib and Trastuzumab for Early Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: Results From the Randomized Phase III Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization Trial. J Clin Oncol. 2016; 34(10): 1034–1042, doi: 10.1200/JCO.2015.62.1797, indexed in Pubmed: 26598744.
105. Piccart M, Procter M, Fumagalli D i wsp. A randomised multi-center, double-blind, placebo-controlled trial comparing chemotherapy plus trastuzumab plus pertuzumab versus chemotherapy plus trastuzumab plus placebo as adjuvant therapy in patients with operable HER2-positive early breast cancer. San Antonio Breast Cancer Symposium 2019; abstract GS1-04.
106. Martin M, Holmes F, Ejlertsen B, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2017; 18(12): 1688–1700, doi: 10.1016/s1470-2045(17)30717-9.
107. Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet. 2015; 386(10001): 1353–1361, doi: 10.1016/s0140-6736(15)60908-4.
108. Muss HB, Berry DA, Cirrincione CT, et al. CALGB Investigators. Adjuvant chemotherapy in older women with early-stage breast cancer. N Engl J Med. 2009; 360(20): 2055–2065, doi: 10.1056/NEJMoa0810266, indexed in Pubmed: 19439741.
109. Perrone F, Nuzzo F, Di Rella F, et al. Weekly docetaxel versus CMF as adjuvant chemotherapy for older women with early breast cancer: final results of the randomized phase III ELDA trial. Ann Oncol. 2015; 26(4): 675–682, doi: 10.1093/annonc/mdu564, indexed in Pubmed: 25488686.
110. Giordano S. Breast Cancer in Men. N Engl J Med. 2018; 378(24): 2311–2320, doi: 10.1056/nejmra1707939.
111. Wu SG, Zhang WW, Sun JY, et al. Comparable survival between additional radiotherapy and local surgery in occult breast cancer after axillary lymph node dissection: a population-based analysis. J Cancer. 2017; 8(18): 3849–3855, doi: 10.7150/jca.21217, indexed in Pubmed: 29151972.
112. Roila F, Molassiotis A, Herrstedt J, et al. participants of the MASCC/ESMO Consensus Conference Copenhagen 2015. 2016 MASCC and ESMO guideline update for the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting and of nausea and vomiting in advanced cancer patients. Ann Oncol. 2016; 27(suppl 5): v119–v133, doi: 10.1093/annonc/mdw270, indexed in Pubmed: 27664248.
113. Nangia J, Wang T, Osborne C, et al. Effect of a Scalp Cooling Device on Alopecia in Women Undergoing Chemotherapy for Breast Cancer: The SCALP Randomized Clinical Trial. JAMA. 2017; 317(6): 596–605, doi: 10.1001/jama.2016.20939, indexed in Pubmed: 28196254.
114. Tsuyuki S, Yamagami K, Yoshibayashi H, et al. Effectiveness and safety of surgical glove compression therapy as a prophylactic method against nanoparticle albumin-bound-paclitaxel-induced peripheral neuropathy. Breast. 2019; 47: 22–27, doi: 10.1016/j.breast.2019.06.008, indexed in Pubmed: 31302389.
115. Grossmann M, Ramchand SK, Milat F, et al. Assessment and Management of Bone Health in Women With Oestrogen Receptor-Positive Breast Cancer Receiving Endocrine Therapy: Position Statement of the Endocrine Society of Australia, the Australian and New Zealand Bone & Mineral Society, the Australasian Menopause Society and the Clinical Oncology Society of Australia. Clin Endocrinol (Oxf). 2018; 89: 280–296.
116. Aebi S, Gelber S, Anderson SJ, et al. CALOR investigators. Chemotherapy for isolated locoregional recurrence of breast cancer (CALOR): a randomised trial. Lancet Oncol. 2014; 15: 156–163, doi: 10.1016/S1470-2045(13)70589-8, indexed in Pubmed: 24439313.
117. Waeber M, Castiglione-Gertsch M, Dietrich D, et al. Swiss Group for Clinical Cancer Research (SAKK). Adjuvant therapy after excision and radiation of isolated postmastectomy locoregional breast cancer recurrence: definitive results of a phase III randomized trial (SAKK 23/82) comparing tamoxifen with observation. Ann Oncol. 2003; 14(8): 1215–1221, doi: 10.1093/annonc/mdg347, indexed in Pubmed: 12881382.
118. Klijn JG, Blamey RW, Boccardo F, et al. Combined Hormone Agents Trialists' Group and the European Organization for Research and

Treatment of Cancer. Combined tamoxifen and luteinizing hormone-releasing hormone (LHRH) agonist versus LHRH agonist alone in premenopausal advanced breast cancer: a meta-analysis of four randomized trials. J Clin Oncol. 2001; 19(2): 343–353, doi: 10.1200/JCO.2001.19.2.343, indexed in Pubmed: 11208825.
119. Dear RF, McGeechan K, Jenkins MC, et al. Combination versus sequential single agent chemotherapy for metastatic breast cancer. Cochrane Database Syst Rev. 2013(12): CD008792, doi: 10.1002/14651858.CD008792.pub2, indexed in Pubmed: 24347031.
120. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001; 344(11): 783–792, doi: 10.1056/NEJM200103153441101, indexed in Pubmed: 11248153.
121. Robertson J, Bondarenko I, Trishkina E, et al. Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. Lancet. 2016; 388: 2997–3005, doi: 10.1016/s0140-6736(16)32389-3.
122. Gibson LJ, Dawson CK, Lawrence DH, et al. Aromatase inhibitors for treatment of advanced breast cancer in postmenopausal women. Cochrane Database Syst Rev. 2007(1): CD003370, doi: 10.1002/14651858.CD003370.pub2, indexed in Pubmed: 17253488.
123. Im SA, Lu YS, Bardia A, et al. Overall survival with ribociclib plus endocrine therapy in breast cancer. N Engl J Med. 2019; 381(4): 307–316, doi: 10.1056/NEJMoa1903765, indexed in Pubmed: 31166679.
124. Turner NC, Finn RS, Martin M, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 2016; 375: 1925–1936, doi: 10.1056/NEJMoa1607303, indexed in Pubmed: 27959613.
125. Turner NC, Ro J, André F, et al. PALOMA3 Study Group. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2015; 373(3): 209–219, doi: 10.1056/NEJMoa1505270, indexed in Pubmed: 26030518.
126. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16: 25–35, doi: 10.1016/S1470-2045(14)71159-3, indexed in Pubmed: 25524798.
127. Goetz MP, Toi M, Campone M, et al. MONARCH 3: abemaciclib as initial therapy for advanced breast cancer. J Clin Oncol. 2017; 35(32): 3638–3646, doi: 10.1200/JCO.2017.75.6155, indexed in Pubmed: 28968163.
128. Cristofanilli M, Turner N, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol. 2016; 17(4): 425–439, doi: 10.1016/s1470-2045(15)00613-0.
129. Slamon DJ, Neven P, Chia S, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol. 2020; 36: 2465–2472.
130. Sledge GW, Toi M, Neven P, et al. The Effect of Abemaciclib Plus Fulvestrant on Overall Survival in Hormone Receptor-Positive, ERBB2-Negative Breast Cancer That Progressed on Endocrine Therapy-MONARCH 2: A Randomized Clinical Trial. JAMA Oncol 2019; 6: 116-124.
131. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med. 2012; 366(6): 520–529, doi: 10.1056/NEJMoa1109653, indexed in Pubmed: 22149876.
132. André F, Ciruelos E, Rubovszky G, et al. SOLAR-1 Study Group. Alpelisib for -Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2019; 380(20): 1929–1940, doi: 10.1056/NEJMoa1813904, indexed in Pubmed: 31091374.
133. Balduzzi S, Mantarro S, Guarneri V, et al. Trastuzumab-containing regimens for metastatic breast cancer. Cochrane Database Syst Rev. 2014(6): CD006242, doi: 10.1002/14651858.CD006242.pub2, indexed in Pubmed: 24919460.
134. Swain S, Miles D, Kim SB, et al. End-of-study analysis from the phase III, randomized, double-blind, placebo (Pla)-controlled CLEOPATRA study of first-line (1L) pertuzumab (P), trastuzumab (H), and docetaxel (D) in patients (pts) with HER2-positive metastatic breast cancer (MBC). J Clin Oncol. 2019; 37(15_suppl): 1020, doi: 10.1200/jco.2019.37.15_suppl.1020.
135. Miles D, Im YH, Fung A, et al. Effect of docetaxel duration on clinical outcomes: exploratory analysis of CLEOPATRA, a phase III randomized controlled trial. Ann Oncol. 2017; 28(11): 2761–2767, doi: 10.1093/annonc/mdx406, indexed in Pubmed: 29112701.
136. Kaufman B, Mackey JR, Clemens MR, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. J Clin Oncol. 2009; 27(33): 5529–5537, doi: 10.1200/JCO.2008.20.6847, indexed in Pubmed: 19786670.
137. Johnston S, Pippen J, Pivot X, et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. J Clin Oncol. 2009; 27(33): 5538–5546, doi: 10.1200/JCO.2009.23.3734, indexed in Pubmed: 19786658.
138. Rimawi M, Ferrero JM, de la Haba-Rodriguez J, et al. PERTAIN Study Group. First-Line Trastuzumab Plus an Aromatase Inhibitor, With or Without Pertuzumab, in Human Epidermal Growth Factor Receptor 2-Positive and Hormone Receptor-Positive Metastatic or Locally Advanced Breast Cancer (PERTAIN): A Randomized, Open-Label Phase II Trial. J Clin Oncol. 2018; 36(28): 2826–2835, doi: 10.1200/JCO.2017.76.7863, indexed in Pubmed: 30106636.
139. Johnston SRD, Hegg R, Im SA, et al. Phase III, Randomized Study of Dual Human Epidermal Growth Factor Receptor 2 (HER2) Blockade With Lapatinib Plus Trastuzumab in Combination With an Aromatase Inhibitor in Postmenopausal Women With HER2-Positive, Hormone Receptor-Positive Metastatic Breast Cancer: ALTERNATIVE. J Clin Oncol. 2018; 36(8): 741–748, doi: 10.1200/JCO.2017.74.7824, indexed in Pubmed: 29244528.
140. Verma S, Miles D, Gianni L, et al. EMILIA Study Group. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med. 2012; 367(19): 1783–1791, doi: 10.1056/NEJMoa1209124, indexed in Pubmed: 23020162.
141. Krop I, Kim SB, Martin A, et al. Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. Lancet Oncol. 2017; 18(6): 743–754, doi: 10.1016/s1470-2045(17)30313-3.
142. Cameron D, Casey M, Press M, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat. 2008; 112(3): 533–543, doi: 10.1007/s10549-007-9885-0, indexed in Pubmed: 18188694.
143. Blackwell KL, Burstein HJ, Storniolo AM, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. J Clin Oncol. 2012; 30(21): 2585–2592, doi: 10.1200/JCO.2011.35.6725, indexed in Pubmed: 22689807.
144. Murthy RK, Loi S, Okines A, et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. N Engl J Med. 2020; 382(7): 597–609, doi: 10.1056/NEJMoa1914609, indexed in Pubmed: 31825569.
145. Modi S, Saura C, Yamashita T, et al. DESTINY-Breast01 Investigators. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. N Engl J Med. 2020; 382(7): 610–621, doi: 10.1056/NEJMoa1914510, indexed in Pubmed: 31825192.
146. Saura C, Oliveira M, Feng YH, et al. Neratinib + capecitabine versus lapatinib + capecitabine in patients with HER2+ metastatic breast cancer previously treated with ≥ 2 HER2-directed regimens: Findings from the multinational, randomized, phase III NALA trial. J Clin Oncol. 2019; 37(15_suppl): 1002, doi: 10.1200/jco.2019.37.15_suppl.1002.
147. Sledge GW, Neuberg D, Bernardo P, et al. Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). J Clin Oncol. 2003; 21(4): 588–592, doi: 10.1200/JCO.2003.08.013, indexed in Pubmed: 12586793.
148. Munzone E, Colleoni M. Clinical overview of metronomic chemotherapy in breast cancer. Nat Rev Clin Oncol. 2015; 12(11): 631–644, doi: 10.1038/nrclinonc.2015.131, indexed in Pubmed: 26241939.
149. Tutt A, Tovey H, Cheang MC, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med. 2018; 24(5): 628–637, doi: 10.1038/s41591-018-0009-7, indexed in Pubmed: 29713086.
150. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med. 2018; 379(8): 753–763, doi: 10.1056/NEJMoa1802905, indexed in Pubmed: 30110579.
151. Bardia A, Mayer IA, Vahdat LT, et al. Sacituzumab govitecan-hziy in refractory metastatic triple-negative breast cancer. N Engl J Med.

2019; 380(8): 741–751, doi: 10.1056/NEJMoa1814213, indexed in Pubmed: 30786188.
152. Ramakrishna N, Temin S, Chandarlapaty S, et al. Recommendations on disease management for patients with advanced human epidermal growth factor receptor 2-positive breast cancer and brain metastases: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol. 2014; 32(19): 2100–2108, doi: 10.1200/JCO.2013.54.0955, indexed in Pubmed: 24799487.
153. Kantarjian H, Yap HY, Hortobagyi G, et al. Hormonal therapy for metastatic male breast cancer. Arch Intern Med. 1983; 143(2): 237–240, doi: 10.1001/archinte.1983.00350020055012, indexed in Pubmed: 6824391.
154. Mauras N, O'Brien KO, Klein KO, et al. Estrogen suppression in males: metabolic effects. J Clin Endocrinol Metab. 2000; 85(7): 2370–2377, doi: 10.1210/jcem.85.7.6676, indexed in Pubmed: 10902781.
155. Zagouri F, Sergentanis TN, Azim HA, et al. Aromatase inhibitors in male breast cancer: a pooled analysis. Breast Cancer Res Treat. 2015; 151(1): 141–147, doi: 10.1007/s10549-015-3356-9, indexed in Pubmed: 25850534.
156. Van Poznak CH, Temin S, Yee GC, et al. American Society of Clinical Oncology. American Society of Clinical Oncology executive summary of the clinical practice guideline update on the role of bone-modifying agents in metastatic breast cancer. J Clin Oncol. 2011; 29(9): 1221––1227, doi: 10.1200/JCO.2010.32.5209, indexed in Pubmed: 21343561.
157. Hortobagyi GN, Van Poznak C, Harker WG, et al. Continued Treatment Effect of Zoledronic Acid Dosing Every 12 vs 4 Weeks in Women With Breast Cancer Metastatic to Bone: The OPTIMIZE-2 Randomized Clinical Trial. JAMA Oncol. 2017; 3(7): 906–912, doi: 10.1001/jamaoncol.2016.6316, indexed in Pubmed: 28125763.
158. Amadori D, Aglietta M, Alessi B, et al. Efficacy and safety of 12-weekly versus 4-weekly zoledronic acid for prolonged treatment of patients with bone metastases from breast cancer (ZOOM): a phase 3, open-label, randomised, non-inferiority trial. Lancet Oncol. 2013; 14(7): 663–670, doi: 10.1016/s1470-2045(13)70174-8.
159. Himelstein AL, Foster JC, Khatcheressian JL, et al. Effect of Longer-Interval vs Standard Dosing of Zoledronic Acid on Skeletal Events in Patients With Bone Metastases: A Randomized Clinical Trial. JAMA. 2017; 317(1): 48–58, doi: 10.1001/jama.2016.19425, indexed in Pubmed: 28030702.
160. Stopeck AT, Lipton A, Body JJ, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol. 2010; 28(35): 5132–5139, doi: 10.1200/JCO.2010.29.7101, indexed in Pubmed: 21060033.
161. Lutz S, Balboni T, Jones J, et al. Palliative radiation therapy for bone metastases: Update of an ASTRO Evidence-Based Guideline. Pract Radiat Oncol. 2017; 7(1): 4–12, doi: 10.1016/j.prro.2016.08.001, indexed in Pubmed: 27663933.
162. Finlay I, Mason M, Shelley M. Radioisotopes for the palliation of metastatic bone cancer: a systematic review. Lancet Oncol. 2005; 6(6): 392–400, doi: 10.1016/s1470-2045(05)70206-0.
163. Thomas A, Khan SA, Chrischilles EA, et al. Initial Surgery and Survival in Stage IV Breast Cancer in the United States, 1988-2011. JAMA Surg. 2016; 151(5): 424–431, doi: 10.1001/jamasurg.2015.4539, indexed in Pubmed: 26629881.
164. Khan SA, Zhao F, Solin LJ, et al. A randomized chase III trial of the value of Elary local therapy for the intact primary tumor in patients with metastatic breast cancer: ECOG-ACRIN 2108. J Clin Oncol 2020; LBA2.
165. Lee J. A meta-analysis of the association between physical activity and breast cancer mortality. Cancer Nurs. 2019; 42(4): 271–285, doi: 10.1097/NCC.0000000000000580, indexed in Pubmed: 29601358.
166. Lahart IM, Metsios GS, Nevill AM, et al. Physical activity for women with breast cancer after adjuvant therapy. Cochrane Database Syst Rev. 2018; 1: CD011292, doi: 10.1002/14651858.CD011292.pub2, indexed in Pubmed: 29376559.
167. Playdon MC, Bracken MB, Sanft TB, et al. Weight gain after breast cancer diagnosis and all-cause mortality: systematic review and meta-analysis. J Natl Cancer Inst. 2015; 107(12): djv275, doi: 10.1093/jnci/djv275, indexed in Pubmed: 26424778.
168. Holmberg L, Iversen OE, Rudenstam CM, et al. HABITS Study Group. Increased risk of recurrence after hormone replacement therapy in breast cancer survivors. J Natl Cancer Inst. 2008; 100(7): 475–482, doi: 10.1093/jnci/djn058, indexed in Pubmed: 18364505.
169. Passarelli MN, Newcomb PA, Hampton JM, et al. Cigarette smoking before and after breast cancer diagnosis: mortality from breast cancer and smoking-related diseases. J Clin Oncol. 2016; 34(12): 1315–1322, doi: 10.1200/JCO.2015.63.9328, indexed in Pubmed: 26811527.
170. de Bock GH, Bonnema J, van der Hage J, et al. Effectiveness of routine visits and routine tests in detecting isolated locoregional recurrences after treatment for early-stage invasive breast cancer: a meta-analysis and systematic review. J Clin Oncol. 2004; 22(19): 4010–4018, doi: 10.1200/JCO.2004.06.080, indexed in Pubmed: 15459225.
171. Montgomery DA, Krupa K, Cooke TG. Follow-up in breast cancer: does routine clinical examination improve outcome? A systematic review of the literature. Br J Cancer. 2007; 97(12): 1632–1641, doi: 10.1038/sj.bjc.6604065, indexed in Pubmed: 18000508.
172. Lu W, de Bock GH, Schaapveld M, et al. The value of routine physical examination in the follow up of women with a history of early breast cancer. Eur J Cancer. 2011; 47(5): 676–682, doi: 10.1016/j.ejca.2010.11.006, indexed in Pubmed: 21130643.
173. Moschetti I, Cinquini M, Lambertini M, et al. Follow-up strategies for women treated for early breast cancer. Cochrane Database Syst Rev. 2016(5): CD001768, doi: 10.1002/14651858.CD001768.pub3, indexed in Pubmed: 27230946.
174. Jassem J, Duchnowska R, Kawecki A, et al. Badania kontrolne po leczeniu w najczęstszych nowotworach litych u dorosłych. Nowotwory J Oncol. 2014; 64(5): 415–435, doi: 10.5603/njo.2014.0070.

Piotr Radziwon[1, 2], Maciej Krzakowski[3], Ewa Kalinka[4], Renata Zaucha[5], Piotr Wysocki[6], Dariusz Kowalski[7], Jerzy Gryglewicz[8], Marek Z. Wojtukiewicz[9]

[1]Regional Center for Transfusion Medicine in Białystok, Poland
[2]Department of Hematology, Medical University of Białystok, Poland
[3]Department of Lungs and Thoracic Cancers, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[4]Polish Mother's Memorial Hospital Research Institute in Lodz, Poland
[5]Department and Clinic of Oncology and Radiotherapy, Medical University of Gdańsk, Poland
[6]Department of Oncology, Jagiellonian University — *Collegium Medicum* in Krakow, Poland
[7]Conservative Department at the Department of Lungs and Thoracic Cancers, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[8]Institute of Management in Healthcare, Lazarski University, Warsaw, Poland
[9]Department of Oncology, Medical University of Białystok, Poland

Anemia in cancer patients — Expert Group recommendations. Revision 2020

Address for correspondence:
Prof. dr hab. n. med. Piotr Radziwon
Klinika Hematologii
Uniwersytet Medyczny w Białymstoku
e-mail: piotr.radziwon@wp.pl

Oncology in Clinical Practice
2020, Vol. 16, No. 5, 261–269
DOI: 10.5603/OCP.2020.0020
Translation: dr n. med. Dariusz Stencel

ISSN 2450–1654

ABSTRACT

Anemia is a common feature in about 40% of patients at the moment of cancer diagnosis and in more than half of patients on anticancer therapy. Therapeutic alternatives in cancer patients with anemia include substitution of lacking agents, red blood cell transfusions, and erythropoiesis-stimulating agents (ESAs). The advantages of red blood cell transfusions are rapid increase of hemoglobin concentration and effectiveness independent of the cause of anemia. However, several adverse reactions may occur after blood component transfusion. ESAs act through stimulation of erythropoietin receptors. Use of ESAs reduces the need for red blood cell transfusions, decreases the risk of post-transfusion adverse reactions, and improves the quality of life of cancer patients with chemotherapy-induced anemia. In accordance with registered indications, ESA may be administered in non-myeloid cancer patients with chemotherapy-induced anemia. Thromboembolic events and arterial hypertension are known risks of ESA treatment. If ESAs are used in accordance with currently approved indications and are not administered when hemoglobin (Hb) concentration is 12 g/dL or above, there is no observed unfavorable effect on survival or thromboembolic risk. The administration of RBC transfusions without delay is justified in patients with Hb under 7–8 g/dL and/or severe anemia-related symptoms (even at higher Hb levels) and the need for immediate Hb and symptom improvement. The goal of ESA treatment is maintenance of the lowest hemoglobin concentration needed to avoid red blood cell transfusion. ESAs may be used in patients with symptomatic chemotherapy-induced anemia and Hb concentration at 10 g/dL or below. There is no indication for ESAs in patients who are not receiving chemotherapy or who are receiving radiotherapy.

Key words: anemia, chemotherapy induced anemia, cancer, blood transfusion, erythropoiesis stimulating agents

Oncol Clin Pract 2020; 16, 5: 261–269

Anemia — morbidity, etiology, classification

Anemia (Lat. *anaemia* — comes from the Greek name *anaimia*, meaning lack of blood) is a reduction of blood's ability to deliver oxygen to tissues and its oxygen-carrying capacity. Anemia very often accompanies cancers, disturbs the anticancer treatment and adversely impact on the patients' quality of life (QoL).

Anemia occurs in approximately 40% of patients at cancer diagnosis and in more than half of patients undergoing anticancer treatment. The influence of anemia on malaise and quality of life has been described since the 1970s, so it is very important to treat all symptomatic patients [1].

Table 1. Reference values of the red cell system

	Women	Men
Hemoglobin level	12.5–15.5 g/dL	13,5–17,5 g/dL
RBC count	4.2–5.4 T/L	4,6–6,2 T/L
Hematocrit	37–47%	40–54%
MCV	80–94 fL	
MCHC	32–38 g/dL	
MCH	27–32 pg	
Reticulocyte percentage	5–15‰ (28–100 G/L)	
RDW*	11.5–14.5%	

*Red blood cell volume variation (anisocytosis); MCV — mean corpuscular volume; MCHC — mean corpuscular hemoglobin concentration; MCH — mean corpuscular hemoglobin; RBC — red blood count; RDW — red cell distribution width

In the prospective European Cancer Anaemia Survey (ECAS) [2], more than half of the 15,367 patients from 24 European countries developed anemia during anticancer treatment. A similar observational POLCAS study [3] involving 999 patients from 13 Polish oncology centers provided almost identical results — anemia was found in more than half of the patients after treatment completion (most often cancer of the female reproductive system, lung cancer and testicular cancer). A decrease in hemoglobin (Hb) levels correlated with a decline in performance status (PS), but only one-third of anemic patients received treatment, the most frequently red cell concentrates (RCC) transfusion.

Abnormal hematopoiesis or too fast red blood cells breakdown, as well as, acute or chronic blood loss lead to decreased Hb level and the number of erythrocytes (red blood cells, RBCs) in the peripheral blood below the normal values (Table 1) [4, 5].

Depending on Hb level, anemia is classified as: mild (Hb > 10 g/dL, but below the normal value), moderate (Hb 8–10 g/dL), severe (Hb 6.5–7.9 g/dL) and life-threatening (Hb < 6.5 g/dL).

The most important causes of anemia are:

— deficiencies of:
  - iron following bleeding in and out of the tumor or following surgery,
  - folic acid due to malnutrition,
  - vitamin B12 associated with malabsorption disorders (e.g. after gastrectomy, in gastrointestinal neoplasms);
— immune (lymphomas, chronic lymphocytic leukemia, adenocarcinomas) and non-immune hemolysis (e.g. microangiopathic hemolytic anemia [MAHA] in mucus-producing tumors or prostate cancer — usual reticulocytes below 2‰);
— bone marrow suppression after systemic use of cytotoxic drugs (especially nephrotoxic) or after irradiation > 20% of the bone marrow volume);
— erythropoiesis inhibition due to tumor infiltration of bone marrow;
— erythrophagocytosis in histiocytic lymphomas;
— erythropoiesis inhibition due to suppression of endogenous erythropoietin production (e.g. by cytokines) or inappropriate iron utilization [the most common cause, i.e. functional iron deficiency, which gives a picture of anemia of chronic disease (ACD)].

