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EXPERTS' OPINION

ORIGINAL ARTICLES

REVIEW ARTICLES

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Expert opinion on adjuvant treatment with osimertinib in patients with non-small cell lung carcinoma after radical tumor resection

Introduction

Lung cancer is the most common cause of cancer-related deaths in Poland, accounting for approximately 18% of deaths in women and 26% in men [1]. Non-small cell lung cancer (NSCLC) accounts for 80–85% of all primary lung cancers. Improving the effectiveness of treatment of NSCLC patients is important to reduce the total absolute number of deaths due to malignancies. The diagnosis of NSCLC in its early stages enables radical resection, which is the most effective treatment method. This is reflected in the 5-year survival rates, which for stages I–III are: I 73–90%, II 56–65%, and III 12–41% [2]. Surgical treatment achieves significantly better results than other methods, but it is not curative in all patients. The reason is the appearance of local recurrences and distant metastases, the frequency of which (25–50%) depends on cancer stage and other factors [3]. The above data justify the use of adjuvant treatment in NSCLC patients undergoing complete resection. Until recently, systemic adjuvant treatment consisted solely of chemotherapy with platinum-based regimens (3–4 cycles). The value of adjuvant chemotherapy was confirmed by the results of the LACE (lung adjuvant cisplatin evaluation) meta-analysis. The use of chemotherapy was associated with a reduction in the risk of death by 11% and an increase in the probability of 5-year survival by 5.3% [4]. Adjuvant postoperative chemotherapy is currently recommended in patients after resection of NSCLC in stages II and III, while adjuvant radiotherapy is only recommended in the case of incomplete tumor resection [5].

Breakthrough discoveries of the last two decades including the identification of specific molecular targets in NSCLC cells, evaluation of tumor cell expression of molecules that block anticancer T-cell activity, and introduction of targeted drugs significantly improved the prognosis of patients with locally advanced (Stage IIIB) and disseminated (Stage IV) NSCLC. These drugs are more effective and associated with a lower risk of side effects than chemotherapy. One of the most important groups is the next generation of tyrosine kinase inhibitors (TKI) targeting the epidermal growth factor receptor (EGFR) [6]. Demonstrating the effectiveness of

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TKI-EGFR in patients with advanced NSCLC naturally raised the question of the possibility of using these drugs in adjuvant treatment in patients with stage I–IIIA undergoing radical surgical resection. To clarify this issue, a multicenter Phase III study was planned and conducted to evaluate the efficacy of adjuvant treatment with osimertinib (ADAURA, Adjuvant Therapy for EGFR Mutant Early-Stage NSCLC). The highest quality of the study (placebo-controlled, randomized, double-blinded) allowed for obtaining reliable and convincing results that are extremely important for clinical practice. In the group of patients with stage II–IIIA, in whom the presence of an activating *EGFR* gene mutation was confirmed in the postoperative material, treatment with osimertinib was associated with a significant increase in the percentage of patients who survived 24 months without recurrence of the disease (osimertinib 90% *versus* placebo 44%) [7]. A similar result was obtained for a wider group with stage IB–IIIA (89% and 49%, respectively) [8].

The unequivocal results of the ADAURA study justified a positive opinion of the Food and Drug Administration (FDA) issued in December 2020 regarding the use of osimertinib in the adjuvant treatment of patients with NSCLC with adenocarcinoma morphology or NSCLC with a predominant adenocarcinoma component undergoing radical resection, with confirmed *EGFR* gene mutations. In April 2021, the European Medicines Agency (EMA) also issued a positive decision.

From January 1, 2023, the National Health Fund introduced reimbursement of osimertinib treatment in the above indication under therapeutic drug program B.6. "Treatment of patients with lung cancer and pleural mesothelioma".