A healthy person has enough iron stores for up to 2-fold increase of erythropoiesis. Blood loss or impaired absorption leads to true iron deficiency with ferritin levels 30 ng/mL and transferrin saturation below 15%. Abnormal values of the above parameters are standard indications for iron preparations use. It should be remembered that in cancer patients, functional iron deficiency is often observed, with ferritin level 800 ng/mL or less and transferrin saturation below 20% [5].

Based on the mean corpuscular volume, anemia could be classified as:

— microcytic (MCV < 80 fL) — with increased red cell distribution width (RDW), most often due to iron deficiency in chronic bleeding or sideroblastic anemia, with normal RDW in the course of ACD and spherocytosis;
— normocytic (MCV 80–100 fL) in the course of ACD (most often), after chemotherapy or irradiation (iatrogenic), as a result of bone marrow infiltration, acute bleeding, in the initial stage of iron deficiency anemia, in mixed vitamin deficiencies, in kidney diseases, hypothyroidism;
— macrocytic (MCV > 100 fL) in hemolysis (often in lymphoproliferative diseases — late autoimmune hemolysis, after fludarabine, after incompatible blood transfusion), due to vitamin B12 and/or folic acid deficiency, in myelodysplastic syndromes, multiple myeloma, liver diseases, hypothyroidism, sideroblastic anemia and during the regeneration of the hematopoietic system after chemotherapy.

In addition to a low MCV, abnormal laboratory parameters in iron deficiency include:

— RDW — increased;
— number of hypochromic erythrocytes — increased;
— reticulocytes Hb content — decreased;
— iron concentration — reduced;
— ferritin concentration — low;
— transferrin saturation — low (Fe/TIBC < 20%);
— concentration of soluble transferrin receptors (sTfR) — increased;
— total iron binding capacity (TIBC) — increased.

Consequences of anemia in cancer patients

Anemia in cancer patients:

— worsens the quality of life;
— precludes the maintenance of the chemotherapy regimen, which directly affects the effectiveness of the therapy;

— reduces radiation-curability;
— has a negative prognostic impact;
— correlates with higher mortality (in particular in patients with lymphomas, head and neck cancers, lung cancer, cervical cancer, prostate cancer).

Diagnostics

Depending on patient's general condition, before deciding on the treatment method, the tests should be performed to determine the etiology of anemia and to enable causative treatment and/or therapy with the lowest risk of adverse reactions.

The following diagnostic tests are recommended:
— complete blood count;
— a reticulocyte count;
— iron concentration;
— TIBC;
— transferrin saturation;
— ferritin concentration;
— folic acid concentration;
— vitamin B12 concentration
— fecal occult blood test (FOBT);
— parameters assessing renal function.

Additional tests could be performed if clinically justified:
— erythropoietin concentration;
— TSH level;
— direct antiglobulin test (CLL, lymphomas, prior autoimmune disease);
— testing for hemoglobinopathy.

When the cause of anemia in cancer patient is not determined, it is classified as cancer induced anemia (CIA).

Treatment

In the management of anemia in cancer patients, causal treatment should be used when available and the diagnosed deficiencies should be corrected first (iron, vitamin B12, folic acid). If deficiencies correction does not bring the expected results and the anemia does not improve despite the anticancer treatment, ESA administration may be considered. RBC transfusions are reserved for the following situations: deficiencies correction has not brought the expected results, there are no indications for ESA, and the level of anemia does not allow the initiation or continuation of anticancer treatment or causes significant symptoms.

Iron supplementation

Criteria for starting iron supplementation are as follow:
— anemia ($8 < Hb < 10$ g/dL) or
— absolute iron deficiency (ferritin < 100 ng/mL and transferrin saturation < 20%);
— relative iron deficiency (ferritin > 100 ng/mL and transferrin saturation < 20%) — iron should be administered before starting ESA.

While using ESA, iron levels should be monitored and supplemented as needed.

Contraindications to iron supplementation — active infection, treatment with drugs with cardiotoxicity related to the generation of free oxygen radicals (anthracyclines, alkylating drugs and *Vinca* alkaloids).

Administration route

Due to frequently reduced iron absorption from the gastrointestinal tract in cancer patients, iron preparations should be administered intravenously.

Dosage
— 1000 μg once or in divided doses, depending on the type of drug.

Red blood cells concentrates transfusion

Preparations containing red blood cells are:
— red cell concentrate (RCC) (packed red cells);
— leukocyte-depleted RCC;
— irradiated RCC;
— irradiated leukocyte-depleted RCC;
— washed RCC.

The advantages of RBC transfusions are that:
— they rapidly increase hemoglobin levels in patients with anemia;
— they are effective regardless of anemia etiology.

Cancer patients receiving a transfusion of blood components are found to have:
— shorter overall survival time [6–10];
— the earlier occurrence of tumor relapse [10–12];
— higher mortality due to recurrence of the neoplastic disease [11, 13];
— a higher number of postoperative complications (including infections) [14–17];
— prolonged hospital stay [18];
— higher risk of developing lymphomas [19];
— higher risk of thromboembolic complications.

The reasons for the adverse effects of blood components transfusions may be:
— changes that occur during the RBC storage;
— immunogenicity of blood cells;
— thrombogenicity of blood component;
— presence of pathogens and/or leukocytes in blood components;
— immunomodulation;
— human error;
— using less safe blood concentrates due to lower costs.

Table 2. Post-transfusion immune-mediated adverse reactions

Post-transfusion immune-mediated adverse reactions	
Early	**Delayed**
Acute hemolytic transfusion reaction (AHTR)	Delayed hemolytic transfusion reaction
Transfusion-related acute lung injury (TRALI)	Transfusion-associated graft versus host disease (TA-GvHD)
Febrile non-hemolytic transfusion reactions (FNHTR)	Post-transfusion purpura (PTP)
Anaphylactic reaction	Alloimmunization to blood cell antigens
Urticaria	Immunosuppression

Due to the increasingly common use of immunotherapy with immune checkpoint inhibitors in cancer patients, the impact of blood component transfusions on the immune system should be taken into account. Cytokine release (including IL-6, IL-8, IL-10) induced by transfusion of blood components has proven pro-inflammatory and immunosuppressive effects, and, its clinically significant interaction with a mechanism of action of immunomodulating drugs cannot be excluded [20].

Therefore, RBC transfusions should not be used as a universal method of treating anemia in cancer patients and should be limited only to situations in which they are the only effective way to raise hemoglobin levels or are indications for immediate elimination or relief of anemia symptoms.

In addition, 2020 has already brought an additional problem in many countries (including Poland) related to a significant reduction in the availability of blood and its components due to the rapidly spreading SARS-CoV-2 pandemic and the need for their rational use.

Due to the possibility of a number of post-transfusion adverse reactions, including fatal ones (Tables 2 and 3), and taking into account that the majority of them are caused by the presence of leukocytes in blood components, it is advisable to use prophylaxis by leukocyte depletion in blood components and/or X-ray irradiation.

Absolute indications to leukocyte-depleted RCC include [19]:
— transfusions in patients with previous non-hemolytic febrile reactions;
— transfusions in patients with previous TRALI;
— transfusions in patients with or suspected to have anti-HLA antibodies;
— prophylaxis of immunization with erythrocyte antigens — multiple recipients (in the course of hematopoietic malignancies or chronic renal failure);
— prophylaxis of immunization with HLA antigens;
  • non-hemolytic febrile reactions,
  • platelet transfusion refractoriness;
— prophylaxis of cytomegalovirus (CMV) infection.

Table 3. Post-transfusion non-immune-mediated adverse reactions

Post-transfusion non-immune-mediated adverse reactions	
Early	**Delayed**
Non-immune hemolysis	Hemosiderosis
Transfusion associated circulatory overload (TACO)	Transmission of viral, bacterial, protozoal infections
Sepsis	Transmission of prions
Air embolism	
Citrate intoxication	

Leukocyte depletion in blood components does not prevent transfusion-associated graft *versus* host disease (TA-GvHD) which is caused by donor lymphocytes. In order to reduce the risk of TA-GvHD, irradiation of RBC concentrates is necessary.

Absolute indication to irradiated RCC include [19]:
— relatedness (1st and 2nd degrees) between donor and recipient;
— HLA compatible blood components;
— immunodeficiency (especially with severe T-cell deficiency syndrome);
— transfusion of granulocyte concentrates;
— hematopoietic cell transplant recipients — from the initiation of conditioning chemotherapy and/or radiotherapy to completion of GvHD prophylaxis related to the transplant, usually for about 3 months (autologous transplant) or 6 months (allogeneic transplant) after the transplant or until the blood lymphocyte count is above 10^9/L;
— chronic GvHD;
— autologous hematopoietic cells collection and within 7 days prior to collection;
— immunosuppressive treatment;
— Hodgkin's disease;
— treatment with purine analogues (e.g. fludarabine, cladribine, deoxycoformicin) or purine antagonists (bendamustine, clofarabine);
— treatment with alemtuzumab (anti-CD52).

Erythropoiesis stimulating agents (ESA)

Erythropoiesis stimulating agents (ESAs) include:
— epoetin (alpha, beta, theta);
— darbepoetin alfa.

ESAs work by stimulating the receptors for erythropoietin.

Aim of ESA treatment

The use of ESA reduces the number of necessary transfusions, reduces the risk of post-transfusion adverse reactions, and improves the quality of life of patients with chemotherapy-induced anemia.

The target hemoglobin level, which obviates the need for RBC transfusion is approximately 12 g/dL. When using ESA a Hb level of 12 g/dL should not be exceeded.

According to the registered indications, ESAs can be used in patients with non-myeloid neoplasms with chemotherapy-induced anemia (CIA). In line with the ESMO recommendations, ESA can also be used in patients with myelodysplastic syndrome [21].

The use of ESA in patients with hypersensitivity to the drug and uncontrolled hypertension is not recommended.

All meta-analyses confirmed the effectiveness of ESA in reducing the frequency of blood transfusions, which is the main goal of ESA use in patients with CIA.

It is worth noting that ESA, unlike RBC concentrate, has a positive effect on the immune system. Among other things, ESA reduces the expression of pro-inflammatory cytokine genes (IL-1β, IL-6, IL-10, TNF-α), lowers the concentration of IL-1α and IL-6 and causes a decrease in the number of suppressive cells (CD8+CD152+) [22–25].

Risk related to the use of ESAs

Using ESA increases the risk of:

1. Thromboembolic complications

It should be highlighted that many factors may contribute to the increased risk of thromboembolic complications in cancer patients. The most important of them are: high hematocrit, advanced patient's age, prolonged immobilization, major surgery, multiple injuries, a history of thromboembolism, chronic heart failure and cancer type [26]. Remarkably higher risk of thromboembolic events occurs in pancreatic and gastric cancer, and in multiple myeloma during immunomodulatory treatment [27, 28]. However, there is no convincing clinical evidence that the use of ESA further increases the risk of thromboembolic events in patients treated with lenalidomide or thalidomide. [29, 30].

Due to the lack of prospective randomized clinical trials (RCTs) proving that anticoagulation treatment reduces the risk of thromboembolic events in patients receiving ESA, and the conclusions from meta-analyzes showing a relatively low risk of thromboembolic complications in patients treated with ESA according to the currently recognized indications, routine thromboprophylaxis during treatment with ESA alone is not recommended [31].

Table 4. Model of risk assessment of thromboembolic complications in outpatients

Risk factors	Points
Gastric cancer, pancreatic cancer	2
Lung cancer, bladder cancer, testicular cancer, kidney cancer, lymphoma	1
Platelet count before chemotherapy ≥ 350,000/μL	1
Hemoglobin level < 10 g/dL **or ESA use**	1
Leukocyte count before chemotherapy > 11,000/μL	1
BMI ≥ 35 kg/m^2	1

High risk — total points ≥ 3
Intermediate risk — total points = 1–2
Low risk — total points = 0

Table 5. Model of risk assessment of thromboembolic complications in in patients treated stationary (authors modification)

Risk factors	Points
Active malignant tumor	3
History of thrombosis (excluding superficial thrombosis)	3
Mobility restrictions	3
Thrombophilia	3
Recent (up to a month) trauma or surgery	2
Age ≥ 70 years	1
Heart and/or lung failure	1
Myocardial infarct and/or ischemic stroke	1
Acute infection and/or rheumatological disease	1
BMI ≥ 30 kg/m^2	1
Current hormone treatment	1
ESA use	1

High risk — total points ≥ 4

However, other risk factors for thromboembolic complications in cancer patients should be considered and the administration of ESA should be included when assessing individualized risk for each patient. The algorithms for calculating the risk indices for outpatients (example in Table 4 [32]) or hospitalized patients (example — Table 5 [33]) may be helpful.

2. Hypertension — patients with chronic renal failure are particularly at risk

When ESAs are used in accordance with the registration and based on recommendations for the treatment of chemotherapy-induced anemia and are not used

when the Hb level is 12 g/dL or higher, then no adverse effect on overall survival is observed, and there is no evidence from clinical trials (neither single studies nor meta-analyzes) of a stimulating effect of ESAs on cancer progression or relapse [34–53].

Recommendations

1. Indications for the initiation of anemia treatment

In most cases of normovolemic anemia with Hb concentration above 7 g/dL, proper oxygenation of tissues is ensured without the need to activate adaptive mechanisms, provided that normal life activities are performed and do not require greater physical effort. Red blood cell transfusion in most people with Hb levels higher than 7 g/dL does not increase the amount of oxygen delivered to the organs. In patients with symptoms of severe anemia (symptoms of ischemic heart disease, tachycardia, dyspnea, orthostatic hypotension, fatigue), red blood cell transfusion is indicated when the Hb concentration is lower than 8 g/dL. Majority of patients — even in a severe general state — tolerate Hb levels in the range of 7–10 g/dL well [54]. In general, the perioperative mortality rate in patients with preoperative Hb levels between 6 and 10 g/dL is not increased compared to patients with Hb levels above 10 g/dL. Moreover, there are reports that a liberal red blood cell transfusion strategy (Hb concentration < 10 g/dL) is associated with higher mortality compared to a restrictive strategy, in which the use of RBC is ordered only after the Hb concentration drops below 7–8 g/dL [55].

It should be emphasized that in the early stages of neoplastic disease, a statistically significant positive correlation was observed between RBC transfusion and shorter overall survival and higher mortality. According to the authors of these recommendations, there is a risk associated with RBC transfusions in the treatment of anemia in early-stage cancers. This is most likely due to the immunomodulatory effect of the transfused blood component, which suppresses the recipient's immune system and weakens its cancer-controlling function.

If the Hb level is above 6 g/dL and there are no symptoms of severe anemia requiring urgent RBC transfusion, it is recommended to diagnose the cause(s) of anemia and apply the procedures appropriate to the diagnosis (e.g., correct iron deficiency, stop bleeding, stop hemolysis). If the above procedure does not bring the expected results (increase in Hb level above 8 g/dL), the use of ESA can be considered (Figure 1). In patients receiving chemotherapy or combined chemoradiotherapy, ESA should be started at Hb levels < 10 g/dL if there are symptoms related to anemia. The use of ESA may be considered in selected asymptomatic patients receiving chemotherapy with Hb levels < 8 g/dL.

In patients with normal Hb levels before chemotherapy prophylactic use of ESA is not recommended.

There is no clear evidence that leukocyte-depleted RBC concentrate transfusions have a more favorable impact on the course of the neoplastic disease than blood cells without a reduced number of leukocytes. However, due to the higher risk of post-transfusion adverse reactions related to the presence of leukocytes in concentrates (febrile non-hemolytic transfusion reactions, TRALI, immunization, CMV transmission), it is advisable to use leukocyte-depleted RBC concentrate in cancer patients who are expected to receive multiple transfusions of blood components.

2. Aim of anemia treatment

The aims of anemia treatment include:

— improvement or resolution of anemia symptoms;

— enabling anticancer treatment;

— improvement of quality of life, taking into account a patient's life expectancy.

This goal should be achieved with the least invasive and safest treatment methods. Table 6 presents a comparison of the advantages and risks related to specific treatment methods.

3. Drug dosing

Iron dosage

Due to very common elevated levels of hepcidin blocking the ferroportin responsible for the iron transport from enterocytes into the blood in cancer patients, orally administered iron will not be effective. In these patients, iron should only be administered intravenously. Currently used iron preparations are safe and, in accordance with the recommendations of the European Medicines Agency, do not require a trial dose administration [56]. When choosing an iron preparation, the deficiency stage and the duration of infusion should be taken into account (Table 7). The recommended dose is 1000 μg as a single or divided dose.

ESA dosage

The starting dose for ESA is:

— epoetin — 150 U/kg three times per week or 30,000 U/week;

— darbepoetin — 2.25 μg/kg/week or 500 μg/3 weeks.

A preliminary evaluation of iron balance is necessary and the use of ESA should only be started after any deficiencies have been corrected. It is advisable to monitor hemoglobin levels and iron stores during treatment [57]. In the case of iron deficiency, appropriate supplementation is necessary, but only by the intravenous route.

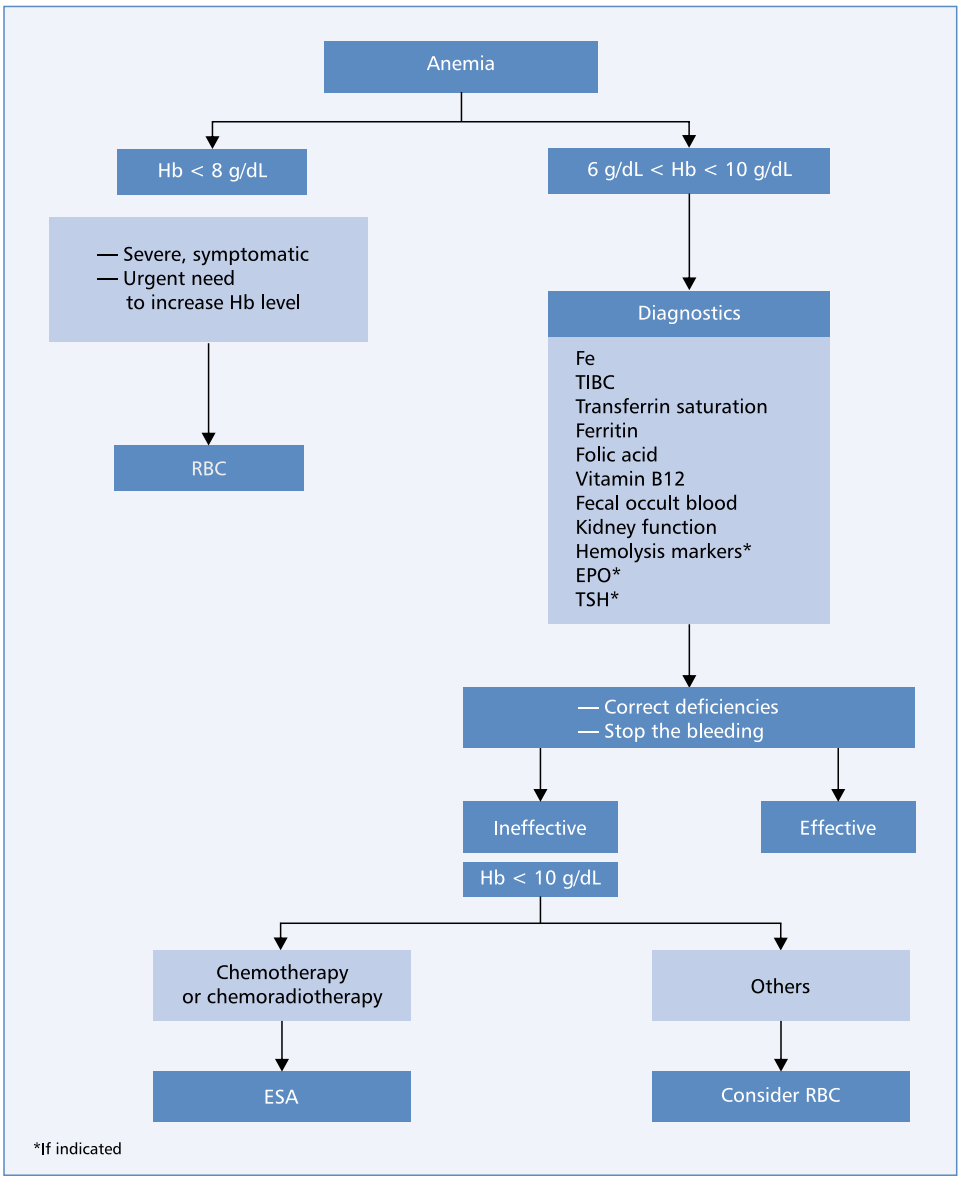

Figure 1. Algorithm for management of anemia in cancer patients. Hb — hemoglobin; ESA — erythropoiesis stimulating agent; RBC — a concentrate of red blood cells

Table 6. Advantages and risks related to specific methods of anemia treatment

	Advantages	Risks and limitations
RBC transfusions	Quickly reduces the symptoms of anemia, regardless of its cause, and increases the Hb level	Can cause many adverse reactions, including fatal ones
		Require pre-transfusion tests
		The necessity of hospitalization
		Hb concentration cannot be kept on stable level
		Adverse effect on the immune system (immunosuppression, possible interaction with immunotherapy)
ESA	Possibility of outpatient treatment	Increased risk of thromboembolic complications
	Stable Hb levels during treatment	The time required to achieve a treatment effect
	Beneficial effect on the immune system	Indications limited to the group of patients receiving chemotherapy or chemoradiotherapy
	Improving patients' quality of life	May be ineffective in some patients

Table 7. Dosages and minimum infusion time for iron preparations.

Iron preparation	Maximum dose	Minimum infusion time
Gluconate	125 mg	60 min
Saccharide	200–500 mg	30–210 min
Dextran	Different	240–360 min
Isomaltoside	20 mg/kg to 1000 mg	60 min
Carboxymatoside	20 mg/kg to 1000 mg	15 min

ESA doses should be reduced by approximately 25–50% if the hemoglobin concentration rises to levels preventing red blood cell transfusions or increases by more than 2 g/dL within 4 weeks.

It is recommended to thoroughly inform patients about the planned use of ESA together with comprehensive information on the purpose and potential adverse reactions associated with the treatment (especially thromboembolic complications). It is also recommended to inform the primary care physician about the use of ESA.

Except patients receiving epoetin theta (deliberately administered at a low initial dose), increases in ESA doses and changes to other ESA preparation in unresponsive patients within 4–8 weeks are not recommended. ESA treatment should be discontinued in patients who have not demonstrated at least initial Hb response after this period.

ESA discontinuation is also recommended after a maximum of 4 weeks after chemotherapy completion and in the case of the appearance of neutralizing anti-ESA antibodies.

Conflicts of interest

The authors declare to have no conflict of interest.

References

1. Curt GA, Breitbart W, Cella D, et al. Impact of cancer-related fatigue on the lives of patients: new findings from the Fatigue Coalition. Oncologist. 2000; 5(5): 353–360, doi: 10.1634/theoncologist.5-5-353, indexed in Pubmed: 11040270.
2. Ludwig H, Van Belle S, Barrett-Lee P, et al. The European Cancer Anaemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. Eur J Cancer. 2004; 40(15): 2293–2306, doi: 10.1016/j.ejca.2004.06.019, indexed in Pubmed: 15454256.
3. Wojtukiewicz MZ, Sierko E, Rybaltowski M, et al. The Polish Cancer Anemia Survey (POLCAS): a retrospective multicenter study of 999 cases. Int J Hematol. 2009; 89(3): 276–284, doi: 10.1007/s12185-009-0273-x, indexed in Pubmed: 19343481.
4. Podolak-Dawidziak M, Szczeklik A. Choroby Wewnętrzne. Kraków 2006: 1441–1464.
5. Auerbach M, Henry DH. Increased importance of intravenous iron in chemotherapy-induced anemia. J Clin Oncol. 2007; 25(15): 2145–2146, doi: 10.1200/JCO.2007.11.8364, indexed in Pubmed: 17513828.
6. Bhide SA, Ahmed M, Rengarajan V, et al. Anemia during sequential induction chemotherapy and chemoradiation for head and neck cancer: the impact of blood transfusion on treatment outcome. Int J Radiat Oncol Biol Phys. 2009; 73(2): 391–398, doi: 10.1016/j.ijrobp.2008.04.052, indexed in Pubmed: 18692326.
7. Chau JKM, Harris JR, Seikaly HR. Transfusion as a predictor of recurrence and survival in head and neck cancer surgery patients. J Otolaryngol Head Neck Surg. 2010; 39(5): 516–522, indexed in Pubmed: 20828514.
8. Panagopoulos ND, Karakantza M, Koletsis E, et al. Influence of blood transfusions and preoperative anemia on long-term survival in patients operated for non-small cell lung cancer. Lung Cancer. 2008; 62(2): 273–280, doi: 10.1016/j.lungcan.2008.02.025, indexed in Pubmed: 18430486.
9. Ling FC, Hoelscher AH, Vallböhmer D, et al. Leukocyte depletion in allogeneic blood transfusion does not change the negative influence on survival following transthoracic resection for esophageal cancer. J Gastrointest Surg. 2009; 13(4): 581–586, doi: 10.1007/s11605-008-0787-1, indexed in Pubmed: 19152023.
10. Yamamoto J, Kosuge T, Takayama T, et al. Perioperative blood transfusion promotes recurrence of hepatocellular carcinoma after hepatectomy. Surgery. 1994; 115(3): 303–309, indexed in Pubmed: 8128355.
11. Schiergens TS, Rentsch M, Kasparek MS, et al. Impact of perioperative allogeneic red blood cell transfusion on recurrence and overall survival after resection of colorectal liver metastases. Dis Colon Rectum. 2015; 58: 74–82.
12. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. Cochrane Database Syst Rev. 2006(1): CD005033, doi: 10.1002/14651858.CD005033.pub2, indexed in Pubmed: 16437512.
13. Vamvakas EC. Perioperative blood transfusion and cancer recurrence: meta-analysis for explanation. Transfusion. 1995; 35(9): 760–768, doi: 10.1046/j.1537-2995.1995.35996029162.x, indexed in Pubmed: 7570938.
14. Thomas P, Michelet P, Barlesi F, et al. Impact of blood transfusions on outcome after pneumonectomy for thoracic malignancies. Eur Respir J. 2007; 29(3): 565–570, doi: 10.1183/09031936.00059506, indexed in Pubmed: 17079259.
15. Kooby DA, Stockman J, Ben-Porat L, et al. Influence of transfusions on perioperative and long-term outcome in patients following hepatic resection for colorectal metastases. Ann Surg. 2003; 237(6): 860–869, doi: 10.1097/01.SLA.0000072371.95588.DA, indexed in Pubmed: 12796583.
16. Cescon M, Vetrone G, Grazi GL, et al. Trends in perioperative outcome after hepatic resection: analysis of 1500 consecutive unselected cases over 20 years. Ann Surg. 2009; 249(6): 995–1002, doi: 10.1097/SLA.0b013e3181a63c74, indexed in Pubmed: 19474679.
17. Sarani B, Dunkman WJ, Dean L, et al. Transfusion of fresh frozen plasma in critically ill surgical patients is associated with an increased risk of infection. Crit Care Med. 2008; 36(4): 1114–1118, doi: 10.1097/CCM.0b013e318168f89d, indexed in Pubmed: 18379235.
18. Castillo JJ, Dalia S, Pascual SK. Association between red blood cell transfusions and development of non-Hodgkin lymphoma: a meta-analysis of observational studies. Blood. 2010; 116: 2897–2907.
19. Radziwon P, Wojtukiewicz MZ. Bezpieczne i skuteczne stosowanie składników krwi w onkologii. W: Wojtukiewicz MZ, Deptała A (red.). Problemy hematologiczne u chorych na nowotwory. Termedia Wydawnictwa Medyczne, Poznań 2014: 449–475.
20. Ydy LR, Slhessarenko N, de Aguilar-Nascimento JE. Effect of perioperative allogeneic red blood cell transfusion on the immune-inflammatory response after colorectal cancer resection. World J Surg. 2007; 31(10): 2044–2051, doi: 10.1007/s00268-007-9159-3, indexed in Pubmed: 17671807.
21. Aapro M, Beguin Y, Bokemeyer C, et al. ESMO Guidelines Committee, ESMO Guidelines Committee. Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines. Ann Oncol. 2018; 29(Suppl 4): iv96–iv9iv110, doi: 10.1093/annonc/mdx758, indexed in Pubmed: 29471514.

22. Zhang J, Zou YR, Zhong X, et al. Erythropoietin pretreatment ameliorates renal ischaemia-reperfusion injury by activating PI3K/Akt signalling. Nephrology (Carlton). 2015; 20(4): 266–272, doi: 10.1111/nep.12384, indexed in Pubmed: 25581532.
23. Rong R, Xijun X. Erythropoietin pretreatment suppresses inflammation by activating the PI3K/Akt signaling pathway in myocardial ischemia-reperfusion injury. Exp Ther Med. 2015; 10(2): 413–418, doi: 10.3892/etm.2015.2534, indexed in Pubmed: 26622330.
24. Lisowska M, Bryl E, Witkowski JM. Wpływ rekombinowanej ludzkiej erytropoetyny na układ odpornościowy. Forum Medycyny Rodzinnej. 2009; 3: 359–366.
25. Li J, Xu J, Yan X, et al. Targeting Interleukin-6 (IL-6) Sensitizes Anti-PD-L1 Treatment in a Colorectal Cancer Preclinical Model. Med Sci Monit. 2018; 24: 5501–5508, doi: 10.12659/MSM.907439, indexed in Pubmed: 30087314.
26. Bokemeyer C, Aapro MS, Courdi A, et al. European Organisation for Research and Treatment of Cancer (EORTC) Taskforce for the Elderly. EORTC guidelines for the use of erythropoietic proteins in anaemic patients with cancer: 2006 update. Eur J Cancer. 2007; 43(2): 258–270, doi: 10.1016/j.ejca.2006.10.014, indexed in Pubmed: 17182241.
27. Musallam KM, Dahdaleh FS, Shamseddine AI, et al. Incidence and prophylaxis of venous thromboembolic events in multiple myeloma patients receiving immunomodulatory therapy. Thromb Res. 2009; 123(5): 679–686, doi: 10.1016/j.thromres.2008.09.008, indexed in Pubmed: 18992924.
28. Zonder JA. Thrombotic complications of myeloma therapy. Hematology Am Soc Hematol Educ Program. 2006: 348–355, doi: 10.1182/asheducation-2006.1.348, indexed in Pubmed: 17124082.
29. Galli M, Elice F, Crippa C, et al. Recombinant human erythropoietin and the risk of thrombosis in patients receiving thalidomide for multiple myeloma. Haematologica. 2004; 89(9): 1141–1142, indexed in Pubmed: 15377478.
30. Knight R, DeLap RJ, Zeldis JB. Lenalidomide and venous thrombosis in multiple myeloma. N Engl J Med. 2006; 354(19): 2079–2080, doi: 10.1056/NEJMc053530, indexed in Pubmed: 16687729.
31. Mulder FI, Candeloro M, Kamphuisen PW, et al. CAT-prediction collaborators. The Khorana score for prediction of venous thromboembolism in cancer patients: a systematic review and meta-analysis. Haematologica. 2019; 104(6): 1277–1287, doi: 10.3324/haematol.2018.209114, indexed in Pubmed: 30606788.
32. Key NS, Khorana AA, Kuderer NM, et al. Venous thromboembolism prophylaxis and treatment in patients with cancer: ASCO clinical practice guideline update. J Clin Oncol. 2020; 38(5): 496–520, doi: 10.1200/JCO.19.01461, indexed in Pubmed: 31381464.
33. Germini F, Agnelli G, Fedele M, et al. Padua prediction score or clinical judgment for decision making on antithrombotic prophylaxis: a quasi-randomized controlled trial. J Thromb Thrombolysis. 2016; 42(3): 336–339, doi: 10.1007/s11239-016-1358-z, indexed in Pubmed: 27052516.
34. Ludwig H, Crawford J, Osterborg A, et al. Pooled analysis of individual patient-level data from all randomized, double-blind, placebo-controlled trials of darbepoetin alfa in the treatment of patients with chemotherapy-induced anemia. J Clin Oncol. 2009; 27(17): 2838–2847, doi: 10.1200/JCO.2008.19.1130, indexed in Pubmed: 19380447.
35. Tonelli M, Hemmelgarn B, Reiman T, et al. Benefits and harms of erythropoiesis-stimulating agents for anemia related to cancer: a meta-analysis. CMAJ. 2009; 180(11): E62–E71, doi: 10.1503/cmaj.090470, indexed in Pubmed: 19407261.
36. Bohlius J, Schmidlin K, Brillant C, et al. Erythropoietin or Darbepoetin for patients with cancer — meta-analysis based on individual patient data. Cochrane Database Syst Rev. 2009(3): CD007303, doi: 10.1002/14651858.CD007303.pub2, indexed in Pubmed: 19588423.
37. Glaspy J, Crawford J, Vansteenkiste J, et al. Erythropoiesis-stimulating agents in oncology: a study-level meta-analysis of survival and other safety outcomes. Br J Cancer. 2010; 102(2): 301–315, doi: 10.1038/sj.bjc.6605498, indexed in Pubmed: 20051958.
38. Tonia T, Mettler A, Robert N, et al. Erythropoietin or darbepoetin for patients with cancer. Cochrane Database Syst Rev. 2012; 12: CD003407, doi: 10.1002/14651858.CD003407.pub5, indexed in Pubmed: 23235597.
39. Bennett CL, Silver SM, Djulbegovic B, et al. Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. JAMA. 2008; 299(8): 914–924, doi: 10.1001/jama.299.8.914, indexed in Pubmed: 18314434.
40. Vansteenkiste J, Glaspy J, Henry D, et al. Benefits and risks of using erythropoiesis-stimulating agents (ESAs) in lung cancer patients: study-level and patient-level meta-analyses. Lung Cancer. 2012; 76(3): 478–485, doi: 10.1016/j.lungcan.2011.12.015, indexed in Pubmed: 22277104.
41. Hedenus M, Osterborg A, Tomita D, et al. Effects of erythropoiesis-stimulating agents on survival and other outcomes in patients with lymphoproliferative malignancies: a study-level meta-analysis. Leuk Lymphoma. 2012; 53(11): 2151–2158, doi: 10.3109/10428194.2012.684347, indexed in Pubmed: 22497574.
42. Moebus V, Jackisch C, Schneeweiss A, et al. AGO Breast Study Group. Adding epoetin alfa to intense dose-dense adjuvant chemotherapy for breast cancer: randomized clinical trial. J Natl Cancer Inst. 2013; 105(14): 1018–1026, doi: 10.1093/jnci/djt145, indexed in Pubmed: 23860204.
43. Nitz U, Oberhoff C, Reimer T, et al. Adjuvant chemotherapy with or without darbepoetin in node-positive breast cancer: a safety analysis from the phase III ARA plus trial. San Antonio Breast Cancer Symposium, San Antonio, Texas 2011.
44. Pirker R, Ramlau RA, Schuette W, et al. Safety and efficacy of darbepoetin alpha in previously untreated extensive-stage small-cell lung cancer treated with platinum plus etoposide. J Clin Oncol. 2008; 26(14): 2342–2349, doi: 10.1200/JCO.2007.15.0748, indexed in Pubmed: 18467726.
45. Delarue R, Haioun C, Coiffier B, et al. Survival effect of darbepoetin alfa in patients with diffuse large B-cell lymphoma (DLBCL) treated with immunochemotherapy: The LNH03-6B study. Journal of Clinical Oncology. 2008; 29(15_suppl): 9048, doi: 10.1200/jco.2011.29.15_suppl.9048.
46. Engert A, Josting A, Haverkamp H, et al. Epoetin alfa in patients with advanced-stage Hodgkin's lymphoma: results of the randomized placebo-controlled GHSG HD15EPO trial. J Clin Oncol. 2010; 28(13): 2239–2245, doi: 10.1200/JCO.2009.25.1835, indexed in Pubmed: 20368566.
47. Aapro M, Jelkmann W, Constantinescu SN, et al. Effects of erythropoietin receptors and erythropoiesis-stimulating agents on disease progression in cancer. Br J Cancer. 2012; 106(7): 1249–1258, doi: 10.1038/bjc.2012.42, indexed in Pubmed: 22395661.
48. Hedenus M, Vansteenkiste J, Kotasek D, et al. Darbepoetin alfa for the treatment of chemotherapy-induced anemia: disease progression and survival analysis from four randomized, double-blind, placebo-controlled trials. J Clin Oncol. 2005; 23(28): 6941–6948, doi: 10.1200/JCO.2005.03.434, indexed in Pubmed: 16192582.
49. Boogaerts M, Oberhoff C, Ten Bokkel Huinink W, et al. Epoetin beta (NeoRecormon) therapy in patients with solid tumours receiving platinum and non-platinum chemotherapy: a meta-analysis. Anticancer Res. 2006; 26(1B): 479–484, indexed in Pubmed: 16739308.
50. Seidenfeld J, Piper M, Bohlius J, et al. Comparative effectiveness of epoetin and darbepoetin for managing anemia in patients undergoing cancer treatment: comparative effectiveness review. https://www.ncbi.nlm.nih.gov/books/NBK42982 (2006).
51. Aapro M, Osterwalder B, Scherhag A, et al. Epoetin-beta treatment in patients with cancer chemotherapy-induced anaemia: the impact of initial haemoglobin and target haemoglobin levels on survival, tumour progression and thromboembolic events. Br J Cancer. 2009; 101(12): 1961–1971, doi: 10.1038/sj.bjc.6605255, indexed in Pubmed: 19997109.
52. Bohlius J, Wilson J, Seidenfeld J, et al. Recombinant human erythropoietins and cancer patients: updated meta-analysis of 57 studies including 9353 patients. J Natl Cancer Inst. 2006; 98(10): 708–714, doi: 10.1093/jnci/djj189, indexed in Pubmed: 16705125.
53. Gascón P, Nagarkar R, Šmakal M, et al. A Randomized, Double-Blind, Placebo-Controlled, Phase III Noninferiority Study of the Long-Term Safety and Efficacy of Darbepoetin Alfa for Chemotherapy-Induced Anemia in Patients With Advanced NSCLC. J Thorac Oncol. 2020; 15(2): 190–202, doi: 10.1016/j.jtho.2019.10.005, indexed in Pubmed: 31629060.
54. Hebert PC, McDonald BJ, Tinmouth A. Clinical consequences of anemia and red cell transfusion in the critically ill. Crit Care Clin. 2004; 20: 225–235.
55. McIntyre LA, Fergusson DA, Hutchison JS, et al. Effect of a liberal versus restrictive transfusion strategy on mortality in patients with moderate to severe head injury. Neurocrit Care. 2006; 5(1): 4–9, doi: 10.1385/ncc:5:1:4, indexed in Pubmed: 16960287.
56. European Medicines Agency: Assessment report for: Iron containing intravenous (IV) medicinal products. EMEA/H/A-31/1322. European Medicines Agency, 2013 [Last accessed: July 15, 2018] Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/IV_iron_31/WC500150771.pdf.
57. Bohlius J, Bohlke K, Castelli R, et al. Management of cancer-associated anemia with erythropoiesis-stimulating agents: ASCO/ASH clinical practice guideline update. Blood Advances. 2019; 3(8): 1197–1210, doi: 10.1182/bloodadvances.2018030387.

VIA MEDICA

ORIGINAL ARTICLE

Kamila Wojas-Krawczyk[1], Patryk Jasielski[1], Paweł Krawczyk[1], Tomasz Jankowski[1], Magdalena Wójcik-Superczyńska[1], Katarzyna Reszka[2], Izabela Chmielewska[1], Jarosław Buczkowski[1], Tomasz Kucharczyk[1], Justyna Szumiło[3], Jarosław Kołb-Sielecki[4], Youssef Sleiman[5], Aleksandra Szczęsna[6], Tomasz Ciszewski[7], Rodryg Ramlau[8], Grażyna Jagiełło[9], Piotr Krudys[10], Janusz Milanowski[1]

[1]Pneumonology, Oncology and Allergology Department, Medical University of Lublin, Poland
[2]Genetics and Immunology Institute GENIM, Lublin, Poland
[3]Clinical Pathomorphology Department, Medical University of Lublin, Poland
[4]The Center for Pulmonary Diseases and Tuberculosis, Olsztyn, Poland
[5]Provincial Specialist St. Raphael Hospital, Czerwona Góra, Poland
[6]Mazowieckie Center for Lung Diseases and Tuberculosis, Otwock, Poland
[7]Provincial Hospital St. Padre Pio, Przemyśl, Poland
[8]Oncology Department, Medical University of Poznan, Poland
[9]Kuyavian-Pomeranian Pulmonology Center, Bydgoszcz, Poland
[10]Jizera Pulmonology and Chemotherapy Center, Szklarska Poręba, Poland

Analysis of *ROS1* gene rearrangement incidence among NSCLC patients with fluorescent *in situ* hybridization technique

Address for correspondence:
Dr hab. n. med. Kamila Wojas-Krawczyk
Katedra i Klinika Pneumonologii, Onkologii i Alergologii
Uniwersytet Medyczny w Lublinie
ul. Jaczewskiego 8, 20–954 Lublin
Phone: +48 81 724 42
e-mail: kamilawojas@wp.pl

Oncology in Clinical Practice
2020, Vol. 16, No. 5, 270–275
DOI: 10.5603/OCP.2020.0024

ISSN 2450–1654

ABSTRACT

Introduction. The rearrangement of the gene encoding ROS protooncogene (ROS1) is observed in a very small percentage (1–2%) of patients with non-small cell lung cancer (NSCLC). The clinical characteristics of *ROS1*-positive patients are similar to those observed in the group of patients with *ALK* gene rearrangement. Detection of *ROS1* gene rearrangement is an extremely important predictive factor enabling the use of crizotinib in the 1st line of NSCLC patients with stage IIIB or IV. Due to the addition of crizotinib to the list of reimbursed drugs from January 2019, the analysis of this genetic change should be part of a molecular tests panel performed in patients with locally advanced and advanced NSCLC in the qualification for molecularly targeted treatment.

Aim of the study. Analysis of *ROS1* gene rearrangement incidence among NSCLC patients in stage IIIB or IV qualified for molecularly targeted therapies. Presentation of methodological difficulties with fluorescent *in situ* hybridization (FISH) technique which is used to detect *ROS1* genetic abnormality.

Materials and methods. The analysis of *ROS1* gene rearrangement was carried out using fluorescent *in situ* hybridization technique in tissue samples taken from 573 NSCLC patients of non-squamous cell type during routine pathomorphological diagnostics.

Results. The material obtained from the tumor was fixed in formalin and archived in paraffin. Histological material was obtained from 408 patients, and 165 — cytological (cytoblock). A reliable (diagnostic) result of the *ROS1* gene rearrangement was obtained in 439 patients (76.61%). The main difficulties for *ROS1* gene analysis were low number of cancer cells, as well as high background fluorescence interference and fragmentation of cell nuclei. *ROS1* gene rearrangement was detected in 9 patients with adenocarcinoma (1.57% among all patients), including 5 men and 4 women. In 19 patients, other abnormalities regarding the *ROS1* gene were observed, primarily the polysomy of the examined *ROS1* gene fragment (3.32%). Polysomy did not coexist with the *ROS1* rearrangement.

Conclusion. Fluorescent *in situ* hybridization is a useful tool in detecting *ROS1* gene rearrangement. The test can be performed in both histological and cytological material (cytoblock). However, the correct fixation of the material and the appropriate number of tumor cells in the tested samples is extremely important for obtaining a reliable result.

Key words: *ROS1* rearangement, fluorescence *in situ* hybridization, non-small cell lung cancer, crizotinib

Oncol Clin Pract 2020; 16, 5: 270–275

Introduction

The initiation of the carcinogenesis process is associated with the appearance of somatic (non-hereditary), single mutation in the oncogene, which results in disruption of basic physiological processes, and consequently leads to uncontrolled cell division. Based on this basic assumption, molecularly targeted therapy is treatment that blocks the abnormal signaling pathway in cancer cells. Therefore, the effectiveness of molecularly targeted therapy depends on the presence (or absence) of the driver mutation [1, 2].

At present, several molecularly targeted therapies are available for the treatment of patients with non-small cell lung cancer (NSCLC). Significant clinical response after the use of EGFR tyrosine kinase inhibitors (TKI) (such as gefitinib, erlotinib, afatinib, osimertinib, dacomitinib) is observed in NSCLC patients with a detected activation mutation in the epidermal growth factor receptor gene — *EGFR*. In Poland, gefitinib, erlotinib, afatinib and, for selected patients, osimertinib are refunded. Another type of molecularly targeted therapy is the use of anaplastic lymphoma kinase (ALK) inhibitors in patients with known *ALK* gene rearrangement. In this group of drugs, reimbursement in Poland covers crizotinib, ceritinib and alectinib, while brigatinib and lorlatinib are also registered in the European Union [3–5]. BRAF and MEK inhibitors: dabrafenib and trametinib are successfully used in NSCLC patients with mutations in the *BRAF* gene, and in the case of *NTRK* gene rearrangement — larotrectinib and entrectinib (non-reimbursed drugs in Poland) [5].

ROS1 inhibitors are another group of molecularly targeted drugs that have been used in NSCLC patients. The *ROS1* gene, located on chromosome 6 (cytogenetic location: 6p22), encodes a receptor with ROS tyrosine kinase activity, belonging to the family of insulin receptors evolutionally related to the ALK receptor [6–8]. The molecular abnormalities found in NSCLC patients are the rearrangement of the *ROS1* gene. This abnormality occurs in only 1–2% of patients diagnosed with adenocarcinoma, and the clinical characteristics of patients with *ROS1* gene rearrangement are similar to patients with NSCLC with a confirmed abnormality in the *ALK* gene [6–8].

In the group of patients with *ROS1* gene rearrangement, it is possible to use the ALK, ROS1 and MET tyrosine kinase inhibitor — crizotinib. In prospective clinical trials, over 70% of NSCLC patients with *ROS1* gene rearrangement receiving crizotinib in the 1st line of treatment responded to the treatment and had a median progression-free survival time of 19.2 months [8, 9]. For these reasons, the diagnosis of *ROS1* gene rearrangement should be immediately included in the panel of molecular tests offered to patients with locally advanced and advanced NSCLC. In Poland, such a diagnostic procedure has been available to an increasing extent since January 2019, when crizotinib was reimbursed for patients with adenocarcinoma of the lung with *ROS1* gene rearrangement.

Aim of the study

The aim of this study is to evaluate the incidence of rearrangement and other molecular abnormalities of the *ROS1* gene determined by fluorescence *in situ* hybridization (FISH) in patients with locally advanced and advanced NSCLC. In addition, methodological difficulties of the FISH test used to detect *ROS1* gene abnormalities were presented.

Materials and methods

Study group characteristics

The material obtained from the tumor was fixed in formalin and archived in paraffin from 573 patients with NSCLC of a type other than squamous cell carcinoma. The *ROS1* gene rearrangement study was performed after excluding the presence of mutations in the *EGFR* gene and the rearrangement of the *ALK* gene. In 408 patients the examination was performed in histological material, and in 165 — cytological (cellblock). The demographic and clinical characteristics of the patients are summarized in Table 1.

Table 1. Demographic analysis of patients undergoing *ROS1* gene rearrangement assessment

Gender (n, %)	
Male	226 (39.44%)
Female	347 (60.56%)
Age (years, mean and standard deviation)	66.19 ± 8.44
Female	65.85 ± 8.89
Male	66.22 ± 8.13
Pathologic diagnosis of NSCLC	
Adenocarcinoma	464 (80.10%)
Other non-squamous NSCLC	109 (19.90%)
Expression of TTF1 on tumor cells	
TTF1 expression present	270 (47.12%)
TTF1 expression absent	77 (13.44%)
TTF1 expression not analyzed	226 (39.44%)
Types of analyzed material	
Histological material (small sections and surgical materials)	408 (71.20%)
Cellblock	165 (28.80%)

TTF1 — thyroid transcription factor type 1

ROS1 gene rearrangement analysis procedure using fluorescence in situ hybridization technique

The method of analyzing of *ROS1* gene rearrangement is analogous to the method of analyzing of *ALK* gene rearrangement. During the study of the rearrangement of the *ROS1* gene, its integrity is assessed, i.e. we examine the fact that a DNA strand breaks and a fragment of the *ROS1* gene moves to another place in the genome, but we do not examine the type of gene fusion that is being formed [8]. In the FISH technique, we use molecular probes — short fragments of DNA complementary to the sequences of interest in the tested DNA. In the diagnosis of *ROS1* gene rearrangement we use 2 probes: a probe with a green fluorochrome, which covers proximal DNA sequences, closer to the region sensitive to *ROS1* gene breaks, and a probe with a red or orange fluorochrome, whose sequences are complementary distally to the region sensitive to cracks in the *ROS1* gene (based on ZytoLight® Spec ROS1 Dual Color Break Apart Probe). When carrying out the FISH test, it should be taken into account that the manufacturers of molecular probes can label them in different ways, which is of great importance when interpreting the obtained results.

The laboratory procedure for handling the material for studying the rearrangement of the *ROS1* gene is based on the use of ready-made kits that allow dewaxing of tissue material, fixation, digestion in a protease buffer, denaturation and hybridization with a specific molecular probe. In this procedure, one should follow the instructions provided by the manufacturer and validate the methodology used in the laboratory. The present study uses the ZytoLight® SPEC ROS1 DualColor Break Apart Probe (ZytoVision, Germany), the Vysis Paraffin Pretreatment and Post-hybridization Wash Buffer Kit (Abbott, USA), while fluorescence signals have been assessed using an Axio Scope microscope (Zeiss, Germany). It should also be remembered that, similarly to the analysis of the *ALK* gene rearrangement, not all materials can be analyzed for the *ROS1* gene rearrangement. Table 2 summarizes the materials that are delivered to laboratories and in which it is possible to perform the FISH technique.