This document presents four key aspects for obtaining a positive therapeutic effect after adjuvant treatment with osimertinib in patients with lung adenocarcinoma or NSCLC with a predominant adenocarcinoma component undergoing surgical resection, such as:

1) surgical treatment and securing postoperative material for further examinations;
2) pathomorphological assessment of postoperative material;
3) identification of activating mutations in the *EGFR* gene;
4) recommendations for adjuvant treatment with osimertinib in the postoperative period.

Surgical treatment of patients with NSCLC. Securing surgical material for further evaluation

Resection of lung parenchyma is the treatment of choice in NSCLC patients in stages I and II and selected patients in stage III, in whom the functional state of the respiratory and cardiovascular systems allows for radical surgery. The recommended type of surgery for patients in stages I–IIIA who are eligible for surgical treatment is lobectomy.

A smaller resection than a lobectomy is indicated only in patients with limited respiratory reserves or with other comorbidities that do not allow for a more extensive procedure. According to the recommendations of the International Association for the Study of Lung Cancer (IASLC), each anatomical resection should be supplemented with the resection of appropriate hilar and mediastinal lymph node stations [9]. The impact of the extent of lymphadenectomy on the results of surgical treatment has not been definitively established, but a more extensive excision of the lymphatic system allows for a more complete postoperative tumor staging and facilitates qualification for adjuvant treatment [9, 10].

Regional lymph nodes for lung cancer include 14 nodal stations located above the diaphragm, in the chest, as well as subscalene and supraclavicular nodes.

The postoperative material should contain at least 6 lymph nodes, including 3 mediastinal (N2) lymph nodes, among them bifurcation (subcarinal) lymph nodes, and 3 hilar and intrapulmonary (N1) lymph nodes.

The required number of removed nodes is related to the assessment of the radicality of the resection.

The main principles of lung cancer radical resection are presented in Table 1.

Principles of sending postoperative material for pathomorphological examination

Postoperative material sent to the Pathomorphological Diagnostics Unit (PDU) requires appropriate protection enabling good fixation of the material and a properly completed referral form.

Table 1. Principles of radical resection of lung cancer

Principles of radical resection of lung cancer
Tumor resection (lobectomy, bilobectomy, less often pneumonectomy or sublobar resection) together with the regional lymphatic system
Block resection in cases of tumor infiltration of adjacent tissue structures with marking the margins, which is important for microscopic radicality assessment
Lymphadenectomy involving at least 6 lymph nodes: hilar (N1) and mediastinal (N2) with marking the lymph node located highest in the mediastinum in relation to the tumor

The material covering a lobe, lobes, a lung, or a fragment of a lung and lymph nodes should be placed in disposable plastic containers intended for this purpose, meeting the requirements of an *in vitro* diagnostic (IVD) medical device adapted to the size of the collected material and enabling proper fixation.

The required fixative is a 10% buffered formalin solution with a neutral pH (7.2–7.4). Depending on the rules agreed with PDU regarding the submission of material for pathomorphological evaluation, it is also possible to send unfixed material immediately after collection.

The resected and secured material must be delivered to the PDU **within** 72 hours of the end of the surgical procedure, preferably within 48 hours [11–13].

Tissue elements of importance for staging and assessment of surgery radicality (e.g. fragments of the pericardium, diaphragm, chest wall) or lesions that may be difficult to find during material preparation by a pathologist (e.g. ground-glass nodules, GGNs) should be marked in a way that allows for identification and proper collection of samples for microscopic evaluation [11, 12].

Each collected lymph node of a given station sent for pathomorphological examination should be placed in a separate container. This applies especially to fragmented material due to the risk of incorrect determination of the number of removed lymph nodes [14].

The attached referral form for pathomorphological examination should contain all data allowing for the identification of the patient and the material sent. Information on the type of procedure performed, the type of material collected, date and time of collection, and placement in the fixative is necessary. Clinical data on the current disease, location of lesions, and past medical history, especially regarding oncological diseases, including pathomorphological diagnosis and treatment, are also necessary [11–13].

Depending on the rules adopted at the center, it is possible to include information in the referral form about the need to provide material for *EGFR* gene status assessment, if required qualification criteria for adjuvant treatment with osimertinib are met.

Principles of sending surgical material for testing mutations in the *EGFR* gene

In patients with primary lung adenocarcinoma or another morphological form of NSCLC diagnosed in the postoperative material with a predominance of adenocarcinoma tissue (≥ 50%) and meeting the eligibility criteria for treatment with osimertinib (disease stage IB–IIIA, radical surgery R0), *EGFR* gene status should be determined. The procedure for sending for *EGFR* gene status testing may vary, which results from different organizational protocols adopted in individual units. Possible protocols include sending for *EGFR* gene status testing by:

— the surgeon who operated, together with attached consent to perform the genetic test or information about consent expressed by the patient, obtained upon admission to the hospital;
— a designated person responsible for analysis of the results of all pathomorphological tests in the thoracic surgery center, together with attached consent to perform the genetic test or information about consent expressed by the patient, obtained upon admission to the hospital;
— a pathologist evaluating the postoperative material, provided that the information about the need to assess *EGFR* gene mutation was included in the referral form for pathomorphological examination.

Pathomorphological examination of surgical material in patients qualified for osimertinib treatment

The pathomorphological examination of surgical material from lung cancer patients aims to determine its morphological form and histological differentiation grade as well as to assess prognostic factors, tumor stage (pTNM, tumor, nodes, metastasis), and radicality of surgical procedure.

A key prerequisite for establishing a pathomorphological diagnosis is compliance with the rules covering the initial preparation of the material and the phase of pathomorphological diagnosis in accordance with the recommendations of the Polish Society of Pathologists (PSP) and accreditation standards developed for PDU by PSP in 2021 in cooperation with the National Centre for Quality Assessment in Healthcare [11–13].

Macroscopic and microscopic examination of postoperative material

The post-operative material submitted to the PDU requires preliminary processing, allowing for proper preservation and preparation for the collection of specimens.

Macroscopic assessment includes examining the tumor with three dimensions in millimeters, determining the exact location in relation to the bronchus and pleura and distance from the edges of bronchus and vessels cutoff and the pulmonary pleura. The assessment of the peripheral lung parenchyma for the presence of atelectasis and inflammation, determining their extent, and the presence of additional nodular lesions is also important for disease staging [11, 15–18].

The number of specimens to be taken for microscopic examination depends on the type of material sent and the size of the lesion. Due to the heterogeneity of lung cancers, especially adenocarcinomas, it is recommended to use the principle of collecting 1 biopsy/1 cm of tumor

Table 2. Microscopic assessment of pleural infiltration [21]

Category	Definition
PL0	No infiltration of pulmonary pleura The tumor is separated from the pleura by the lung parenchyma or does not cross the elastic lamina of the pulmonary pleura
PL1	The cancer infiltration exceeds the elastic lamina of the pulmonary pleura
PL2	The cancer infiltration covers the entire thickness of the lung pleura and exceeds its surface
PL3	The cancer infiltration penetrates the parietal pleura or chest wall

[15, 16]. Tumors up to 3 cm in diameter, which on computed tomography (CT) of the chest are described as GGN or ground-glass nodules with consolidation, suggesting the possibility of proliferation of adenocarcinoma in situ (AIS) or minimally invasive adenocarcinoma (MIA) require examination of the entire lesion.

The material should be taken both from all places that are important for cancer staging as well as from the areas constituting the edges of the surgical resection and, if relevant, also the margin covering the resection edge with the tumor [15–18].

In the material covering the lobe, lobes, or lung, it is important to find and assess the lymph nodes in the area of the bronchovascular border and intrapulmonary (station N1) [16–18].