The tumor cell nucleus is rated as positive (with the present rearrangement of the *ROS1* gene) when the gap between the orange or red and green signal is greater than the diameter of the largest signal in the pair, or when there is an isolated green signal in the presence of fusion signals (based on ZytoLight® Spec ROS1 Dual Color Break Apart Probe). Diagrams of observable signals from fluorescent probes are presented in Figure 1.

The result of the *ROS1* gene rearrangement study is considered positive when the described signal abnormalities are found in 15% of the examined tumor cell nuclei. However, to prevent bias error, it is recommended that the test be performed by two screeners [8]. A diagram of the diagnostic procedure for assessing the *ROS1* gene rearrangement is presented in Figure 2.

In order to compare means from two independent groups, the Student's T-test and Statistica v. 13.1 program were used. The assessment whether the observed distribution of a given feature depends on another variable was carried out using the Pearson χ^2 test. Survival analysis was performed using the Kaplan-Meier method using MedCalc v. 18.11.6.

Results

Analysis of the incidence of *ROS1* gene abnormalities

The *ROS1* gene rearrangement study using FISH was performed in 573 patients with non-squamous NSCLC. In 439 cases (76.61%) a reliable test result was obtained, while in 134 (23.39%) cases no diagnostic result was obtained. Among the non-diagnostic materials, there were 55 cytological materials fixed in the form of cellblocks (which constituted 33% of all cellblocks sent for examination) and 79 histological materials (which constituted 19.4% of all histological materials). Hence, the non-diagnostic result of the *ROS1* gene rearrangement study was obtained significantly more frequently in cytological than histological materials ($P = 0.00035$, $\chi^2 = 12.798$).

Table 2. Possibilities of performing the ROS1 gene rearrangement assay in various materials

Tissue material — FFPE block	Thick needle biopsy material — FFPE block	Cryobiopsy	Cytological material — cellblock	Cytological material H+E or Papanicolaou — microscopic glass slide	Liquid biopsy — peripheral blood sample
+++	+++	+ (the method must be validated by the laboratory)	++	– (only medical experiment using DNA stability in some cytological preparations stained with Papanicolau or H + E technique)	– (only medical experiment using free circulating cancer cells)

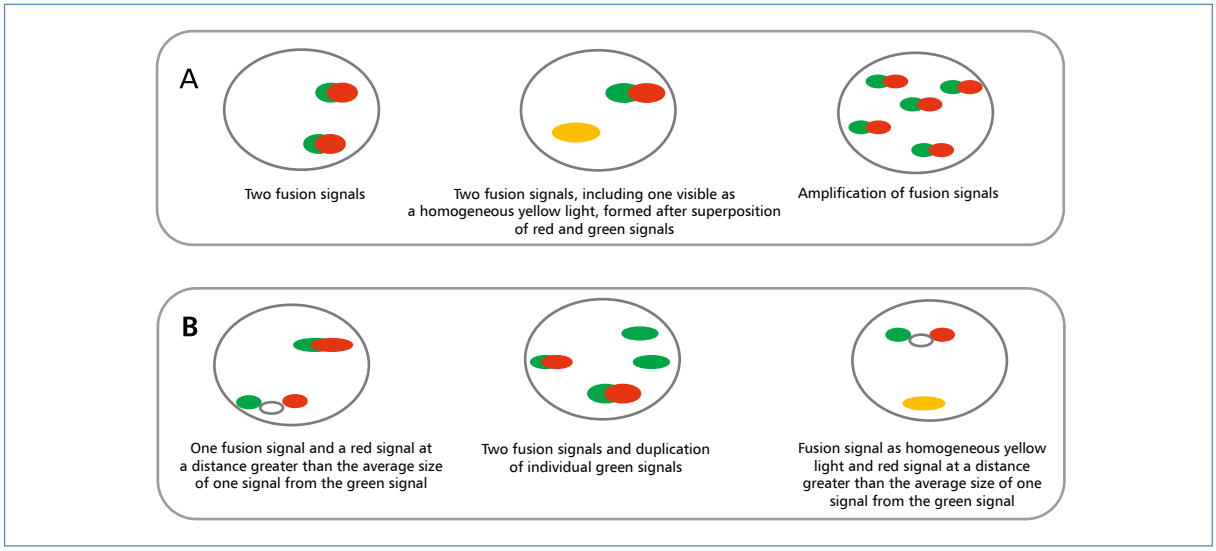

Figure 1. The representative diagrams of signals from fluorescent probes in the case of: **A** — tumor cell nuclei without rearrangement of the *ROS1* gene; **B** — nuclei of cancer cells with the current rearrangement of the *ROS1* gene

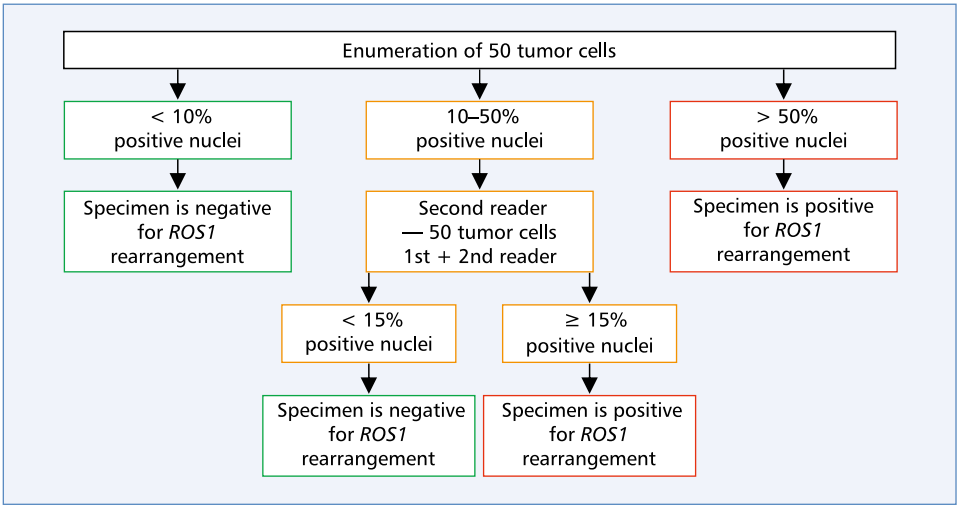

Figure 2. The scoring algorithm recommended for *ROS1* testing with FISH technique [8]

The limitations of the *ROS1* gene rearrangement analysis using FISH method resulted mainly from the insufficient number of cancer cells in the examined material and the lack of molecular probe signals due to the most likely incorrect fixation of the materials sent for testing. Pre-laboratory treatment of histological and cytological material has an extremely important impact on the possibility of obtaining a diagnostic result of FISH gene rearrangement testing.

In the examined group, rearrangement of the *ROS1* gene was detected in 9 cases, which constituted 1.57% of all examined samples. Rearrangement was detected in 5 men and 4 women ($P = 0.757$, $\chi^2 = 0.096$). Lung adenocarcinoma was diagnosed in all *ROS1*-positive patients (1.94% among patients with adenocarcinoma). In 6 *ROS1*-positive patients, expression of TTF1 protein on tumor cells was observed, and in the remaining three there was no expression of this adenocarcinoma marker ($P = 0.415$, $\chi^2 = 0.664$). In *ROS1-positive* patients, the median percentage of cancer cell nuclei with *ROS1* gene rearrangement was 18% and the median copy number of the *ROS1* gene was 2.6.

In 138 (24.08%) patients, cancer cell nuclei with *ROS1* gene rearrangement were observed, however, with a result that did not meet the criteria for inclusion for a molecularly targeted treatment (< 15% of cancer cell nuclei with *ROS1* gene rearrangement). In 19 patients (3.32% of analyzed cases) *ROS1* gene polysomy was observed (≥ 4 copies of the *ROS1* gene in the cell nucleus), however, in no case did this abnormality coexist with *ROS1* gene rearrangement. In the whole study group, the median copy number of the *ROS1* gene was

2.4. The number of copies of the *ROS1* gene did not significantly depend on sex, age, pathomorphological diagnosis, as well as the presence of TTF1 and CK7 expression on cancer cells.

Life expectancy of patients with known ROS1 gene status

In the studied group, 6 patients with *ROS1* gene rearrangement received molecularly targeted treatment with crizotinib (the remaining three patients in this group had an adverse course of the disease which prevented systemic treatment). In patients without *ROS1* gene rearrangement, 54 patients with PD-L1 expression on over 50% of cancer cells (9.57%) received first-line treatment with pembrolizumab, and 412 patients received chemotherapy (73.05%), among whom 2nd line immunotherapy received 16 patients (this number will increase significantly during observation of patients, since we began observing patients from January 2019). 98 patients did not receive any systemic treatment due to poor fitness and the presence of concomitant diseases (17.38%).

The median of overall survival (mOS) did not depend on sex, age, pathological diagnosis, presence of rearrangement of the *ROS1* gene and the number of copies of the *ROS1* gene in cancer cell nuclei. mOS in patients with non-squamous NSCLC type with TTF1 expression on cancer cells was 13 months, and in patients without this marker only 7 months (HR = 0.5634, P = 0.01). mOS in patients receiving first-line chemotherapy followed by second-line immunotherapy was 29 months (95% CI: 20.0–29.0), in patients receiving only chemotherapy — 14 months (95% CI: 10.0–30.0) and in patients without systemic treatment (due to poor performance) — 2 months. mOS in patients with *ROS1* gene rearrangement treated with crizotinib and in patients with PD-L1 expression on more than 50% of cancer cells receiving 1st line immunotherapy with pembrolizumab was not achieved. These differences were statistically significant (P < 0.0001). In the group of patients treated with crizotinib, at the time of statistical analysis, five patients were still alive (from 2 to 13 months of treatment), and one patient died 7 months after the implementation of molecularly targeted treatment.

Discussion

Rearrangement of the *ROS1* gene was first detected in a patient with lung cancer in 2007 [10]. Currently, this change is relatively well known — it is estimated that this rearrangement occurs in 1–2% of NSCLC patients. Patients with *ROS1* gene rearrangement are usually a group of young patients with adenocarcinoma (around 40–50 years old), however, there is a noticeable increase in the incidence of *ROS1* rearrangement also in patients over 70 years of age. 70% of patients with *ROS1* gene rearrangement have never smoked and 30% still smoke or smoked in the past [9, 11]. No significant differences were observed in the occurrence of rearrangement depending on the race of patients with NSCLC — in a study conducted by the IASLC (International Association for the Study of Lung Cancer) the rearrangement of the ROS1 gene was found in 2.3% of Asian patients, in 2% of patients of the race Caucasian and 1.6% of patients living in North America. However, local differences are described in the incidence of *ROS1* rearrangement — in a study conducted in northern India, this abnormality was found in 2.8% of NSCLC patients [12]. To date, it is difficult to determine the frequency of this genetic abnormality in the Polish population of NSCLC patients. In the presented study, *ROS1* gene rearrangement was detected in 1.57% of NSCLC patients with non-squamous cell type and in 1.94% of patients with adenocarcinoma, which confirms the worldwide incidence of this genetic abnormality.

Despite the sporadic occurrence of this rearrangement of the *ROS1* gene, the benefits of its diagnosis and the introduction of molecularly targeted therapy in *ROS1*-positive patients can be significant. In the PROFILE1001 clinical trial, 53 patients with locally advanced and advanced NSCLC with detected *ROS1* gene rearrangement were treated with crizotinib. The response rate to treatment was 72%, and the median overall survival was 51.4 months [13]. In another study, the efficacy of crizotinib in 1st line of treatment (n = 30) was compared to chemotherapy based on platinum and pemetrexed (n = 47) in NSCLC patients with *ROS1* gene rearrangement. The median follow-up was 28.1 months. The objective response rate in the crizotinib group was higher than in the group receiving chemotherapy (86.7% *vs.* 44.7%, respectively; P < 0.001). In addition, a significant increase in progression-free survival time (18.4 months) was observed in patients treated with crizotinib compared to patients receiving chemotherapy (8.6 months; P < 0.001). The median overall survival was not reached for patients receiving crizotinib, but it was 28.4 months for patients receiving chemotherapy (cross-over effect) [14].

From January 2019, crizotinib was reimbursed in Poland as a molecularly targeted therapy for patients with stage IIIB or IV NSCLC with *ROS1* gene rearrangement. The problem that clinicians planning therapy with crizotinib in ROS1-positive patients may encounter is the development of resistance to this drug during treatment. Gainor et al. observed that as many as 53% of patients undergoing crizotinib treatment develop resistance, which is most likely associated with the appearance of new mutations in the *ROS1* gene [15].

The problem of crizotinib resistance may be solved by research into the 2nd generation of ROS1 inhibitors. An example of the usefulness of this group of drugs may be the proven efficacy of lorlatinib and repotrectinib observed in *ROS1*-positive patients progressing after the use of crizotinib [16].

In Poland, the fluorescence in situ hybridization method using specific molecular probes is used to diagnose the *ROS1* gene rearrangement. It is an effective and proven diagnostic method, characterized by high sensitivity and specificity, and the kits for this diagnostic method have CE-IVD (*in vitro* diagnostic) certificates. The false-positive results described in the literature may result from the detection of an inactive fusion in the *ROS1* gene resulting from post-transcriptional processing, but this is a casuistic situation. As a result of the rearrangement taking place, the *ROS1* gene may fuse with other genes, e.g. *TPD52L1*, present near the location of the *ROS1* gene. The existence of such a partner gene fusion may be a diagnostic problem in the FISH method [8, 17]. However, to date, it is the technique most widely used in the diagnosis of rearrangement of the *ROS1* gene, and the only limitation of this method is the possibility of damage to the genetic material of cancer cells during improper fixation and protection of tissue material, and too low number of cancer cells in the assessed materials. Research is ongoing on the possibility of detecting the presence of an abnormal fusion protein containing *ROS1* on the surface of cancer cells by immunohistochemistry (IHC) [8]. The IHC method has obtained the CE-IVD certificate in recent months. In some laboratories, it is already routinely used for screening for *ROS1* gene abnormalities. However, it should be remembered that all positive IHC results for the presence of a *ROS1*-containing fusion protein must still be confirmed by FISH. Another technique that can be used in analyzing *ROS1* gene abnormalities is next-generation sequencing (NGS) [8].

In summary, analysis of the *ROS1* gene rearrangement among patients with locally advanced or advanced non-small cell lung cancer should be the standard in the diagnosis of predictive factors. Patients with *ROS1* gene rearrangement, thanks to new generations of drugs, have a chance to significantly extend life expectancy and improve its quality. The technique of fluorescence in situ hybridization is the basic diagnostic method, but it should be remembered that pre-laboratory treatment of histological and cytological material has an extremely important impact on the possibility of obtaining a diagnostic and reliable result of gene rearrangement testing using this method.

Conflicts of interest

The authors declare to have no conflict of interest.

References

1. Raparia K, Villa C, DeCamp MM, et al. Molecular profiling in non-small cell lung cancer: a step toward personalized medicine. Arch Pathol Lab Med. 2013; 137(4): 481–491, doi: 10.5858/arpa.2012-0287-RA, indexed in Pubmed: 23544937.
2. Rosell R, Bivona TG, Karachaliou N. Genetics and biomarkers in personalisation of lung cancer treatment. Lancet. 2013; 382(9893): 720–731, doi: 10.1016/S0140-6736(13)61715-8, indexed in Pubmed: 23972815.
3. Sasaki T, Rodig SJ, Chirieac LR, et al. The biology and treatment of EML4-ALK non-small cell lung cancer. Eur J Cancer. 2010; 46(10): 1773–1780, doi: 10.1016/j.ejca.2010.04.002, indexed in Pubmed: 20418096.
4. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med. 2012; 18(3): 378–381, doi: 10.1038/nm.2658, indexed in Pubmed: 22327623.
5. Jassem J, Krzakowski M. Nowotwory klatki piersiowej. Praktyczny przewodnik dla lekarzy. Wydanie III. Via Medica, Gdańsk 2018.
6. Birchmeier C, O'Neill K, Riggs M , et al. Characterization of ROS1 cDNA from a human glioblastoma cell line. Proc Natl Acad Sci U S A. 1990; 87(12): 4799–4803, doi: 10.1073/pnas.87.12.4799, indexed in Pubmed: 2352949.
7. Charest A, Lane K, McMahon K, et al. Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). Gen Chrom Cancer. 2003; 37(1): 58–71, doi: 10.1002/gcc.10207, indexed in Pubmed: 12661006.
8. Tsao MS, Hirsch RR, Yatabe Y, et al. IASLC Atlas of ALK and ROS1 testing in lung cancer. Second Edition. 2016.
9. Shaw AT, Ou SHI, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med. 2014; 371(21): 1963–1971, doi: 10.1056/NEJMoa1406766, indexed in Pubmed: 25264305.
10. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell. 2007; 131(6): 1190–1203, doi: 10.1016/j.cell.2007.11.025, indexed in Pubmed: 18083107.
11. Bergethon K, Shaw AT, Ou SHI, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol. 2012; 30(8): 863–870, doi: 10.1200/JCO.2011.35.6345, indexed in Pubmed: 22215748.
12. Mehta A, Saifi M, Batra U, et al. Incidence of -rearranged non-small-cell lung carcinoma in india and efficacy of crizotinib in lung adenocarcinoma patients. Lung Cancer (Auckl). 2020; 11: 19–25, doi: 10.2147/LCTT.S244366, indexed in Pubmed: 32158297.
13. Shaw AT, Riely GJ, Bang YJ, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. Ann Oncol. 2019; 30(7): 1121–1126, doi: 10.1093/annonc/mdz131, indexed in Pubmed: 30980071.
14. Shen L, Qiang T, Li Z, et al. First-line crizotinib versus platinum-pemetrexed chemotherapy in patients with advanced ROS1-rearranged non-small-cell lung cancer. Cancer Med. 2020; 13: 1–9, doi: 10.1002/cam4.2972, indexed in Pubmed: 32167664.
15. Gainor JF, Tseng D, Yoda S, et al. Patterns of metastatic spread and mechanisms of resistance to crizotinib in ROS1-positive non-small-cell lung cancer. JCO Precis Oncol. 2017; 10, doi: 10.1200/PO.17.00063, indexed in Pubmed: 29333528.
16. Shaw A, Felip E, Bauer T, et al. Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. Lancet Oncol. 2017; 18(12): 1590–1599, doi: 10.1016/s1470-2045(17)30680-0.
17. Zhu VW, Upadhyay D, Schrock AB, et al. TPD52L1-ROS1, a new ROS1 fusion variant in lung adenosquamous cell carcinoma identified by comprehensive genomic profiling. Lung Cancer. 2016; 97: 48–50, doi: 10.1016/j.lungcan.2016.04.013, indexed in Pubmed: 27237027.

VIA MEDICA

ORIGINAL ARTICLE

Elżbieta Bylina, Czesław Osuch, Piotr Rutkowski

Department of Melanoma and Soft Tissue and Bone Sarcomas, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland

Analysis of reliability of different risk classifications for assessment of relapses of gastrointestinal stromal tumors (GIST) — the impact of primary tumor genotyping

Address for correspondence:
Prof. dr hab. n. med. Piotr Rutkowski
Klinika Nowotworów Tkanek Miękkich, Kości i Czerniaków
Narodowy Instytut Onkologii
im. Marii Skłodowskiej-Curie
— Państwowy Instytut Badawczy
ul. Roentgena 5, 02–781 Warszawa
e-mail: piotr.rutkowski@pib-nio.pl

ABSTRACT

Background. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Radical surgery is the primary treatment for GIST. Unfortunately, 40–50% of patients relapse, mainly due to hepatic and peritoneal metastases. Currently, the treatment of choice for locally advanced, inoperable or metastatic GIST is the use of tyrosine kinase inhibitors, including imatinib. GISTs are a group of tumors with various morphological, pathological and molecular features as well as different clinical courses, therefore their biological course is difficult to determine. Nevertheless, we currently have 5 classifications that assess the risk of relapse after surgery. The aim of this study was to analyze prognostic factors with regard to the risk of recurrence and overall survival, and to compare the clinical reliability of the recurrence risk classifications developed so far with an attempt to present a new classification including the genotype of primary GIST.

Patients and methods. The material consisted of a group of 697 patients with primary GIST treated with the intention to cure, collected prospectively as part of the GIST clinical registry, Department of Melanoma and Soft Tissue and Bone Sarcomas, Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw. All patients were classified based on 5 existing recurrence risk classifications. Univariate and multivariate analysis were performed for disease-free survival (DFS) and overall survival (OS). The relationships of the following factors with DFS and OS were assessed: sex, age, primary tumor mutational status, primary tumor location, primary tumor size, number of mitoses/50 HPF, surgical margins and the presence of tumor rupture. The next analysis concerned the comparison of the accuracy of existing recurrence risk classifications. The analysis was performed using ROC curves and a new classification model was proposed including mutation analysis as well as factors such as gender and age for selected existing recurrence risk assessment models.

Results. Univariate and multivariate analyses showed statistical significance of variables such as male sex ($P = 0.02$), mitotic index 5–10/50 HPF and > 10/50 HPF ($P < 0.001$), primary tumor size 5–10 cm and > 10 cm ($P < 0.001$), primary tumor location outside of the stomach ($P < 0.001$), R1 surgery ($P < 0.001$), tumor rupture ($P < 0.001$), and the presence of mutations in the *KIT* gene exon 11 including deletion 557–558 and the *KIT* gene exon 9 ($P = 0.009$) as negative prognostic factors affecting disease recurrence. Five-year disease-free survival rate was 57.3%. Median DFS was 76 months. Negative prognostic factors for OS are: age < 40 ($P = 0.045$), mitotic index 5–10/50 and > 10/50 HPF ($P < 0.001$), primary tumor size 5–10 cm and > 10 cm ($P < 0.001$), R1 surgery and tumor rupture ($P < 0.001$). All existing recurrence risk classifications showed prognostic value for assessing differences in DFS and OS, no significant differences were found between individual recurrence risk classifications. In addition, the reliability of all these classifications was improved by adding gender, age and mutation status. The value added of mutation status for better risk assessment was most significant when used in intermediate risk groups according to different classifications ($P < 0.01$).

Conclusion. All current GIST recurrence risk classifications allow for reliable assessment of recurrence risk. Mutations involving deletions (557–558) in the *KIT* gene exon 11 are most often present in the group at high risk of recurrence. Patients with confirmed mutations in the *PDGFRA* gene exon 18 and wild-type genotype have a favorable prognostic effect. The reliability of existing classifications for assessing the risk of relapse after GIST resection can be improved by adding mutation status, especially in groups at intermediate risk of relapse, which should facilitate therapeutic decisions in the context of adjuvant therapy.

Key words: GIST, risk classification, genotyping

Oncol Clin Pract 2020; 16, 5: 276–294

Oncology in Clinical Practice
2020, Vol. 16, No. 5, 276–294
DOI: 10.5603/OCP.2020.0032
Translation: prof. Ewa Bartnik

ISSN 2450–1654

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. These neoplasms most commonly occur in the stomach (40–70%), the small intestine (20–40%) much less frequently in the large intestine (5–15%), and rarely (> 5%) in the esophagus and intraperitoneally [1–3]. The majority of GIST is characterized by the occurrence of a mutation activating the *KIT* protooncogene (about 70–80%), and the *PDGFRA* gene that is the platelet-derived growth factor receptor alpha (approximately 5–15%). The remaining GIST (approximately 15%) is the so-called wild type (WT), in which no mutations are found in the *KIT* or *PDGFRA* genes. A characteristic immunochemical marker for GIST is CD117 and a positive reaction indicating the presence of this antigen occurs in about 95% cases, which is the most important criterium in differential diagnosis [2, 4, 5].

At this moment we do not have reliable and clear data which would answer the question about the frequency of occurrence and incidence for these tumors, but clinically significant cases are calculated at 3–4 per million inhabitants per year [6–11].

The basic method of treating GIST is radical resection based on removing the tumor within the borders of healthy tissues. Radical surgery allows for 5-year survivals without relapse in 35–65% patients [12–16]. Unfortunately, in 40–50% patients after potentially therapeutic resection a relapse occurs, mainly in the form of metastases to the liver and peritoneum [5, 17].

Because of relapses in such a large group of patients and the therapeutic success of the low molecular weight tyrosine kinase inhibitor (IKT) imatinib monosulphate in the therapy of locally non-resectable and/or metastatic GIST [18–23], adjuvant therapy with imatinib was introduced to clinical practice in order to reduce disease recurrence/improve patient cures [19, 24–28]. These analyses also indicated that the effect of adjuvant treatment is associated with the tumor genotype and the effectiveness of longer adjuvant treatment with imatinib was most clearly seen in the group of GIST patients with deletion or insertion/deletion in exon 11 of *KIT*. Of course, it remains to be discussed whether imatinib should be used in GIST patients with an intermediate recurrence risk and also which of the existing recurrence risk classifications should be used, as well as whether adjuvant therapy should be used for GIST with genotypes with low susceptibility to imatinib [26–28].

GIST is a group of tumors with diverse morphological and pathological characteristics and varied clinical course [2]. Their biological course is difficult to define and as is known from analyses conducted so far it depends on several basic criteria: the size and localization of the primary tumor and the mitotic index [29]. A consensus elaborated by the NIH (National Institutes of Health) in the United States in 2001, presented for the first time a practical scheme for evaluating the risk of a clinical course taking into consideration the size of the primary tumor and the mitotic index of GIST (Table 1) [13, 30].

The next classification evaluating recurrence risk and the tightly associated prognosis for the patients is the classification based on the location of the primary tumor proposed by Miettinen and Lasota from AFIP (Armed Forces Institute of Pathology). They proved by analyzing about 1600 GIST cases that large (> 10 cm) neoplasms in the stomach with a low mitotic index have only a 12% recurrence risk whereas for GIST located in the small intestine for similar parameters the recurrence risk increases to > 50% [31, 32].

A successive additional negative prognostic factor of GIST recurrence risk after resection is perforation of the primary tumor (regardless of whether it is spontaneous or a result of surgery). This idea became the basis for the next classification proposed by Joensuu who modified the NIH classification including the neoplasm location (stomach *vs*. other) and tumor perforation as a prognostic factor independent of size and mitotic index. Patients with tumor perforation have a high recurrence risk due to the possibility of formation of intraperitoneal implantation during perforation [33].

One of the last classifications proposed by AJCC (American Joint Committee on Cancer) based to a large extent on the classification of Miettinen and Lasota was presented in January 2010 and the current TNM system

Table 1. Factors taken into consideration in the classification of recurrence risk plus a model with added mutation evaluation

Characteristic	National Institutes of Health (NIH)	Miettinen and Lasota (AFIP-NCCN)	NIH according to Joensuu	TNM (according to AJCC 2010 and 2017)	Nomogram according to Gold	Model with mutation evaluated in this work
Tumor size	X	X	X	X	X	X
Mitotic number	X	X	X	X	X	X
Tumor location		X	X	X	X	X
Tumor rupture			X			X
Presence of metastases characteristics N and M				X		
Probable progression — ree survival 2 and 5 years in %					X	
Addition of mutation						X

was created especially for GIST. This classification divides the localization of the primary tumor into those derived from the stomach and others [34, 35].

A nomogram presented at the end of 2009 by Gold et al. is the next classification evaluating recurrence risk taking into consideration the mitotic index, the size of the primary tumor and localization. On the basis of the number of points it evaluates and expresses in percent the probable survival time (2 and 5 years) without GIST recurrence. The nomogram is suggested to better evaluate the recurrence risk in comparison with the NIH classification and is similar to the classification proposed by AFIP Mettienen and Lasota and as the earlier systems can be used to qualify patients and to make decision on adjuvant treatment [36]. However, it does not take possible tumor perforation into consideration and takes tumor size as a continuous variable.

Molecular analysis of GIST detected the presence of two mutually exclusive mutations in the *KIT* and *PDGFRA* genes. These mutations cause excessive expression and activation of the *KIT* and *PDGFRA* protooncogenes. GIST mutations are commonly observed in the *KIT* gene (80–90%) and most of them occur in exon 11 and less frequently in exon 9 and sporadically in exons 13 and 17. A mutation in the *PDGFRA* gene is less common and is found in about 5 to 10% GIST and most often is in exon 18 and less frequently exon 12. In about 10–15% GIST no mutations in these two genes are observed, they are WT (wild type) [37–40]. Analyses performed so far indicate that the presence of mutations in the *KIT* or *PDGFRA* genes is important for predicting responses to imatinib treatment, moreover the data show that a significant role is also played by a mutation in a defined exon. Patients with mutations in exon 11 of *KIT* respond better to imatinib treatment while patients with mutations in exon 9 are more often resistant to therapy with this drug. The results of analyses confirm the idea of using a dose of 800 mg/day in patients with mutations in exon 9 of *KIT* [41–43].

It seems that determining the type of mutation may also be of prognostic significance in primary GIST, though at present we do not have data which would allow unequivocal confirmation of this idea. Difficulties in showing such relations are due to GIST pathogenesis as *KIT* mutations are a very early stage in the formation of these neoplasms and cannot be an independent factor determining an aggressive course of GIST. Several investigations have confirmed the association between some *KIT* mutations and a more aggressive course. However other analyses confirmed that these mutations also occur in very small GIST with a clinically benign course [44–46]. The results of analyses performed so far suggest further investigations are required in order to evaluate the prognostic significance of *KIT* mutations in larger patient groups [47]. There are also suggestions that the *PDGFRA* mutation in the primary tumor occurs almost exclusively in GIST originating in the stomach and is characterized by a more indolent course of the disease [48].

The most important problem after treatment of primary GIST is to determine significant and independent prognostic factors. This statement is important as at present we know about at least several clinical and/or molecular parameters which can affect the prognosis and treatment of GIST patients.

We currently have 5 systems of evaluating the recurrence risk for GIST after resection (Table 1), none of the proposed systems encompasses the mutation status as one of the factors which could affect recurrence risk. An attempt to include the mutation status was made during

the creation of the TNM AJCC system, but it was finally not included because of the small amount of data [34].

Determining which of the present systems which are used for evaluating risk on the basis of prognostic factors is the best for foreseeing recurrence risk so that it can be used in clinical practice and whether and if so what would be the significance of including the status of the mutation in primary GIST is the subject of this paper (Table 1).

Material and methods

The analysis was performed for a group of 697 patients with primary GIST treated with the intention to cure (R0/R1 resection), collected prospectively in the Department of Melanoma and Soft Tissue and Bone Sarcomas, Maria Skłodowska-Curie National Research Institute of Oncology from 2001. The analyzed group consisted of 375 (53.8%) women and 322 (46.2%) men, treated by radical resection in the years 2001–2011. Data about the patients and their treatment were obtained on the basis of the analysis of the patients' medical records and data concerning their survival from the National Neoplasm Registry. The analyzed group encompassed only patients after surgical resection of primary GIST without metastases at the moment of diagnosis and with a confirmed histopathological analysis. All patients in the analyzed group underwent radical (macroscopically) resection and did not receive adjuvant treatment. After resection of the primary GIST the patients were subjected to careful medical observation during which a physical examination and computer tomography of the abdominal cavity and pelvis were performed according to recommendations for GIST patients with a high and intermediate recurrence risk, every 3–4 months for the first 2 years after resection of the primary GIST, and subsequently every 6 months until 5 years after the original resection and after 5 years once a year in the case of resection of a GIST with a low degree of aggressiveness [48]. In 291 cases mutations in *KIT* and *PDGRFA* genes were analyzed. The material for molecular analysis was taken from paraffin blocks and/or freshly frozen tumor tissue. Molecular analysis was performed for exons 9, 11, 13, 14 and 17 of the *KIT* gene and exons 12 and 18 of the *PDGFRA* gene.

All patients were classified based on 5 existing recurrence risk classifications. Detailed clinical, pathological and molecular data are presented in Table 2.

Statistical analysis

The analyzed patients were observed from 2001 (date associated with the creation of the Clinical GIST Registry) until August 2013. The final date of the surgery of the patients included in the analysis was December 2010. The frequency of recurrence was evaluated on the basis of computer tomography during the period of observation. Disease-free survival (DFS) was calculated from the date of GIST resection to the date of local recurrence, date of distant metastases or date of the last observation. Overall survival (OS) was calculated from the resection to the date of the last observation or the date of death.

Univariate analysis was performed overall survival and for disease-free survival using Kaplan-Meier and the log-rank test (univariate analyses). Survival of the patients was expressed in the form of probability of death during 5 years from the operation (with a 95% confidence interval) and graphically on figures showing survival curves. In order to identify independent variables affecting the patients' survival a multivariate Cox model was used. Significant variables were selected by a progressive stepwise approach. The results are presented as a hazard ratio (HR) with a 95% confidence interval. In the next step of the analysis using methods of logistic regression, a model was constructed in which probability of disease-free survival and overall survival was estimated for 1 and 5 years. We checked whether taking the mutation code into consideration significantly improved the predictive abilities of the model. To models selected *a priori* variables signifying the mutation code were added and then ROC curves were constructed and then ROC curves constructed on the basis of values calculated from the models were compared. The same method was used to compare different classifications.

The calculations were performed using the software package R 3.0.1 (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/), the survival package (Therneau T (2013). _A Package for Survival Analysis in S_. R package version 2.37-4, URL: http://CRAN.R-project.org/package=survival) and pROC (Xavier Robin, Natacha Turck, Alexandre Hainard, Natalia Tiberti, Frédérique Lisacek, Jean-Charles Sanchez and Markus Müller (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics, 12, p. 77. DOI: 10.1186/1471-2105-12-77 URL: http://www.biomedcentral.com/1471-2105/12/77/).

Results

Univariate analysis

Progression-free survival

The basic evaluated parameter was disease-free survival (DFS). During the observations, 265 (38.3%) recurrences were observed. Median disease-free survival

Table 2. Characteristics of the analyzed patients

Characteristic		Number (%)
Sex	Women	375 (53.8%)
	Men	322 (46.2%)
Age at diagnosis (years)	< 40	62 (8.9%)
	41–65	422 (60.6%)
	> 65	213 (30.5%)
Localization	Stomach	373 (53.5%)
	Duodenum	36 (5.2%)
	Small intestine	237 (34.0%)
	Large intestine/rectum	25 (3.6%)
	Other	26 (3.7%)
Size of primary tumor [cm]	< 5	266 (39.1%)
	> 5–10	254 (37.4%)
	> 10	160 (23.5%)
	No data	17 (2.4%)
Number of mitoses in 50 visual fields at large magnification	≤ 5	401 (63%)
	> 5–10	98 (15.4%)
	> 10	138 (21.7%)
	No data	60 (8.6%)
Surgical margins	R0	554 (80.4%)
	R1	135 (19.5%)
	No data	8 (1.1%)
Tumor rupture	No	596 (92.5%)
	Yes	48 (7.5%)
	No data	53 (7.6%)
Mutation analysis	*KIT* 11 deletion 557–558	65 (22.3%)
	KIT 11 point mutation or insertion	63 (21.6%)
	KIT 11 other deletions	45 (15.5%)
	KIT 9	23 (7.9%)
	PDGFRA 18 D842V	25 (8.6%)
	Other *PDGFRA* mutations	21 (7.2%)
	Other *KIT* mutations	11 (3.8%)
	Wild type (WT) — no *KIT* or *PDGFRA* mutations	38 (13.1%)
	No data	406 (58.2%)
Recurrence risk according to NIH (National Institutes of Health Classification)	Very low	32 (4.9%)
	Low	171 (26.1%)
	Intermediate	150 (22.9%)
	High	303 (46.1%)
	No data	41 (5.9%)

→

Table 2 cont. Characteristics of the analyzed patients

Characteristic		Number (%)
Recurrence risk according to Joensuu	Very low	49 (7.4%)
	Low	162 (24.4%)
	Intermediate	98 (14.8%)
	High	355 (53.5%)
	No data	33 (4.7%)
Recurrence risk according to Miettinen and Lasota (AFIP-NCCN)	Very low	153 (23.9%)
	Low	135 (21.1%)
	Intermediate	105 (16.4%)
	High	246 (38.5%)
	No data	58 (8.3%)
Recurrence risk according to AJCC	Tumor stage I	281 (40.3%)
	Tumor stage II	115 (16.5%)
	Tumor stage IIIA	79 (11.3%)
	Tumor stage IIIB	160 (23.0%)
	No data	62 (8.9%)
Recurrence risk within 2 years according to Gold's nomogram	[1,25)	148 (21.2%)
	[26,50)	56 (8.0%)
	[51,75)	72 (10.3%)
	[76,98]	324 (46.5%)
	No data	97 (13.9%)
Recurrence risk within 5 years according to Gold's nomogram	[1,8)	160 (23.0%)
	[9,68)	149 (21.4%)
	[69,91)	179 (25.7%)
	[92,96]	112 (16.1%)
	No data	97 (13.9%)

was 76 months. In Table 3 univariate analysis for particular variables which could affect disease recurrence is presented. The variables for which statistical significance was demonstrated were: male sex ($P = 0.02$), mitotic index 5–10/50 HPF and > 10/50 HPF ($P < 0.001$), size 5–10 cm and > 10 cm ($P < 0.001$), localization outside the stomach ($P < 0.001$), extent of surgery R1 ($P < 0.001$), tumor rupture $P < 0.001$), and presence of a mutation in the *KIT* gene in exon 11 encompassing the 557–558 deletion an in the *KIT* gene in exon 9 ($P = 0.009$).

Overall survival

The next parameter evaluated during the analysis was overall survival (OS) estimated by the Kaplan-Meier method. 118 (17.2%) of the patients died and the OS median was not attained.

Based on univariate analysis, the following factors were found to have a negative effect on OS: the number of mitoses > 10/HPF ($P < 0.001$), size of the primary tumor > 10 cm ($P < 0.001$), surgical margins R1 ($P = 0.004$), tumor rupture ($P < 0.001$) and age < 40 ($P = 0.045$). Detailed results for individual variables are presented in Table 4.

Multivariate analyses

In order to identify independent variables affecting progression-free survival and overall survival of the patients, Cox's multiparameter model was used. Significant variables were selected by the progressive stepwise approach. 2 models were constructed: the first one for variables without adding the mutations and the second

Table 3. Results of univariate analysis for disease-free survival (DFS)

Characteristic		Number of cases	5 year survival	95% confidence interval	p
Sex	Women	372	59.7	(53.7–66.5)	0.024
	Men	320	54.2	(48.1–61.1)	
Age (years)	< 40	61	56.6	(44.0–72.8)	0.389
	41–65	421	55.9	(50.4–62.0)	
	> 65	210	60.3	(52.4–69.5)	
Localization	Stomach	370	74.5	(69.3 - 80.2)	< 0.001
	Duodenum	36	48.5	(31.8–73.9)	
	Small intestine	236	41.7	(35.0–49.6)	
	Large intestine/rectum	24	45.8	(28.1–74.7)	
	Other	26	17.4	(5.5–55.0)	
Size of primary tumor [cm]	< 5	263	86.2	(79.8–93.1)	< 0.001
	> 5–10	253	55.2	(48.5–62.7)	
	> 10	169	27.8	(21.1–36.5)	
	No data	17	41.8	(22.8–76.6)	
Number of mitoses in 50 visual fields (mitotic index, MI)	≤ 5	398	80.2	(75.3–85.4)	< 0.001
	> 5–10	98	44.5	(33.8–58.5)	
	> 10	138	16.8	(10.8–26.0)	
	No data	58	46.4	(33.8–63.8)	
Surgical margins (R0, R1)	R0	551	63.2	(58.4–68.4)	< 0.001
	R1	133	34.7	(26.3–45.7)	
	No data	8	55.6	(23.1–100.0)	
Tumor rupture	No	592	60.9	(56.3–65.9)	< 0.001
	Yes	48	24.3	(13.8–43.0)	
	No data	52	45.3	(26.4–78.0)	
Mutation evaluation	*KIT* 11 deletion 557–558	65	35.1	(23.8–51.8)	0.009
	KIT 11 PM/INS	63	59.2	(46.5–75.4)	
	KIT 11 other deletions	45	50.4	(35.5–71.6)	
	KIT 9	23	38.5	(21.2–69.9)	
	PDGFRA 18 D842V	25	83.6	(68.2–100.0)	
	Other mutations of the *PDGFRA* gene	21	87.8	(73.4–100.0)	
	Other mutations of the *KIT* gene	10	50.6	(24.6–100.0)	
	Wild type (WT)	38	44.3	(29.5–66.6)	
	No data	402	61.2	(55.4–67.6)	
Recurrence risk evaluation according to NIH (National Institutes of Health Classification)	High	302	30.8	(25.5–37.2)	< 0.001
	Intermediate	150	79.3	(71.5–88.0)	
	Low	169	94.7	(88.9–100.0)	
	Very low	31	100	(100.0–100.)	
	No data	40	55.7	(40.0–77.5)	

→

Table 3 cont. Results of univariate analysis for disease-free survival (DFS)

Characteristic		Number of cases	5 year survival	95% confidence interval	p
Recurrence risk evaluation according to Joensuu	High	354	34.2	(29.1–40.3)	< 0.001
	Intermediate	98	90.2	(83.9–96.9)	
	Low	207	94.1	(88.9–99.7)	
	No data	33	68.2	(52.7–88.3)	
Recurrence risk evaluation according to Miettinen and Lasota (AFIP-NCCN)	High	254	25.6	(20.1 – 32.6)	< 0.001
	Intermediate	105	66.9	(57.0–78.5)	
	Low	133	89.7	(83.0–96.8)	
	Very low	151	95.4	(91.0–99.9)	
	No data	58	48.4	(35.3–66.5)	
Recurrence risk evaluation according to AJCC	Tumor stage I	277	93.8	(89.8–97.9)	< 0.001
	Tumor stage II	115	68.7	(59.2–79.7)	
	Tumor stage IIIA	79	34.4	(24.2–48.9)	
	Tumor stage IIIB	160	20.4	(14.5–28.8)	
	No data	61	45.4	(33.1–62.3)	
Recurrence risk evaluation within 2 years according to Gold's nomogram	(1,28)	155	22.7	(16.5–31.3)	< 0.001
	(28,83)	154	58.1	(49.4–69.4)	
	(83,96)	169	85.5	(78.8–92.8)	
	(96,98)	119	96.9	(92.7–100.0)	
	No data	95	39.4	(29.4–52.9)	
Recurrence risk evaluation within 5 years according to Gold's nomogram	(1,8)	155	22.7	(16.5–31.3)	< 0.001
	(8,68)	153	58	(49.2–68.2)	
	(68,91)	147	83.3	(75.7–91.7)	
	(91,96)	142	97.4	(93.9–100.0)	
	No data	95	39.4	(29.4–52.9)	

one with the mutations added. Risk classification was not taken into consideration in multivariate analyses as they link individually analyzed parameters.

The factors with a negative effect on the probability of disease recurrence in the Cox regression analysis were: the mitotic index > 5/50 HPF, localization of the primary tumor outside the stomach, the size of the primary tumor > 5 cm and the male sex (Table 5).

In the model taking the GIST genotype into consideration the negative factors were the presence of a mutation other than in *PDGFRA*, the mitotic index > 5/50 HPF, male sex and the size of the primary tumor > 5 cm (Table 6). Similar results were obtained for the evaluation of factors affecting OS (Table 7).

Comparison of the accuracy of classification of GIST recurrence risk

The comparison of the existing recurrence risk classifications was performed using ROC curves. They were compared in the context of 5-year DFS and also 5-year OS. None of the statistically significant differences were found between individual classifications both for 5-year DFS and for 5-year OS.

The comparison of the prognostic value of recurrence risk for 5-year DFS is presented in Figures 1–3.

All analyses indicate a lack of statistically significant differences between individual risk classifications, the graphs are nearly identical. This is due to the fact that

Table 4. Results of univariate analysis for overall survival (OS)

Characteristic		Number of cases	5 year survival	95% confidence interval	p
Sex	Women	369	87.3	(83.0–91.8)	0.141
	Men	318	83.7	(78.8–88.9)	
Age	< 40	61	88.3	(79.9–97.6)	0.045
	41–65	417	86.8	(82.9–91.0)	
	> 65	209	81.5	(74.5–89.0)	
Localization	Stomach	367	89.1	(84.9–93.4)	0.06
	Duodenum	36	81.2	(67.2–98.2)	
	Small intestine	234	84.1	(78.7–89.9)	
	Large intestine/rectum	24	79.9	(64.0–99.7)	
	Other	26	74.3	(56.8–97.0)	
Size of primary tumor	< 5	261	99.5	(98.5–100.0)	< 0.001
	> 5–10	252	82.9	(77.4–88.7)	
	> 10	157	75.6	(68.5–83.5)	
	No data	17	84.6	(67.1–100.0)	
Number of mitoses in 50 visual fields (mitotic index MI/HPF)	≤ 5	395	92.8	(83.3–96.4)	< 0.001
	> 5–10	98	87.2	(79.5–95.7)	
	> 10	136	68.8	(60.5–78.3)	
	No data	58	85.1	(75.5–96.0)	
Surgical margins (R0, R1)	R0	545	89.3	(86.0–92.7)	0.004
	R1	134	72.1	(63.6–81.8)	
	No data	8	100	(100.0–100.0)	
Tumor rupture	No	588	86.6	(83.2–90.2)	0.005
	Yes	48	81.4	(69.6–95.2)	
	No data	51	76.9	(62.1–95.2)	
Mutation evaluation	*KIT* 11 deletion 557–558	65	81.8	(70.0–94.3)	0.75
	KIT 11 PM/INS	62	87	(77.7–97.5)	
	KIT 11 other deletions	45	86.6	(75.1–99.9)	
	KIT 9	23	87.7	(73.0–100.0)	
	PDGFRA 18 D842V	25	82	(65.1–100.0)	
	Other mutations of the *PDGFRA* gene	21	87.4	(72.4–100.0)	
	Other mutations of the *KIT* gene	10	87.5	(67.3–100.0)	
	Wild type (WT)	37	66.4	(51.3–86.0)	
	No data	399	88.5	(84.5–92.6)	

→

Table 4 cont. Results of univariate analysis for overall survival (OS)

Characteristic		Number of cases	5 year survival	95% confidence interval	p
Recurrence risk evaluation according to NIH (National Institute of Health Classification)	High	299	75.7	(70.4–81.4)	< 0.001
	Intermediate	150	97.5	(94.0–100.0)	
	Low	166	100	(100.0–100.0)	
	Very low	32	100	(100.0–100.0)	
	No data	40	82	(68.6–97.9)	
Recurrence risk evaluation according to Joensuu	High	351	78.4	(73.6–83.5)	< 0.001
	Intermediate	98	98.6	(96.0–100.0)	
	Low	205	99.2	(97.5–100.0)	
	No data	33	84	(70.5–99.9)	
Recurrence risk evaluation according to Miettinen and Lasota (AFIP-NCCN)	High	243	76.5	(70.6–82.9)	< 0.001
	Intermediate	104	89.5	(82.7–96.9)	
	Low	133	98.9	(96.9–100.0)	
	Very low	149	96	(91.4–100.0)	
	No data	58	78.9	(70.5–99.9)	
Recurrence risk evaluation according to AJCC	Tumor stage I	275	98.9	(97.3–100.0)	< 0.001
	Tumor stage II	114	91	(84.7–97.8)	
	Tumor stage IIIA	78	75.9	(65.8–87.6)	
	Tumor stage IIIB	159	73.5	(66.1–81.8)	
	No data	61	83	(72.8–94.6)	
Recurrence risk evaluation within 2 years according to Gold's nomogram	[1,28)	154	75.2	(67.9–83.4)	< 0.001
	[28,83)	153	85.9	(79.5–92.8)	
	[83,96)	167	95	(90.7–99.6)	
	[96,98]	118	100	(100.0–100.0)	
	No data	95	80.4	(71.6–90.3)	
Recurrence risk evaluation within 5 years according to Gold's nomogram	[1,8)	154	75.2	(67.9–83.4)	< 0.001
	[8,68)	152	85.9	(79.4–92.8)	
	[68,91)	145	94.4	(89.4–99.6)	
	[91,96]	141	100	(100.0–100.0)	
	No data	95	80.4	(71.6–90.3)	

all classifications include the most significant prognostic factors.

New classification model including mutation analysis for progression-free survival

In the next step of the analysis using logistic regression, a model was constructed in which probability of survival for 1 and 5 years was calculated. The effect of including the mutation code on the predictive value of the model was tested. Variables marking the mutation code were added to models selected *a priori*, and then ROC curves were prepared on the basis of predicted values calculated from the models. The analysis indicated that adding variables such as sex, age and mutation status to the existing classifications improved their reliability.