Pathomorphological classification of lung adenocarcinoma

More than 50% of non-small cell carcinomas are adenocarcinomas. The adenocarcinoma component is also present in adenosquamous NSCLC, which accounts for 2–3% of all lung cancers; it can occur both in the so-called pleomorphic carcinomas (approximately 1%) and combined large-cell neuroendocrine carcinomas. The criteria for the diagnosis of individual morphological forms of lung cancer are strictly defined by the current 5th edition of 2021World Health Organization (WHO) classification (Thoracic Tumours) [19].

Pathomorphological diagnosis of lung adenocarcinoma should take into account all morphological components present in its structure and determine the degree of histological differentiation [grading (G)].

The microscopic diagnosis of lung adenocarcinomas is based on:
- finding morphological features of glandular differentiation (the presence of papillae, micropapillary and acinar structures visible on standard H+E staining) and/or
- the presence of mucus in tumor cells detected by histochemical examination (e.g. mucicarmine) and/or
- expression of immunohistochemical markers of glandular differentiation (TTF-1, napsin A) [19].

The principles for determining the malignancy grade of lung adenocarcinomas refer to non-mucous forms and take into account the dominant morphological type and component of cancer tissue considered poorly differentiated, that is micropapillary, solid, with a complex glandular pattern. This term includes adenocarcinomas with the structure containing the so-called cribriform and fine-tubular, trabecular structures, often trapped in the fibrosing stroma [20].

The assessment of pleural infiltration is important in cancer staging. Therefore, in cancers located peripherally and adjacent to the pleura, it is necessary to perform an additional examination that stains the elastic fibers (e.g. elastic van Gieson method, EvG), enabling a precise assessment of the relationship of the tumor to elastic membranes of pleura, determining its possible infiltration (Tab. 2). The examination also visualizes blood vessels, which facilitates the identification of neoplastic emboli in the vessel lumen [21].

System of clinical (cTNM) and pathomorphological (pTNM) staging of lung cancer

Selection of the optimal therapeutic option for patients with lung cancer requires accurate staging based on the classification system (8th edition) that includes three important elements:
— T (tumor) determination of tumor size and its localization in relation to anatomical structures (Tab. 3);
— N (nodes) assessment of the condition of lymph nodes;
— M (metastasis) information about the presence or absence of distant tumor metastases.

Clinical (c) and pathomorphological (p) TNM classifications do not differ from each other and are based on similar assumptions, and the final staging of the disease requires a correlation of both systems [2, 22].

Additional morphological features affecting the assessment of tumor size pT

— With regard to non-mucinous lepidic adenocarcinomas, the 8th edition of the TNM classification recommends assessment of the invasive component as corresponding to pT with the simultaneous specification of the total size of the lesion (invasive

Table 3. Assessment of primary tumor (T feature)

Category	Definition	
TX	Primary tumor cannot be assessed, or tumor is indicated by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy	
T0	No evidence of primary tumor	
Tis	Carcinoma *in situ*	
T1	Tumor 3 cm or less in greatest dimension, surrounded by the lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e. not in the main bronchus)	
T1mi	Minimally invasive adenocarcinoma (MIA)	Solitary adenocarcinoma (≤ 3 cm) with a predominant lepidic pattern with an invasive component ≤ 5 mm in the greatest dimension, without necrosis, pleural infiltration, alveolar filling (STAS)
T1a	Tumor 1 cm or less in greatest dimension	This includes superficially spreading tumor of any size with its invasive component limited to the bronchial wall, which may extend proximal to the main bronchus
T1b	Tumor more than 1 cm but not more than 2 cm in greatest dimension	
T1c	Tumor more than 2 cm but not more than 3 cm in greatest dimension	
T2	Tumor more than 3 cm but not more than 5 cm or tumor with any of the following features: • involves the main bronchus, regardless of distance to the carina, but without involvement of the carina • invades the visceral pleura • associated with atelectasis or obstructive pneumonitis that extends to the hilar region either involving part of or the entire lung	
T2a	Tumor more than 3 cm but not more than 4 cm in greatest dimension	• Infiltration of adjacent lobe through an interlobar fissure or directly if the fissure is not developed unless higher stage T criteria are met • Hilar adipose tissue infiltration unless higher stage T criteria are met
T2b	Tumor more than 4 cm but not more than 5 cm in greatest dimension	
T3	Tumor more than 5 cm but not more than 7 cm in greatest dimension or one that directly invades any of the following: • parietal pleura • chest wall (including superior sulcus tumors) • rib or ribs • phrenic nerve • parietal pericardium or separate tumor nodule(s) in the same lobe as the primary	
T4	Tumor more than 7 cm or of any size that invades any of the following: • diaphragm, mediastinum, parietal pericardium, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, spine, carina or • tumor nodule(s) in a different ipsilateral lobe separate from that of the primary one	Mediastinal adipose tissue infiltration The term "great vessels" includes: • aorta • superior and inferior vena cava • pulmonary trunk • intrapericardial segments of the right/left pulmonary artery • intrapericardial segments of the upper and lower pulmonary veins