Table 5. Results of multivariate analysis of factors affecting DFS

Characteristic		HR	95% CI	P
Sex	Women	1		
	Men	1.3	(0.99–1.71)	0.6
Age	< 40	1		
	41–65	1.21	(0.76–1.95)	0.42
	> 65	1.13	(0.68–1.88)	0.64
Localization	Duodenum	1		
	Small intestine	0.79	(0.45–1.4)	0.43
	Large intestine/rectum	0.97	(0.42–2.23)	0.94
	Stomach	0.5	(0.28–0.88)	0.02
	Other	1.09	(0.51–2.34)	0.83
Tumor size	< 5	1		
	> 5–10	3.36	(2.09–5.4)	0
	> 10	6.25	(3.84–10.18)	0
Number of mitoses in 50 visual fields (mitotic index MI/HPF)	≤ 5	1		
	> 5–10	2.86	(1.95–4.19)	0
	> 10	5.08	3.67–7.01)	0

Table 6. The results of multiparameter analysis of factors affecting DFS including the type of mutation

Characteristic		HR	95% CI	P
Sex	Women	1		
	Men	1.62	(1.07–2.46)	0.02
Age	< 40	1		
	41–65	1.67	(0.89–3.15)	0.11
	> 65	1.46	(0.71–3.02)	0.31
Localization	Duodenum	1		
	Small intestine	0.98	(0.29–3.34)	0.98
	Large intestine/rectum	1.26	(0.31–5.03)	0.75
	Stomach	0.93	(0.27–3.15)	0.9
	Other	1.24	(0.3–5.13)	0.76
Tumor size	< 5	1		
	> 5–10	2.12	(1.08–4.18)	0.03
	> 10	5.86	(2.84–12.07)	0
Number of mitoses in 50 visual fields (mitotic index MI/HPF)	≤ 5	1		
	> 5–10	3.07	(1.69–5.58)	0
	> 10	4.38	(2.61–7.36)	0
Genotype (mutation evaluation)	*KIT* 11 deletion 557–558	1		
	KIT 11 PM/INS	1.03	(0.58–1.81)	0.92
	KIT 11 other deletions	1.13	(0.63–2.03)	0.69
	KIT 9	1.38	(0.68–2.77)	0.37
	PDGFRA 18 D842V	0.41	(1.14–1.23)	0.05
	Other mutations of the *PDGFRA* gene	0.61	(0.18–2.13)	0.44
	Other mutations of the *KIT* gene	0.76	(0.26–2.25)	0.63
	Wild type (WT)	1.66	(0.86–3.21)	0.13

Table 7. The results of the multiparameter analysis of factors affecting OS including the type of mutation

Characteristic		HR	95% CI	P
Age	< 40	1		
	41–65	2.84	(0.89–9.06)	0.08
	> 65	6.23	(1.83–21.26)	0
Tumor size	< 5	1		
	> 5–10	4.81	(1.1–20.95)	0.04
	> 10	7.31	(1.67–31.97)	0.01
Number of mitoses in 50 visual fields (mitotic index MI/HPF)	≤ 5	1		
	> 5–10	1.91	(0.79–4.62)	0.15
	> 10	3.2	(1.64–6.24)	0
Genotype (mutation evaluation)	*KIT* 11 deletion 557–558	1		
	KIT 11 PM/INS	0.89	(0.37–2.15)	0.79
	KIT 11 Other deletions	0.84	(0.34–2.09)	0.71
	KIT 9	1.12	(0.43–2.92)	0.82
	PDGFRA 18 D842V	2	(0.55–7.34)	0.3
	Other mutations of the *PDGFRA* gene	1.4	(0.3–6.64)	0.67
	Other mutations of the *KIT* gene	0.85	(0.11–6.7)	0.88
	Wild type (WT)	2.59	(1.13–5.96)	0.03

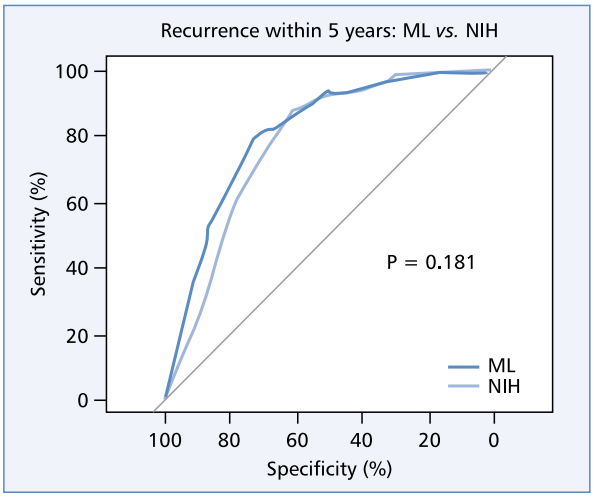

Figure 1. The prognostic value of the Miettinen and Lasota AFIP-NCCN (ML) classification in comparison to National Institutes of Health (NIH) in comparing recurrence risk within 5 years

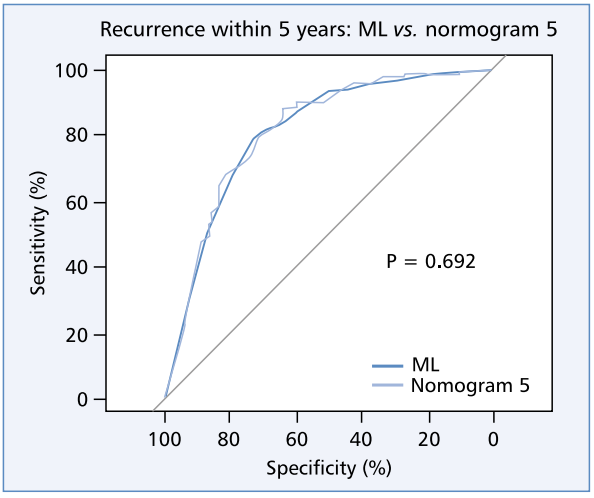

Figure 2. The prognostic value of the Miettinen and Lasota AFIP-NCCN (ML) classification in comparison to Nomogram 5 in comparing recurrence risk within 5 years

Moreover, the addition of the mutation status was the most significant in groups with intermediate risk in individual classifications (Figures 4–9).

In groups with intermediate risk a model taking into consideration sex, age and additionally the type of mutation is the closest to reality (Figures 7–9).

Discussion

The evaluation of recurrence risk after surgical treatment of GIST is very important in the context of adjuvant treatment and planning control examinations during observation after surgery [49]. The present clas-

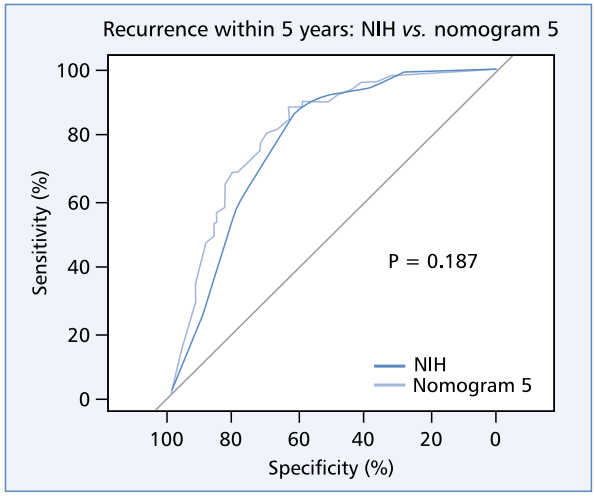

Figure 3. The prognostic value of the National Institutes of Health (NIH) classification in comparison to Nomogram 5 in comparing recurrence risk within 5 years

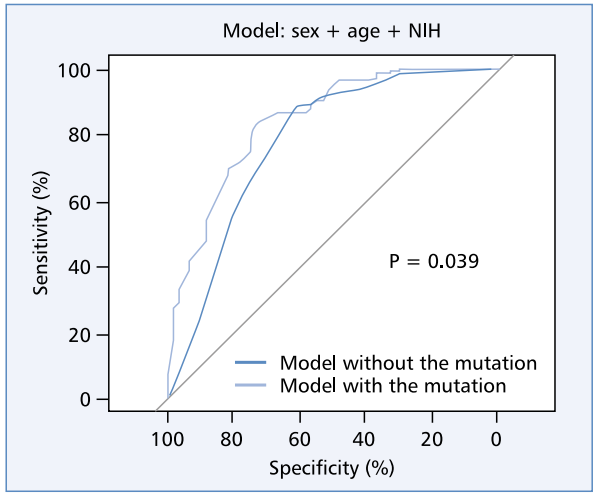

Figure 5. Model taking into consideration: sex, age and classification according to NIH

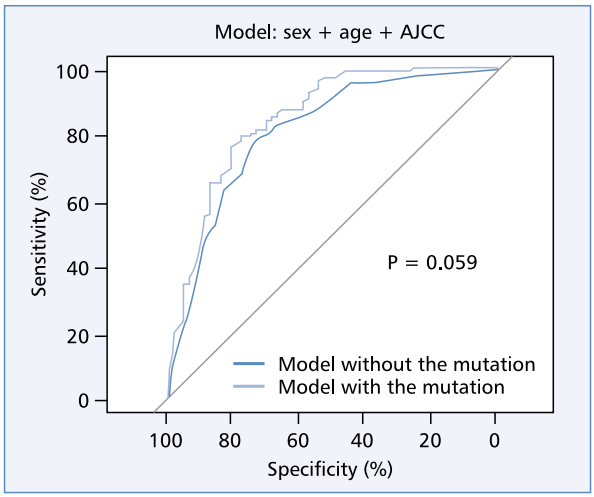

Figure 4. Model taking into consideration: sex, age and classification according to AJCC

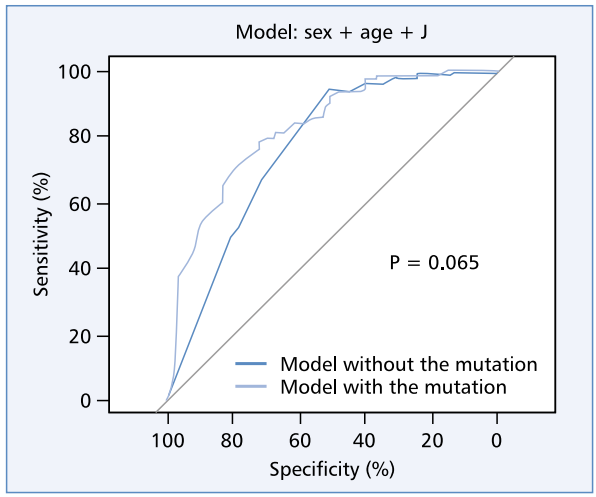

Figure 6. Model taking into consideration: sex, age and classification according to Joensuu (J)

sifications of recurrence risk based on such factors as tumor size, localization, mitotic index and tumor rupture allow reliable estimation of recurrence risk and are used in clinical practice [50, 51]. In recent years mutation status as a factor affecting recurrence risk has also been discussed [49, 51, 52].

In the presented group of patients, the basic evaluated parameter was disease-free survival DFS. During the observations, 265 (38.3%) of disease recurrences were observed. It should be stressed that the analyzed group consisted of patients not receiving imatinib adjuvant therapy after tumor resection, thus DFS represents the natural course of the disease. Median disease-free survival was 76 months. Other authors obtained similar results [17, 53, 54]. The following prognostic factors were found to be statistically significant in the present analysis: mitotic index 5–10/50 HPF and > 10/50 HPF ($P < 0.001$), size 5–10 cm and >10 cm ($P < 0.001$), MI > 5/50 HPF and tumor size > 5 cm are associated with a much shorter disease-free survival, which has also been demonstrated in all previous analyses [55–58]. The results of the analysis also confirm the effect of the tumor location for prognosis in GIST, which is in agreement with the results of other investigations [31, 32, 55–57, 59]. Localization of GIST outside the stomach (mainly in the intestine) gives a much worse prognosis than GIST localized in the stomach which has been reflected in the classification modified by Mietinnenn and Lasota [57, 59]. At present these factors are the basis of existing classifications, including the TNM staging

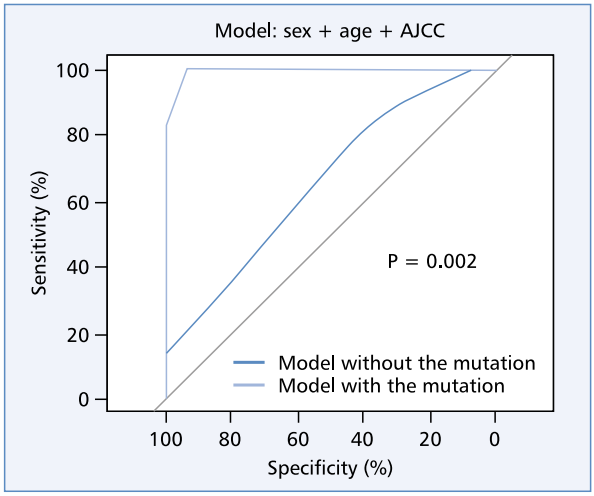

Figure 7. Model taking into consideration: sex, age and classification according to AJCC in an intermediate risk group

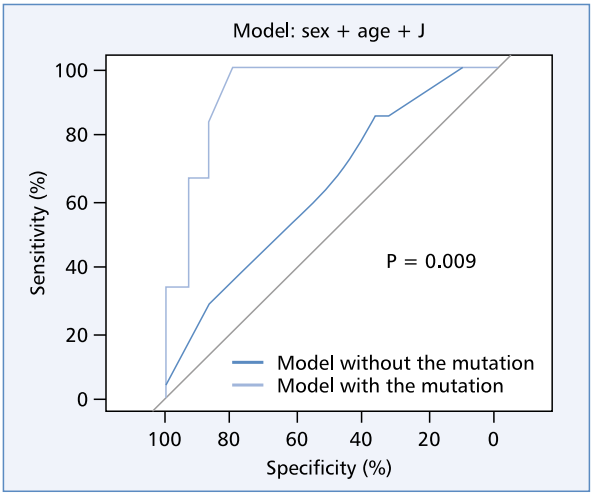

Figure 9. Model taking into consideration: sex, age and classification according to Joensuu (J) in an intermediate risk group

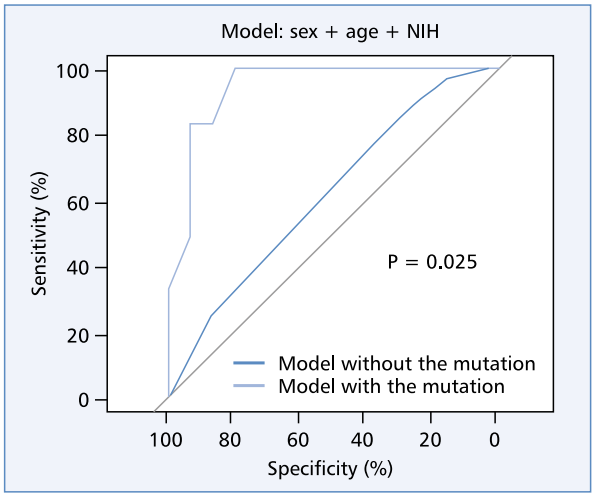

Figure 8. Model taking into consideration: sex, age and classification according to NIH in an intermediate risk group

according to AJCC. The presented relationships also confirm that the group of patients with primary GIST is representative. Other factors which significantly increase GIST recurrence risk are the extent of resection R1 ($P < 0.001$), tumor rupture ($P < 0.001$), male sex ($P = 0.02$). Radical resection (R0) in the microscopic evaluation and lack of tumor rupture regardless of whether spontaneous or linked to surgery, is extremely important during GIST surgery. Tumor rupture, regardless of tumor size and mitotic index, is a factor placing the patients in a high recurrence risk group according to the NIH classification modified according to Joensuu [33, 59–61]. The presented results also indicate an association between male sex and recurrence risk. Male sex in the analyzed group was a negative prognostic factor. Data from the literature concerning this factor are not as unequivocal as those presented above [56, 58]. All the above-mentioned factors (with the exception of resection R1) are statistically significant in uni- and multivariate analysis. So far only a few papers have analyzed the prognostic significance of the genotype of the primary tumor in patients with GIST, as many more were focused on the predictive role of mutations in response to treatment with tyrosine kinase inhibitors [62–64]. The next factor important for evaluation is the presence of a mutation in exon 11 of the *KIT* gene encompassing deletion 557–558, which turned out to be a factor associated with short disease-free survival time. In the light of available data, this mutation most commonly occurs in tumors originating outside the stomach, > 5 cm and with MI > 5/50 HPF, which automatically qualifies the patients for the high recurrence risk group and should be an additional factor in qualification for adjuvant treatment with imatinib [65], moreover, the present data unequivocally indicate that this is the mutation which is the most sensitive to imatinib [66–68]. The results of the univariate analysis indicate that the presence of an exon 9 mutation in the *KIT* gene also significantly shortens the time to disease recurrence. Summing up, the presence of a mutation in the *KIT* gene, regardless of whether it is in exon 11 encompassing deletion 557–558 or exon 9 ($P = 0.009$), is associated with a shorter DFS, comparing to a mutation in the *PDGFR* gene where the estimated 5-year disease-free survival with a mutation in exon 11 encompassing deletion 557–558 is 35.1% (95% CI: 23.8–51.8%) or exon 9 — 38.5% (95% CI: 21.2–69.95) *vs.* 83.6% (95% CI: 68.2–100.0) in the presence of a mutation in the *PDGFRA* gene. Longer DFS for GIST with a *PDGFRA* mutation has already been presented by other authors [69, 70]. The first papers at

the end of the 20th beginning of the 21st century only indicated that patients with GIST with a *KIT* mutation have more aggressive forms of the tumor than patients without the mutation, or with a mutation in the *PDGFRA* gene but the types of mutations were not analyzed in detail [38, 71, 72]. A Spanish group [73] was the first to observe the negative prognostic significance of a deletion encompassing codon 557 and/or 558 of the *KIT* gene. DeMatteo and co-authors also suggested that specific *KIT* mutations can have a prognostic value in univariate, but not multivariate analysis [68, 74] — indicating that GIST with a point mutation or a *KIT* insertion can have a better clinical course than exon 9 *KIT* mutations or deletions encompassing amino acids W557 and/or K558 of *KIT*, whereas tumors without *KIT* mutations are associated with an intermediate prognosis. The presented work is a confirmation of these factors as independent prognostic biomarkers for a much larger group of patients. The biological basis of these associations has not been explained but it is suggested that the mutated form of the KIT protein generated by substitution of proline for lysine in position 558 leads to ahigher constitutive phosphorylation of the receptor and greater cellular proliferation [75]. Several papers have also indicated a more favorable course of the disease in patients with primary GIST with a *PDGFRA* mutation (especially in exon 18, occurring mainly in tumors with a stomach localization and interestingly with a point mutation D842V characterized by resistance to used tyrosine kinase inhibitors in the case of nonresectable/metastatic tumors) [39, 46, 70, 76–78], this was also confirmed by the analysis of trial ACOSOG Z9001 in the placebo group [68].

On the basis of univariate analysis the following factors were found to have a negative effect on OS: the number of mitoses > 10/HPF ($P < 0.001$), size of primary tumor > 10 cm ($P < 0.001$). surgical margins R1 ($P = 0.004$), tumor rupture ($P < 0.001$) and age < 40 ($P = 0.045$). These factors, with the exception of surgical margins, were also found to be statistically significant in multivariate analysis. In the presented analysis no significant effect of mutation status on OS was observed. The analysis of factors affecting OS, after resection of the primary GIST, is one of the few in the literature and indicates significantly good survival even in high-risk groups which is associated with the high activity of imatinib and other tyrosine kinase inhibitors used to treat the recurrence of this disease [14, 79]. The currently used adjuvant therapy imatinib after resection of high-risk GIST can be expected to contribute to further improvement of the patients' survival.

Analysis of existing classifications of recurrence risk (expressed as ROC curves) for 5-year DFS and OS confirmed the prognostic significance of these classifications. The presented results demonstrate that the currently available and used classifications allow a reliable evaluation of recurrence risk, which is in agreement with the results from other authors [80–82]. No statistically significant differences were found when comparing particular classifications. This may be due to the fact that each of them encompasses two characteristics, tumor size and the number of mitoses, which as has also been demonstrated in the present analysis are the most important risk factors. Of course, each of the classifications has limitations, and the results of Goh et al. (2008) indicate that the AFIP classification of Miettinen and Lasota is better at predicting recurrence in comparison with NIH, which is due to the addition of the criterion of tumor localization, which as has been proved also in this analysis is an unfavorable prognostic factor for tumors localized outside the stomach. At the same time, the application of a division into low and very low recurrence risk appears to be insignificant in the context of deciding about adjuvant treatment and the planned scheme of control visits, which is also reflected in the present analysis where no statistically significant difference was found between low and very low recurrence risk both for DFS and OS. Similar results of analyses are also presented by other authors [51]. In the literature, it is difficult to find a comparison of all the current existing classifications. In the analysis performed by Yanagimoto et al. comparing NIH, AFIP, NIH according to Joensuu, AJCC and „Japanese modified NIH", where tumor rupture and/or organ infiltration were added in a group of 712 patients, the NIH classification according to Joensuu was found to be the most sensitive in predicting GIST recurrence. On the basis of this analysis, the NIH classification was selected for qualifying patients for adjuvant treatment [69]. However, this analysis did not take into consideration mutation analysis nor the nomogram according to Gold.

None of the papers published so far has attempted to include mutation analysis in the prognostic system after resection of primary GIST. The results presented in this paper unequivocally demonstrate an improvement in the prognostic accuracy of risk classification after including genotyping in addition to classical prognostic factors — this can affect the proper classification of patients with intermediate recurrence risk for adjuvant treatment with imatinib after resection of the primary tumor. The paper by Wozniak et al. encompassing multicenter clinical, pathological and molecular data of patients with localized GIST after resection collected in the database European ConticaGIST confirms the favorable prognostic significance of the exon 18 *PDGFRA* mutation and the negative effect of duplication in exon 9 of *KIT* (occurring mainly in the small intestine) and deletion 557–558 in exon 11 of *KIT*. Interestingly, according to the authors of that paper, the presence of a deletion encompassing codons 557 and/or 558 of *KIT*

was a significant, independent negative prognostic factor only for GIST originating in the stomach. The authors state that the presence of this genetic perturbation in patients with GIST derived from the stomach even with a theoretically lower risk evaluated on the basis of existing classifications should be an additional indication for adjuvant treatment with imatinib. In the presented work a statistical significance ($P < 0.001$) was also found for the presence of mutations in particular risk groups with an unfavorable indication for the high-risk group. Over 40% of all determined mutations are mutations determined in tumors which were evaluated as a high-risk group, moreover, an exon 11 deletion in *KIT* (W557–K558) is the most common mutation in the group with high recurrence risk. Because of the size of the examined group it was not possible to conduct such a detailed analysis of subgroups of patients depending on the localization as had been done by the ConticaGIST group. In the newest extended analysis by the ConticaGIST research team of a group of 1844 patients where the most common types of mutations were analyzed two prognostic classes were distinguished: class 1 (C1, good prognosis), this group included mutations of exon 11 of *KIT*, duplications, deletions with the exception of codons 557–558 and exon 18 of PDGFRA 18, whereas class 2 (C2, poor prognosis) encompassed deletions of codons 557–558 of *KIT* exon 11. When in a multivariate model the correlation between tumor localization and the mutation status were taken into consideration an unfavorable effect of tumor size > 10 cm, mitotic index 6–10 > 10/50 HPF, were observed but class 2 mutations gave a poorer prognosis only in the case of stomach tumors in contrast to GIST localized outside the stomach [83]. Even though the group analyzed in the present work is smaller and the evaluation of the mutation type was not as precise, similar conclusions can be drawn from the results — a tumor localized in the stomach and the presence of a *KIT* mutation in codons 557–588 is a poor prognostic factor and should be important in updating the current classifications of recurrence risk.

Similar conclusions were reached by the authors on an analysis of a group of 451 patients, during which multivariate Cox regression models allowed three molecular risk groups to be identified: group I had the best result and encompassed mutations of exon 12 of *PDGFRA*, a *BRAF* mutation and exon 13 *KIT* mutations; group II, with an intermediate clinical phenotype (HR = 3.06), encompassed triple-negative cases, mutations in exon 17 of *KIT*, codon D842V in exon 18 of *PDGFRA* and in exon 14 *PDGFRA*; group III had the poorest result (HR = 4.52) and encompassed mutations in exon 9 of *KIT* and exon 11 of *KIT* and in exon 18 of *PDGFRA* other than *D842V*. The mutation was a significant prognostic factor for overall survival in localized GIST not subjected to systemic treatment ($P < 0.001$): in patients with a *KIT* mutation the results were worse than in the case of a *PDGFRA* mutation or triple-negative (wild type *KIT*, *PDGFRA*, *BRAF*). This analysis underlines the prognostic effect of mutation status on the natural course of GIST and suggests that molecular prognostic grouping can supplement clinical stratification criteria when making decisions on adjuvant treatment and responds to the question whether the mutation status affects the prognosis of localized untreated GIST [84].

The Scandinavian Sarcoma Group performed an analysis aimed at determining the effects of *KIT* and *PDGFRA* mutations on recurrence-free survival (RFS) in patients with GIST treated by surgery and with imantinib adjuvant treatment. 400 patients treated by resection in whom recurrence risk was evaluated as high were included in the analysis. They were divided into 2 groups receiving imatinib for one or 3 years. The end-point was disease-free survival. The mutations were grouped according to the gene and exon. Mutations in exon 11 of *KIT* were then grouped into deletion mutations or insertion-deletion mutations, substitution mutations, insertion or duplication mutations and mutations encompassing codons 557 and/or 558. Mutations in *PDGFRA* and insertion or duplication mutations in exon 11 of the *KIT* gene were linked with a favorable DFS, whereas mutations in exon 9 of the *KIT* gene were associated with an unfavorable outcome. Patients with a deletion in exon 11 of the *KIT* gene or an insertion/deletion mutation had a better DFS when they were assigned to a 3-year group in comparison with a one year group (5-year RFS 71.0% *vs.* 41.3%; $P < 0.001$), whereas a lack of positive effects of 3-year treatment was observed in other examined mutation subgroups. Deletion mutations in exon 11 of the *KIT* gene, deletions encompassing codons 557 and/or 558 were linked with short DFS in the one year group but not in the 3-year group. The results of the analysis presented above confirm that the benefits for patients from adjuvant treatment depend on the type of occurring mutation. Patients included in the analysis in whom deletion mutations in exon 11 of the *KIT* gene were confirmed profited the most from a longer duration of adjuvant treatment with imatinib. Thus the time of adjuvant treatment with imatinib modifies the risk of GIST recurrence linked to some *KIT* mutations including deletions, which affect the codons 557 and/or 558 [85] of exon 11.

Conclusions

In this analysis the most important prognostic factors linked to disease-free survival were found to be: tumor size, mitotic index, localization outside the stomach and the presence of a mutation in exon 11 of the *KIT* gene encompassing deletion 557–558 and in exon 9 of the

same gene. The factors which significantly affect overall survival are: number of mitoses > 10/HPF ($P < 0.001$), size of primary tumor > 10 cm ($P < 0.001$), surgical margins R1 ($P = 0.004$), tumor rupture ($P < 0.001$) and age < 40 ($P = 0.045$). Over 40% of all determined mutations were determined in tumors which were classified into the high-risk group, moreover, mutations encompassing deletion (557–558) in exon 11 of the *KIT* gene are most commonly present in the group with a high recurrence risk which should cause initiation of adjuvant therapy. The presence of a mutation in exon 18 of *PDGFRA* has a favorable prognostic significance in GIST after resection of the primary tumor. All presently used classifications of evaluation of GIST recurrence risk allow a reliable evaluation of this risk. The reliability of the existing classifications of GIST recurrence after resection can be improved by including the mutation status especially in groups with intermediate recurrence risk. The use of treatment molecularly directed at the presence of a specific mutation appears to be critical not only in the context of adjuvant treatment but also in the treatment of advanced and/or metastatic disease.

Conflict of interest

PR has received honoraria for Advisory Board from Novartis, Roche, MSD, BMS, Pierre Fabre, Amgen, Blueprint Medicine, Eli Lilly, and honoraria for lectures from Novartis, Roche, MSD, Pfizer, BMS, and travel grant from Orphan Drugs.

References

1. Joensuu H, Fletcher C, Dimitrijevic S, et al. Management of malignant gastrointestinal stromal tumours. Lancet Oncol. 2002; 3(11): 655–664, doi: 10.1016/s1470-2045(02)00899-9, indexed in Pubmed: 12424067.
2. Miettinen M, Lasota J. Gastrointestinal stromal tumors — definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. Virchows Arch. 2001; 438(1): 1–12, doi: 10.1007/s004280000338, indexed in Pubmed: 11213830.
3. Joensuu H, DeMatteo RP. The management of gastrointestinal stromal tumors: a model for targeted and multidisciplinary therapy of malignancy. Annu Rev Med. 2012; 63: 247–258, doi: 10.1146/annurev-med-043010-091813, indexed in Pubmed: 22017446.
4. Kindblom LG, Remotti HE, Aldenborg F, et al. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol. 1998; 152(5): 1259–1269, indexed in Pubmed: 9588894.
5. Zhao X, Yue C. Gastrointestinal stromal tumor. J Gastrointest Oncol. 2012; 3(3): 189–208.
6. Sandvik OM, Søreide K, Kvaløy JT, et al. Epidemiology of gastrointestinal stromal tumours: single-institution experience and clinical presentation over three decades. Cancer Epidemiol. 2011; 35(6): 515–520, doi: 10.1016/j.canep.2011.03.002, indexed in Pubmed: 21489899.
7. Goettsch WG, Bos SD, Breekveldt-Postma N, et al. Incidence of gastrointestinal stromal tumours is underestimated: results of a nation-wide study. Eur J Cancer. 2005; 41(18): 2868–2872, doi: 10.1016/j.ejca.2005.09.009, indexed in Pubmed: 16293410.
8. Rubin JL, Sanon M, Taylor DCa, et al. Epidemiology, survival, and costs of localized gastrointestinal stromal tumors. Int J Gen Med. 2011; 4: 121–130, doi: 10.2147/IJGM.S16090, indexed in Pubmed: 21475624.
9. Tran T, Davila JA, El-Serag HB. The epidemiology of malignant gastrointestinal stromal tumors: an analysis of 1,458 cases from 1992 to 2000. Am J Gastroenterol. 2005; 100(1): 162–168, doi: 10.1111/j.1572-0241.2005.40709.x, indexed in Pubmed: 15654796.
10. Rutkowski P, Kulig J, Krzakowski M. Zasady postępowania diagnostyczno terapeutycznego u chorych na nowotwory podścieliskowe przewodu pokarmowego (GIST) . Nowotwory J Oncol. 2011; 61: 70–80.
11. Ruka W, Rutkowski P, Nowecki Z. Przeżycia chorych na rozsiany i nieoperacyjny GIST leczonych imatinibem – analiza etapowa Klinicznego rejestru GIST. Nowotwory J Oncol. 2005; 55: 195–199.
12. Blanke CD. Therapeutic options for gastrointestinal stroma tumors. ASCO 2003. Educational Book : 266–272.
13. Blay JY, Bonvalot S, Casali P, et al. GIST consensus meeting panelists. Consensus meeting for the management of gastrointestinal stromal tumors. Report of the GIST Consensus Conference of 20-21 March 2004, under the auspices of ESMO. Ann Oncol. 2005; 16(4): 566–578, doi: 10.1093/annonc/mdi127, indexed in Pubmed: 15781488.
14. Trent JC, Benjamin RS. New developments in gastrointestinal stromal tumor. Curr Opin Oncol. 2006; 18(4): 386–395, doi: 10.1097/01.cco.0000228747.02660.e2, indexed in Pubmed: 16721136.
15. Rutkowski P, Nowecki ZI, Michej W, et al. Risk criteria and prognostic factors for predicting recurrences after resection of primary gastrointestinal stromal tumor. Ann Surg Oncol. 2007; 14(7): 2018–2027, doi: 10.1245/s10434-007-9377-9, indexed in Pubmed: 17473953.
16. DeMatteo RP, Lewis JJ, Leung D, et al. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. Ann Surg. 2000; 231(1): 51–58, doi: 10.1097/00000658-200001000-00008, indexed in Pubmed: 10636102.
17. Samiian L, Weaver M, Velanovich V. Evaluation of gastrointestinal stromal tumors for recurrence rates and patterns of long-term follow-up. Am Surg. 2004; 70(3): 187–191, indexed in Pubmed: 15055839.
18. Learn PA, Sicklick JK, DeMatteo RP. Randomized clinical trials in gastrointestinal stromal tumors. Surg Oncol Clin N Am. 2010; 19(1): 101–113, doi: 10.1016/j.soc.2009.09.004, indexed in Pubmed: 19914562.
19. DeMatteo R, Ballman K, Antonescu C, et al. Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: a randomised, double-blind, placebo-controlled trial. Lancet. 2009; 373(9669): 1097–1104, doi: 10.1016/s0140-6736(09)60500-6.
20. Casali PG, Jost L, Reichardt P, et al. ESMO Guidelines Working Group. Gastrointestinal stromal tumors: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol. 2008; 19 Suppl 2: ii35–ii38, doi: 10.1093/annonc/mdn080, indexed in Pubmed: 18456761.
21. van Oosterom AT, Judson I, Verweij J, et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet. 2001; 358(9291): 1421–1423, doi: 10.1016/s0140-6736(01)06535-7.
22. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med. 2002; 347(7): 472–480, doi: 10.1056/NEJMoa020461, indexed in Pubmed: 12181401.
23. Blanke CD, Rankin C, Demetri GD, et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol. 2008; 26(4): 626–632, doi: 10.1200/JCO.2007.13.4452, indexed in Pubmed: 18235122.
24. Eisenberg BL, Smith KD. Adjuvant and neoadjuvant therapy for primary GIST. Cancer Chemother Pharmacol. 2011; 67 Suppl 1: S3–S8, doi: 10.1007/s00280-010-1516-5, indexed in Pubmed: 21116626.
25. Casali PG, Le Cesne A, Poveda Velasco A, et al. Time to Definitive Failure to the First Tyrosine Kinase Inhibitor in Localized GI Stromal Tumors Treated With Imatinib As an Adjuvant: A European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Intergroup Randomized Trial in Collaboration With the Australasian Gastro-Intestinal Trials Group, UNICANCER, French Sarcoma Group, Italian Sarcoma Group, and Spanish Group for Research on Sarcomas. J Clin Oncol. 2015; 33(36): 4276–4283, doi: 10.1200/JCO.2015.62.4304, indexed in Pubmed: 26573069.
26. Reichardt P, Blay JY, Boukovinas I, et al. Adjuvant therapy in primary GIST: state-of-the-art. Ann Oncol. 2012; 23(11): 2776–2781, doi: 10.1093/annonc/mds198, indexed in Pubmed: 22831984.
27. Rutkowski P, Przybył J, Zdzienicki M. Extended adjuvant therapy with imatinib in patients with gastrointestinal stromal tumors: recommendations for patient selection, risk assessment, and molecular response monitoring. Mol Diagn Ther. 2013; 17(1): 9–19, doi: 10.1007/s40291-013-0018-7, indexed in Pubmed: 23355099.
28. Essat M, Cooper K. Imatinib as adjuvant therapy for gastrointestinal stromal tumors: a systematic review. Int J Cancer. 2011; 128(9): 2202–2214, doi: 10.1002/ijc.25827, indexed in Pubmed: 21387287.

29. Miettinen M, El-Rifai W, H L Sobin L, et al. Evaluation of malignancy and prognosis of gastrointestinal stromal tumors: a review. Hum Pathol. 2002; 33(5): 478–483, doi: 10.1053/hupa.2002.124123, indexed in Pubmed: 12094372.
30. Fletcher CDM, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol. 2002; 33(5): 459–465, doi: 10.1053/hupa.2002.123545, indexed in Pubmed: 12094370.
31. Miettinen M, Makhlouf H, Sobin LH, et al. Gastrointestinal stromal tumors of the jejunum and ileum: a clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up. Am J Surg Pathol. 2006; 30(4): 477–489, doi: 10.1097/00000478-200604000-00008, indexed in Pubmed: 16625094.
32. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol. 2005; 29(1): 52–68, doi: 10.1097/01.pas.0000146010.92933.de, indexed in Pubmed: 15613856.
33. Joensuu H. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. Hum Pathol. 2008; 39(10): 1411–1419, doi: 10.1016/j.humpath.2008.06.025, indexed in Pubmed: 18774375.
34. Park CH, Kim GHa, Lee BE, et al. Two staging systems for gastrointestinal stromal tumors in the stomach: which is better? BMC Gastroenterol. 2017; 17(1): 141, doi: 10.1186/s12876-017-0705-7, indexed in Pubmed: 29207963.
35. Sobin LH, Wittekind Ch (ed). International union against cancer (UICC). 7th ed. TNM classification of malignant tumors. Wiley, New York 2010.
36. Gold JS, Gönen M, Gutiérrez A, et al. Development and validation of a prognostic nomogram for recurrence-free survival after complete surgical resection of localised primary gastrointestinal stromal tumour: a retrospective analysis. Lancet Oncol. 2009; 10(11): 1045–1052, doi: 10.1016/S1470-2045(09)70242-6, indexed in Pubmed: 19793678.
37. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998; 279(5350): 577–580, doi: 10.1126/science.279.5350.577, indexed in Pubmed: 9438854.
38. Lasota J, Jasinski M, Sarlomo-Rikala M, et al. Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. Am J Pathol. 1999; 154(1): 53–60, doi: 10.1016/S0002-9440(10)65250-9, indexed in Pubmed: 9916918.
39. Corless CL, Schroeder A, Griffith D, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol. 2005; 23(23): 5357–5364, doi: 10.1200/JCO.2005.14.068, indexed in Pubmed: 15928335.
40. Debiec-Rychter M, Wasag B, Stul M, et al. Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. J Pathol. 2004; 202(4): 430–438, doi: 10.1002/path.1546, indexed in Pubmed: 15095270.
41. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003; 21(23): 4342–4349, doi: 10.1200/JCO.2003.04.190, indexed in Pubmed: 14645423.
42. Wozniak A, Floris G, Debiec-Rychter M, et al. Implications of mutational analysis for the management of patients with gastrointestinal stromal tumors and the application of targeted therapies. Cancer Invest. 2010; 28(8): 839–848, doi: 10.3109/07357907.2010.494322, indexed in Pubmed: 20690803.
43. Debiec-Rychter M, Sciot R, Le Cesne A, et al. EORTC Soft Tissue and Bone Sarcoma Group, Italian Sarcoma Group, Australasian GastroIntestinal Trials Group. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer. 2006; 42(8): 1093–1103, doi: 10.1016/j.ejca.2006.01.030, indexed in Pubmed: 16624552.
44. Andersson J, Bümming P, Meis-Kindblom JM, et al. Gastrointestinal stromal tumors with KIT exon 11 deletions are associated with poor prognosis. Gastroenterology. 2006; 130(6): 1573–1581, doi: 10.1053/j.gastro.2006.01.043, indexed in Pubmed: 16697720.
45. Lasota J, vel Dobosz AJ, Wasag B, et al. Presence of homozygous KIT exon 11 mutations is strongly associated with malignant clinical behavior in gastrointestinal stromal tumors. Lab Invest. 2007; 87(10): 1029–1041, doi: 10.1038/labinvest.3700628, indexed in Pubmed: 17632543.
46. Lasota J, Dansonka-Mieszkowska A, Sobin LH, et al. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. Lab Invest. 2004; 84(7): 874–883, doi: 10.1038/labinvest.3700122, indexed in Pubmed: 15146165.
47. Lasota J, Miettinen M. KIT and PDGFRA mutations in gastrointestinal stromal tumors (GISTs). Semin Diagn Pathol. 2006; 23(2): 91–102, doi: 10.1053/j.semdp.2006.08.006, indexed in Pubmed: 17193822.
48. Wozniak A, Rutkowski P, Bylina E, et al. Correlation between KIT exon 11 mutation site and imatinib treatment outcome in patients with advanced gastrointestinal stromal tumors (GIST). CTOS annual meeting 2012.
49. Gronchi A. Risk stratification models and mutational analysis: keys to optimising adjuvant therapy in patients with gastrointestinal stromal tumour. Eur J Cancer. 2013; 49(4): 884–892, doi: 10.1016/j.ejca.2012.10.025, indexed in Pubmed: 23206668.
50. Rutkowski P, Bylina E, Wozniak A, et al. Validation of the Joensuu risk criteria for primary resectable gastrointestinal stromal tumour - the impact of tumour rupture on patient outcomes. Eur J Surg Oncol. 2011; 37(10): 890–896, doi: 10.1016/j.ejso.2011.06.005, indexed in Pubmed: 21737227.
51. Goh BKP, Chow PKH, Yap WM, et al. Which is the optimal risk stratification system for surgically treated localized primary GIST? Comparison of three contemporary prognostic criteria in 171 tumors and a proposal for a modified Armed Forces Institute of Pathology risk criteria. Ann Surg Oncol. 2008; 15(8): 2153–2163, doi: 10.1245/s10434-008-9969-z, indexed in Pubmed: 18546045.
52. Kim TW, Lee H, Kang YK, et al. Prognostic significance of c-kit mutation in localized gastrointestinal stromal tumors. Clin Cancer Res. 2004; 10(9): 3076–3081, doi: 10.1158/1078-0432.ccr-03-0581, indexed in Pubmed: 15131046.
53. Joensuu H, Vehtari A, Riihimäki J, et al. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. Lancet Oncol. 2012; 13(3): 265–274, doi: 10.1016/S1470-2045(11)70299-6, indexed in Pubmed: 22153892.
54. Kapoor R, Khosla D, Kumar P, et al. Five-year follow up of patients with gastrointestinal stromal tumor: recurrence-free survival by risk group. Asia Pac J Clin Oncol. 2013; 9(1): 40–46, doi: 10.1111/j.1743-7563.2011.01494.x, indexed in Pubmed: 22897235.
55. Bearzi I, Mandolesi A, Arduini F, et al. Gastrointestinal stromal tumor. A study of 158 cases: clinicopathological features and prognostic factors. Anal Quant Cytol Histol. 2006; 28(3): 137–147, indexed in Pubmed: 16786723.
56. Ning L, Zhang Df, Zhou Yb, et al. [Prognosis analysis of 247 cases of gastrointestinal stromal tumor]. Zhonghua Wei Chang Wai Ke Za Zhi. 2013; 16(3): 247–250, indexed in Pubmed: 23536345.
57. Wu XJ, Zhang RX, Chen G, et al. Prognostic factors in surgically managed gastrointestinal stromal tumours. J Int Med Res. 2012; 40(5): 1904–1911, doi: 10.1177/030006051204000531, indexed in Pubmed: 23206474.
58. Fujimoto Y, Nakanishi Y, Yoshimura K, et al. Clinicopathologic study of primary malignant gastrointestinal stromal tumor of the stomach, with special reference to prognostic factors: analysis of results in 140 surgically resected patients. Gastric Cancer. 2003; 6(1): 39–48, doi: 10.1007/s101200300005, indexed in Pubmed: 12673425.
59. Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol. 2006; 23(2): 70–83, doi: 10.1053/j.semdp.2006.09.001, indexed in Pubmed: 17193820.
60. Nishida T, Cho H, Hirota S, et al. Kinki GIST Study Group. Clinicopathological features and prognosis of primary GISTs with tumor rupture in the real world. Ann Surg Oncol. 2018; 25(7): 1961–1969, doi: 10.1245/s10434-018-6505-7, indexed in Pubmed: 29752602.
61. Hohenberger P, Ronellenfitsch U, Oladeji O, et al. Pattern of recurrence in patients with ruptured primary gastrointestinal stromal tumour. Br J Surg. 2010; 97(12): 1854–1859, doi: 10.1002/bjs.7222, indexed in Pubmed: 20730857.
62. Debiec-Rychter M, Dumez H, Judson I, et al. EORTC Soft Tissue and Bone Sarcoma Group. Use of c-KIT/PDGFRA mutational analysis to predict the clinical response to imatinib in patients with advanced gastrointestinal stromal tumours entered on phase I and II studies of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer. 2004; 40(5): 689–695, doi: 10.1016/j.ejca.2003.11.025, indexed in Pubmed: 15010069.
63. Heinrich MC, Corless CL, Blanke CD, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol. 2006; 24(29): 4764–4774, doi: 10.1200/JCO.2006.06.2265, indexed in Pubmed: 16954519.
64. Heinrich MC, Owzar K, Corless CL, et al. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. J Clin Oncol. 2008; 26(33): 5360–5367, doi: 10.1200/JCO.2008.17.4284, indexed in Pubmed: 18955451.
65. Wozniak A, Rutkowski P, Schöffski P, et al. Tumor genotype is an independent prognostic factor in primary gastrointestinal stromal tumors of gastric origin: a european multicenter analysis based on ConticaGIST. Clin Cancer Res. 2014; 20(23): 6105–6116, doi: 10.1158/1078-0432.CCR-14-1677, indexed in Pubmed: 25294914.

66. Bachet JB, Hostein I, Le Cesne A, et al. Prognosis and predictive value of KIT exon 11 deletion in GISTs. Br J Cancer. 2009; 101(1): 7–11, doi: 10.1038/sj.bjc.6605117, indexed in Pubmed: 19536093.
67. Wang M, Xu J, Zhao W, et al. Prognostic value of mutational characteristics in gastrointestinal stromal tumors: a single-center experience in 275 cases. Med Oncol. 2014; 31(1): 819, doi: 10.1007/s12032-013-0819-x, indexed in Pubmed: 24338275.
68. Corless C, Ballman K, Antonescu C, et al. Pathologic and molecular features correlate with long-term outcome after adjuvant therapy of resected primary GI stromal tumor: the ACOSOG Z9001 trial. J Clin Oncol. 2014; 32(15): 1563–1570, doi: 10.1200/jco.2013.51.2046.
69. Yanagimoto Y, Takahashi T, Muguruma K, et al. Re-appraisal of risk classifications for primary gastrointestinal stromal tumors (GISTs) after complete resection: indications for adjuvant therapy. Gastric Cancer. 2015; 18(2): 426–433, doi: 10.1007/s10120-014-0386-7, indexed in Pubmed: 24853473.
70. Wozniak A, Rutkowski P, Piskorz A, et al. Polish Clinical GIST Registry. Prognostic value of KIT/PDGFRA mutations in gastrointestinal stromal tumours (GIST): Polish Clinical GIST Registry experience. Ann Oncol. 2012; 23(2): 353–360, doi: 10.1093/annonc/mdr127, indexed in Pubmed: 21527588.
71. Taniguchi M, Nishida T, Hirota S, et al. Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. Cancer Res. 1999; 59(17): 4297–4300, indexed in Pubmed: 10485475.
72. Singer S, Rubin BP, Lux ML, et al. Prognostic value of KIT mutation type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. J Clin Oncol. 2002; 20(18): 3898–3905, doi: 10.1200/JCO.2002.03.095, indexed in Pubmed: 12228211.
73. Martín J, Poveda A, Llombart-Bosch A, et al. Spanish Group for Sarcoma Research. Deletions affecting codons 557-558 of the c-KIT gene indicate a poor prognosis in patients with completely resected gastrointestinal stromal tumors: a study by the Spanish Group for Sarcoma Research (GEIS). J Clin Oncol. 2005; 23(25): 6190–6198, doi: 10.1200/JCO.2005.19.554, indexed in Pubmed: 16135486.
74. Dematteo RP, Gold JS, Saran L, et al. Tumor mitotic rate, size, and location independently predict recurrence after resection of primary gastrointestinal stromal tumor (GIST). Cancer. 2008; 112(3): 608–615, doi: 10.1002/cncr.23199, indexed in Pubmed: 18076015.
75. Ma Y, Longley BJ, Wang X, et al. Clustering of activating mutations in c-KIT's juxtamembrane coding region in canine mast cell neoplasms. J Invest Dermatol. 1999; 112(2): 165–170, doi: 10.1046/j.1523-1747.1999.00488.x, indexed in Pubmed: 9989791.
76. Zong L, Chen P. Prognostic value of KIT/PDGFRA mutations in gastrointestinal stromal tumors: a meta-analysis. World J Surg Oncol. 2014; 12: 71, doi: 10.1186/1477-7819-12-71, indexed in Pubmed: 24674052.
77. Joensuu H, Rutkowski P, Nishida T, et al. KIT and PDGFRA mutations and the risk of GI stromal tumor recurrence. J Clin Oncol. 2015; 33(6): 634–642, doi: 10.1200/JCO.2014.57.4970, indexed in Pubmed: 25605837.
78. Künstlinger H, Binot E, Merkelbach-Bruse S, et al. High-resolution melting analysis is a sensitive diagnostic tool to detect imatinib-resistant and imatinib-sensitive PDGFRA exon 18 mutations in gastrointestinal stromal tumors. Hum Pathol. 2014; 45(3): 573–582, doi: 10.1016/j.humpath.2013.10.025, indexed in Pubmed: 24444465.
79. Wardelmann E, Thomas N, Merkelbach-Bruse S, et al. Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. Lancet Oncol. 2005; 6(4): 249–251, doi: 10.1016/S1470-2045(05)70097-8, indexed in Pubmed: 15811621.
80. Schmieder M, Henne-Bruns D, Mayer B, et al. Comparison of different risk classification systems in 558 patients with gastrointestinal stromal tumors after R0-resection. Front Pharmacol. 2016; 7: 504, doi: 10.3389/fphar.2016.00504, indexed in Pubmed: 28082898.
81. Chen T, Qiou H, Feng X, et al. Comparison of modified NIH and AFIP risk-stratification criteria for gastrointestinal stromal tumors: A multicenter retrospective study. Zhonghua Wei Chang Wai Ke Za Zhi. 2017; 20(9): 1020–1024, indexed in Pubmed: 28900993.
82. Chen T, Xu L, Ye L, et al. A new nomogram for recurrence-free survival prediction of gastrointestinal stromal tumors: Comparison with current risk classification methods. Eur J Surg Oncol. 2019; 45(6): 1109–1114, doi: 10.1016/j.ejso.2018.12.014, indexed in Pubmed: 30594406.
83. Casali PG, Abecassis N, Bauer S, et al. Gastrointestinal stromal tumours: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018.
84. Rossi S, Gasparotto D, Miceli R, et al. KIT, PDGFRA, and BRAF mutational spectrum impacts on the natural history of imatinib-naive localized GIST: a population-based study. Am J Surg Pathol. 2015; 39(7): 922–930, doi: 10.1097/PAS.0000000000000418, indexed in Pubmed: 25970686.
85. Joensuu H, Wardelmann E, Sihto H, et al. Effect of KIT and PDGFRA Mutations on Survival in Patients With Gastrointestinal Stromal Tumors Treated With Adjuvant Imatinib: An Exploratory Analysis of a Randomized Clinical Trial. JAMA Oncol. 2017; 3(5): 602–609, doi: 10.1001/jamaoncol.2016.5751, indexed in Pubmed: 28334365.
86. Nilsson B, Bümming P, Meis-Kindblom JM, et al. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era — a population-based study in western Sweden. Cancer. 2005; 103(4): 821–829, doi: 10.1002/cncr.20862, indexed in Pubmed: 15648083.
87. Choi H, Charnsangavej C, de Castro Faria S, et al. CT evaluation of the response of gastrointestinal stromal tumors after imatinib mesylate treatment: a quantitative analysis correlated with FDG PET findings. AJR Am J Roentgenol. 2004; 183(6): 1619–1628, doi: 10.2214/ajr.183.6.01831619, indexed in Pubmed: 15547201.
88. Dagher R, Cohen M, Williams G, et al. Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clin Cancer Res. 2002; 8(10): 3034–3038, indexed in Pubmed: 12374669.
89. Le Cesne A, Van Glabbeke M, Verweij J, et al. Absence of progression as assessed by response evaluation criteria in solid tumors predicts survival in advanced GI stromal tumors treated with imatinib mesylate: the intergroup EORTC-ISG-AGITG phase III trial. J Clin Oncol. 2009; 27(24): 3969–3974, doi: 10.1200/JCO.2008.21.3330, indexed in Pubmed: 19620483.

VIA MEDICA

ORIGINAL ARTICLE

Bożena Cybulska-Stopa[1], Marcin Ziętek[2, 3], Grażyna Kamińska-Winciorek[4], Anna M. Czarnecka[5, 6], Karolina Piejko[1], Łukasz Galus[7, 8], Barbara Ziółkowska[9], Stanisław Kieszko[10], Natasza Kempa-Kamińska[2], Jacek Calik[2], Tomasz Kubiatowski[10], Rafał Suwiński[9], Jacek Mackiewicz[7, 11], Piotr Rutkowski[5]

[1]Clinical Oncology Department, Maria Sklodowska-Curie National Research Institute of Oncology, Cracow Branch, Poland
[2]Wrocław Comprehensive Cancer Center, Wroclaw, Poland
[3]Department of Oncological Surgery, Wroclaw Medical University, Wroclaw, Poland
[4]Department of Bone Marrow Transplantation and Hematology-Oncology, Skin Cancer and Melanoma Team, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Poland
[5]Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[6]Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
[7]Department of Medical and Experimental Oncology, Heliodor Swiecicki Clinical Hospital, University of Medical Sciences, Poznan, Poland
[8]Chemotherapy Department, Greater Poland Cancer Centre, Poznan, Poland
[9]Radiotherapy and Chemotherapy Clinic and Teaching Hospital, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Poland
[10]Saint Jan of Dukla Oncology Centre of the Lublin Region, Lublin, Poland
[11]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland

Indirect comparison of treating patients with advanced/metastatic melanoma with nivolumab or pembrolizumab — multicenter analysis

Address for correspondence:
Dr n. med. Bożena Cybulska-Stopa
Narodowy Instytut Onkologii
im. Marii Skłodowskiej-Curie
— Państwowy Instytut Badawczy,
Oddział w Krakowie
ul. Garncarska 11, 31–115 Kraków
Phone: +48 12 63 48 260
e-mail: bcybulskastopa@vp.pl

Oncology in Clinical Practice
2020, Vol. 16, No. 5, 295–300
DOI: 10.5603/OCP.2020.0037
Translation: prof. Ewa Bartnik

ISSN 2450–1654

ABSTRACT

Introduction. The development of a new class of drug — checkpoint inhibitors has changed the prognosis of cancer patients. A particular class of drugs are antibodies against the programmed cell death type 1 receptor/ligand of the programmed cell death type 1 receptor (nivolumab and pembrolizumab). There are, however, no trials with a random selection of the patients which directly compare nivolumab and pembrolizumab. Because of the development of immunotherapy and many new drugs registered as anti-PD-1, it is important to determine whether there are differences in respect to effectiveness and safety in using nivolumab and pembrolizumab.

Material and method. 499 patients with non-resectable or metastatic melanoma treated in the years 2016–2019 in five oncological reference centers in Poland (Cracow, Gliwice, Lublin, Poznań, Wrocław) were included in the analysis. The criterion for inclusion in the analysis was first-line treatment with anti-PD-1 (nivolumab or pembrolizumab).

Results. Median OS and PFS in the whole analyzed group were 19.9 and 7.9 months, respectively. Estimated median OS and PFS were 20.1 and 18.1 months and 8.5 and 6.0 months for nivolumab and pembrolizumab, respectively. No statistically significant difference was observed in median OS and PFS in the group of patients receiving nivolumab and pembrolizumab (respectively P = 0.6291 [HR = 1.06; CI 95% 0.8–1.4] and P = 0.0956 [HR = 1.20; CI 95% 0.97–1.48]). The percentage of grade G3 or/and G4 irAEs was similar in both groups treated with nivolumab or pembrolizumab, 5.8 and 5.2%, respectively.

Conclusions: No differences in the range of OS, PFS and ORR was observed between therapy with nivolumab and pembrolizumab in previously untreated patients with advanced/metastatic melanoma. No differences were found in the frequency of irAEs of grade G3 or G4. The treatment with a specific preparation should be based on the preferences of the patient and the clinician.

Key words: melanoma, immunotherapy, antiPD-1 therapy, pembrolizumab, nivolumab

Oncol Clin Pract 2020; 16, 5: 295–300

Introduction

In recent years the treatment of patients with diagnosed melanoma has changed greatly due to the development of a new class of drugs: antibodies against the programmed cell death type 1 receptor/ligand of the programmed cell death type 1 receptor (anti-PD-1/anti-PD-L1, anti-programmed death receptor-1/ligand-1). The mechanism of action of anti-PD-1 antibodies, which include nivolumab and pembrolizumab, is based on binding of the drug to the PD-1 receptor and blocking interactions with the PD-L1 and PD-L2 ligands, which in turn activates T lymphocytes to an immunological response against neoplastic cells [1–3]. Nivolumab has the structure of a human monoclonal IgG4 antibody with a half-life of about 26 days and shows specificity for the PD-1 receptor [2]. Pembrolizumab is a humanized monoclonal IgG4 antibody with a half-life of about 27 days [3]. Another difference is the dosing of the two drugs. Nivolumab is currently used at a constant dose of 240 mg every 2 weeks or 480 mg every 4 weeks, whereas pembrolizumab is given in a dose of 200 mg every 3 weeks or 400 mg every 6 weeks [4]. The comparison (indirect) of the results of trials with randomization in patients with melanoma treated with nivolumab or pembrolizumab indicates similar effectiveness of both these drugs. However, in patients with metastatic non-small cell lung carcinoma (NSCLC) differences in the effectiveness of nivolumab and pembrolizumab depending on PD-L1 expression were observed [5, 6], which could suggest some differences in the action of the two drugs. There are, however, no trials with a randomized selection of patients which directly compare nivolumab and pembrolizumab. Because of the development of immunotherapy and many new registrations for anti-PD-1 drugs, it is important to determine whether there are differences in the range of effectiveness and safety in using nivolumab and pembrolizumab.

According to the best knowledge of the authors, this is the first and largest analysis comparing the effectiveness and toxicity of nivolumab and pembrolizumab in everyday practice.

Material and method

499 patients with non-resectable or metastatic melanoma treated in the years 2016–2019 in five oncological reference centers in Poland (Cracow, Gliwice, Lublin, Poznań, Wrocław) were included in the analysis. The criterion for inclusion in the analysis was first-line treatment with anti-PD-1 (nivolumab or pembrolizumab). All patients were treated according to the indications of the national drug program: treating skin or mucous membrane melanoma. A detailed description in Polish is available on https://www.gov.pl/web/zdrowie/choroby-onkologiczne [7]. All patients received nivolumab or pembrolizumab, in doses in agreement with the drug characteristics currently in force and the guidelines of the drug program. In all analyzed patients data were collected on age, sex, localization of the primary lesion, degree of disease progression according to TNM (AJCC 8th edition), localization and number of metastases, level of lactate dehydrogenase (LDH), ECOG (Eastern Cooperative Oncology Group) performance status and type of therapy used in first-line and second-line treatment. Information on the degree of disease progression, localization and number of metastases, level of lactate dehydrogenase (LDH) and ECOG performance status [8] were collected at the moment of initiating first-ine systemic treatment. No data on PD-L1 expression were collected as the assays were not available. All patients were treated until disease progression, unacceptable therapy toxicity, death or withdrawal of consent for treatment. The first radiological evaluation was performed after 12 weeks from initiating anti-PD-1 therapy, and then the radiological evaluations of the patients were performed every 3 months according to the requirements of the drug program. Evaluation of the response to treatment was performed according to z RECIST 1.1 criteria [9], according to the requirements of the National Melanoma Treatment Program [7]. Data on the safety of the applied treatment were also collected.

Statistical analysis

The statistical analysis encompassed comparison of nivolumab and pembrolizumab therapy. Endpoints encompassed the comparison of median time to disease progression (PFS, progression-free survival), overall survival (OS) and evaluation of indices of objective response to therapy (ORR, overall response rate) and disease control rate (DCR) defined by the RECIST 1.1. criteria. PFS or OS were evaluated from the beginning of nivolumab or pembrolizumab therapy until disease progression according to RECIST, death or last documented contact. The Kaplan-Meier method was used for estimating PFS and OS with a 95% confidence interval (CI), and the survival curves were analyzed using log-rank. To determine in the multivariate model the significance of the effects of the prognostic variables on PFS and OS at the moment of initiation of anti-PD-1 therapy the Cox proportional hazard model was used. Differences were considered to be statistically significant if the P value was < 0.05. All statistical analyses were performed using STATISTICA 12.

Results

General characteristics of the analyzed group

In the group of 499 patients receiving anti-PD-1 therapy 308 (62%), patients received nivolumab and 191 (38%) pembrolizumab. No statistically signifi-

Table 1. Characteristics of the analyzed group

Variable	Category	Nivolumab n = 308	Pembrolizumab n = 191	P-value
Age (years)	Median (range)	66 (23–93)	68 (27–92)	
	< 65 years	132 (43%)	76 (40%)	0.5
Gender	Male	184 (60%)	111 (58%)	0.72
	Female	124 (40%)	80 (42%)	
BRAF mutation	No mutation	244 (80%)	156 (83%)	0.34
	Mutated	61 (20%)	31 (17%)	
Location of the primary tumor	Skin	272 (89%)	175 (92%)	0.34
	Mucosal	20 (7%)	10 (5%)	
	Unknown	13 (4%)	5 (3%)	
ECOG	0	117 (38%)	77 (40%)	0.57
	1	188 (61%)	111 (58%)	
	2	3 (1%)	2 (2%)	
LDH level	Normal	189 (62%)	108 (56%)	0.43
	> normal	118 (38%)	82 (44%)	
Brain metastasis	No	257 (83%)	155 (81%)	0.51
	Yes	51 (17%)	36 (19%)	
TNM stage (AJCC 8th edition)	III	22 (7%)	6 (3%)	0.25
	M1a	62 (20%)	33 (17%)	
	M1b	65 (21%)	38 (20%)	
	M1c	108 (35%)	78 (41%)	
	M1d	51 (17%)	36 (19%)	
Number of metastatic sites	< 2	85 (28%)	49 (26%)	0.63
	≥ 2	223 (72%)	142 (74%)	

AJCC — American Joint Committee on Cancer; ECOG — Eastern Cooperative Oncology Group; LDH — lactate dehydrogenase; TNM — tumor, node, metastasis

cant differences between the two groups were found in the general characteristics of patients. In the group receiving pembrolizumab there were slightly more patients with metastases to the brain (19% *vs.* 17%) and elevated LDH levels (44% *vs*. 38%) A detailed characterization of the analyzed groups is presented in Table 1.

Treatment results depending on the used therapy

Estimated median OS and PFS in the whole analyzed group were 19.9 and 7.9 months, respectively. Estimated median OS was 20.1 and 18.1 months for nivolumab and pembrolizumab, respectively. No statistically significant difference in median OS was observed between the groups of patients receiving nivolumab and pembrolizumab (P = 0.6291, HR = 1.06; Cl 95% 0.8–1.4) (Fig. 1). The estimated median PFS was 8.5 and 6.0 months for nivolumab and pembrolizumab, respectively and no statistically significant difference in median PFS was found between the groups of patients receiving nivolumab and pembrolizumab (P = 0.0956, HR = 1.20; Cl 95% 0.97–1.48) (Fig. 2). 1-, 2- and 3-year survivals were similar in both groups. No differences were observed in responses to treatment. Detailed data on the results of treatment are presented in Table 2.

Adverse effects

A slightly higher percentage of patients with immunological complications (irAE) were noted in the group of patients receiving nivolumab (25% *vs.* 21.6%). However, the percentage of grade 3 and/or 4 irAEs was similar in both groups treated with nivolumab or pembrolizumab, 5.8 and 5.2%, respectively. In the group receiving pembrolizumab skin, hematological and kidney complications were more common. In the group with nivolumab liver, lung and neurological complications were more common. Endocrinological complications concerning thyroid function were different in both groups of patients. In the group receiving nivolumab, there was more hyperthyroidism, but in 60% (12 patients) of cases, the hyperthyroidism changed into hypothyroidism. In the group receiving pembrolizumab one serious G3 complication related to the drug was observed. No irAE related death was observed in either of the groups. The irAEs are presented in detail in Table 3.

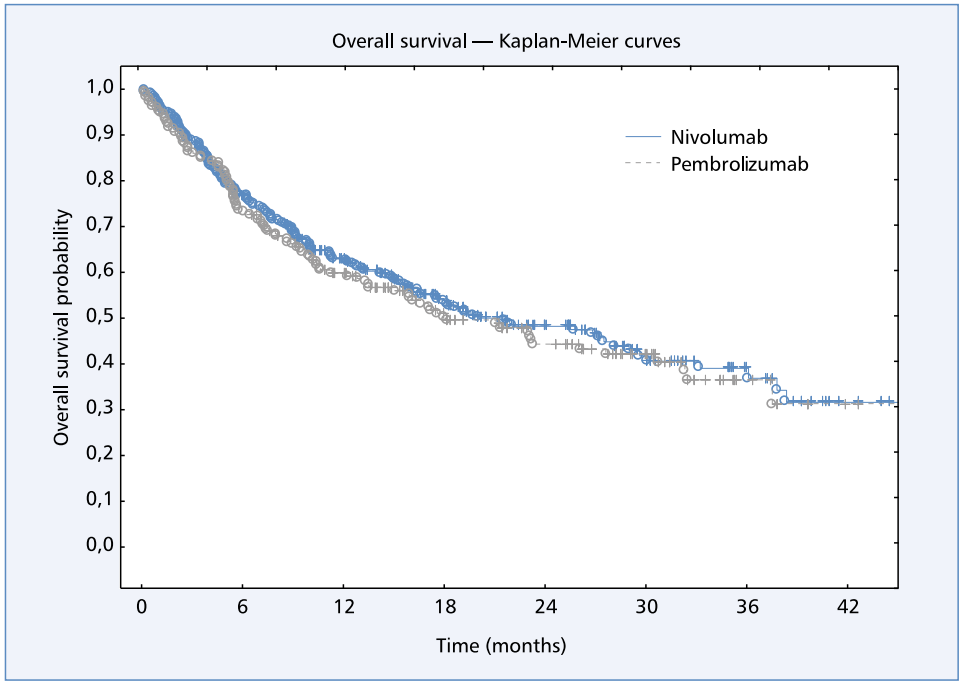

Figure 1. Overall survival depending on the used anti-PD-1 therapy

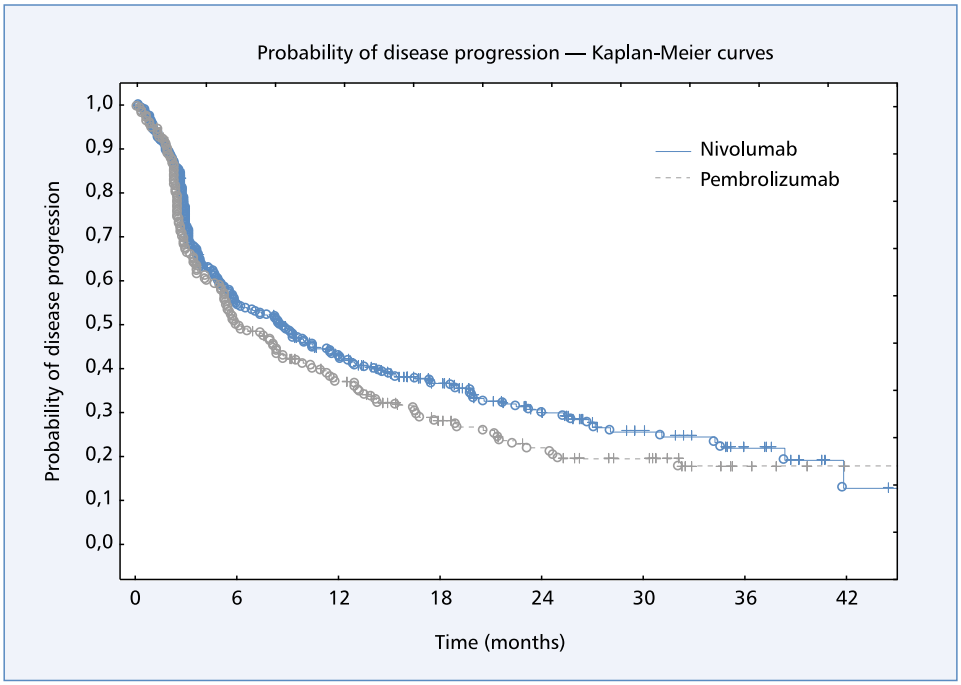

Figure 2. Time to disease progression depending on the anti-PD-1 therapy used

Discussion

In the presented retrospective analysis encompassing treatment results in everyday medical practice (real-world data), no differences were observed in OS and PFS, nor in responses to treatment between nivolumab and pembrolizumab therapy. Also in the analysis by Moser et al., where the results were compared in everyday medical practice of 486 patients treated with pembrolizumab and 402 treated with nivolumab no differences in OS were found between nivolumab and pembrolizumab [10]. Median OS in the whole analyzed

Table 2. Effectiveness of therapy depending on the used drug

Factors		Nivolumab n = 308	Pembrolizumab n = 191
Median OS (months)		20.1	18.1
Estimated overall survival	1 year OS	62%	59%
	2 years OS	48%	44%
	3 years OS	36%	36%
Median PFS (months)		8.5	6.0
Best overall tumor response	CR	6%	5%
	PR	27%	31%
	SD	30%	27%
	PD	37%	37%
	ORR (CR+PR)	33%	36%
	DCR (CR+PR+SD)	63%	63%
Duration of treatment	Median (range) months	6.3 (0.1–41)	5.1 (0.1–43)
irAEs	Patients with irAEs	77 (25%)	41 (21.5%)
The next line of treatment	All	111 (36%)	75 (39%)
	Immunotherapy	80 (72%)	58 (77%)
	Targeted therapy	19 (17%)	12 (16%)
	Chemotherapy	11 (10%)	5 (7%)
	Other	1 (1%)	0

CR — complete response; DCR — disease control rate; irAEs — immune related adverse events; NE — not evaluated; OS — overall survival; ORR — objective response rate; PD — progression disease; PFS — progression free survival; PR — partial response; SD — stable disease

Table 3. Immunological complications during anti-PD-1 therapy

	Nivolumab, n = 308		Pembrolizumab, n = 191	
irAEs	**All grade, n (%)**	**G3 or G4, n (%)**	**All grade, n (%)**	**G3 or G4, n (%)**
Patients with irAEs	77 (25%)		41 (21.5%)	
Overall irAEs	121 (39%)	18 (5.8%)	63 (33%)	10 (5.2%)
Dermatitis (rash)	8 (2.6%)	1 (0.3%)	10 (5.2%)	2 (1%)
Vitiligo	3 (1%)	0	3 (1.6%)	0
Diarrhea/colitis	8 (2.6%)	3 (1%)	4 (2.1%)	1 (0.5%)
Hepatitis or AST/ALT elevation	29 (9.4%)	9 (3%)	10 (5.2%)	2 (1%)
Hypothyroidism	24 (7.8%)	0	16 (8.4%)	0
Hyperthyroidism	21 (6.8%)	0	1 (0.5%)	0
Hypopituitarism/hypophysitis	1 (0.3%)	0	0	0
Pneumonitis	8 (2.6%)	1 (0.3%)	1 (0.5%)	0
Neurological/neuropathy	1 (0.3%)	1 (0.3%)	0	0
Hematological (neutropenia, anemia)	0	0	5 (2.6%)	3 (1.6%)
Cardiological	2 (0.6%)	0	1 (0.5%)	0
Arthralgia/myalgia	3 (1%)	0	2 (1%)	0
Nephritis	2 (0.6%)	0	3 (1.6%)	0
Other	11 (3.6%)	4 (1.3%)	7 (3.7%)	2 (1%)

ALT — alanine aminotransferase; AST — aspartate aminotransferase; irAEs — immune related adverse events

group was 19.9 months and was similar to the median OS in the analysis by Moser et al. [10] (22.6 months). Median OS in both analyses is shorter than in clinical trials for nivolumab and pembrolizumab, 37.5 and 32.7 months, respectively [11–14]. This is probably due to the fact that in our trial almost 20% of patients has metastases to the central nervous system (CNS), which is a known poor prognostic factor. In the Checkmate-066 trial patients with metastases to the brain constituted only 3.6% and in the Keynote-006 trial 9% [11–14]. It should also be noted that the criteria for inclusion in drug programs require additional examinations in patients with metastases to the CNS, which significantly delays the initiation of anti-PD-1 therapy.

In our analysis, PFS and response to treatment were also evaluated, which was not done in the work of Moser et al. because of the lack of data. Median PFS and the number of responses were close to those presented in clinical trials of nivolumab (Checkmate-066) and pembrolizumab (Keynote-006), in which they were 5.1 and 8.4 months, respectively, and the number of objective responses (ORR) 40 and 33–34%, respectively [11, 12].

One of the more important aspects of our analysis is the analysis of immunological toxicity of nivolumab and pembrolizumab therapy. The number of irAEs is smaller than in clinical trials, which may be due to the retrospective character of the presented results and the lack of reporting of especially grade 1 irAEs in everyday clinical practice. It should be also pointed out that in our trial there are slightly fewer G3 and G4 adverse effects than in clinical trials. No irAE related deaths were observed. This may be related to the increasingly common use of anti-PD-1 in clinical practice and thus better management of immunological toxicities, the so-called learning curve. However, the number of grade G3 and G4 irAEs was similar in the group with nivolumab and with pembrolizumab. However, it should be observed that nivolumab and pembrolizumab have a slightly different toxicity profile — irAEs. This is particularly clear in thyroid-associated endocrinological perturbations. It is not clear why hyperthyroidism, which in most cases became hypothyroidism was more frequent in the nivolumab group. This could be linked to the size of both analyzed groups (the group with nivolumab was much larger). Further observations and trials are certainly necessary.

Conclusions

No differences were observed in OS, PFS and ORR between nivolumab and pembrolizumab treatment in previously untreated patients with advanced/metastatic melanoma. No differences were observed in the frequency of grade G3 or G4 irAEs. The choice of treatment with a specific preparation should be based on the preferences of the patient and the clinician.

Conflict of interest

PR — honoraria from BMS, MSD, Novartis, Amgen, Pierre Fabre, Sanofi, Merck and Blueprint Medicines for lectures and Advisory Boards outside of the scope of the study. JM — grants and consultancies — BMS, MSD. Fees and honoraria: AMC, TK, JC — Bristol-Myers Squibb, Novartis, Roche, Merck; BCS — BMS, Novartis, Roche, Pierre Fabre, MSD; RS — BMS, MSD, Astellas Pharma; JM — BMS, GlaxoSmithKline, Roche, MSD, Novartis, Pierre Fabre.

References

1. Wang C, Thudium KB, Han M, et al. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res. 2014; 2(9): 846–856, doi: 10.1158/2326-6066.CIR-14-0040, indexed in Pubmed: 24872026.
2. Guo L, Zhang H, Chen B. Nivolumab as programmed death-1 (PD-1) inhibitor for targeted immunotherapy in tumor. J Cancer. 2017; 8(3): 410–416, doi: 10.7150/jca.17144, indexed in Pubmed: 28261342.
3. Longoria TC, Tewari KS. Evaluation of the pharmacokinetics and metabolism of pembrolizumab in the treatment of melanoma. Expert Opin Drug Metab Toxicol. 2016; 12(10): 1247–1253, doi: 10.1080/17425255.2016.1216976, indexed in Pubmed: 27485741.
4. Rutkowski P, Wysocki PJ, Nasierowska-Guttmejer A, et al. Cutaneous melanomas. Oncol Clin Pract. 2020; 16(4): 163–182, doi: 10.5603/OCP2020.0021.
5. Mok TSK, Wu YL, Kudaba I, et al. KEYNOTE-042 Investigators. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019; 393(10183): 1819–1830, doi: 10.1016/S0140-6736(18)32409-7, indexed in Pubmed: 30955977.
6. Carbone DP, Reck M, Paz-Ares L, et al. CheckMate 026 Investigators. First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer. N Engl J Med. 2017; 376(25): 2415–2426, doi: 10.1056/NEJMoa1613493, indexed in Pubmed: 28636851.
7. https://www.gov.pl/web/zdrowie/choroby-onkologiczne (last access: 26th Jun 2020).
8. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45(2): 228–247, doi: 10.1016/j.ejca.2008.10.026, indexed in Pubmed: 19097774.
9. Common Terminology Criteria for Adverse Events version 4.0) published: May 28, 2009 U.S. Department of Health and Human Services National Institutes of Health, National Cancer Institute.
10. Moser JC, Wei G, Colonna SV, et al. Comparative-effectiveness of pembrolizumab vs. nivolumab for patients with metastatic melanoma. Acta Oncol. 2020; 59(4): 434–437, doi: 10.1080/0284186X.2020.1712473, indexed in Pubmed: 31920132.
11. Robert C, Schachter J, Long GV, et al. KEYNOTE-006 investigators. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015; 372(26): 2521–2532, doi: 10.1056/NEJMoa1503093, indexed in Pubmed: 25891173.
12. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015; 372(4): 320–330, doi: 10.1056/NEJMoa1412082, indexed in Pubmed: 25399552.
13. Ascierto PA, Long GV, Robert C, et al. Survival outcomes in patients with previously untreated BRAF wild-type advanced melanoma treated with nivolumab therapy: three-year follow-up of a randomized phase 3 trial. JAMA Oncol. 2019; 5(2): 187–194, doi: 10.1001/jamaoncol.2018.4514, indexed in Pubmed: 30422243.
14. Robert C, Schachter J, Long G, et al. Abstract CT188: 5-year survival and other long-term outcomes from KEYNOTE-006 study of pembrolizumab (pembro) for ipilimumab (ipi)-naive advanced melanoma. Clinical Trials (13 Supplement). 2019, doi: 10.1158/1538-7445.am2019-ct188.