component/total tumor size). In the assessment of the invasive component and the determination of tumor size (pT), the correlation of microscopic changes with the CT image is helpful. The CT examination also facilitates the determination of tumor size in cases of fragmentation of the lesion and difficulties in distinguishing irregular foci that raise the suspicion of two separate foci [23].

— Multifocal lesions:
  • with similar morphology should be treated as a separate additional (satellite) lesion or metastasis (depending on the location);

Table 4. Assessment of lymph nodes (N disease)

Category	Definition
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in the intrapulmonary lymph nodes, including involvement by direct extension (lymph nodes of 10–14 stations)
N2	Metastasis in the ipsilateral mediastinal and/or subcarinal lymph node(s) (lymph nodes of 2–9 stations)
N3	Metastasis in the: • contralateral mediastinal • or contralateral hilar • or ipsilateral or contralateral scalene • or ipsilateral or contralateral supraclavicular lymph node(s) (lymph nodes of 1 and 2, 4–6, and 8–14 contralateral stations)

- with different morphology and different histological components, should be treated as separate primary (synchronous) lesions and classified separately;
- multifocal adenocarcinoma with AIS, MIA, and lepidic foci should be classified based on the largest lesion with assessing the number of foci;
- diffuse pneumonic-type adenocarcinoma is usually characterized by mucinous or mixed mucinous and serous adenocarcinoma foci (pT3 if unilateral; pT4 if multiple ipsilateral lobes; M1a if applies to the lobes on the opposite side).

Assessment of regional lymph nodes (N)

The assessment of regional lymph nodes (N disease) is presented in Table 4.

Metastases in lymph nodes 10–14 on the primary tumor side are classified as N1.

Metastases limited to midline nodes and mediastinal lymph nodes on tumor side (stations 2–9) are classified as N2.

Involvement of lymph nodes on the primary tumor side and contralateral side within station 1 and stations 2, 4–6, and 8–14 on the contralateral side is classified as N3.

Pathomorphological evaluation of lymph nodes requires determination of the number of lymph nodes examined at a given station and size of individual nodes, assessment of the condition of the node capsule (including possible tumor infiltration), the extent of metastases, the identification of the so-called micrometastases and isolated tumor cells, and the presence of necrotic foci [16, 17]. Involvement of the lymph node(s) by neoplastic infiltration, the so-called "through--continuity" infiltration, is treated as a metastasis to the lymph node [2, 22].

According to the American Joint Committee on Cancer (AJCC) TNM recommendations specifying the required number of collected lymph nodes essential to determine the radicality of the surgical procedure, it is necessary to find at least 3 lymph nodes of the N1 station in the surgical material covering the lobe, lobes, or lung.

Micrometastases are defined as neoplastic foci > 0.2 to ≤ 2 mm in size, which in the pathomorphological examination report are described as "mi" (pNmi).

Single tumor cells or small clusters not larger than 0.2 mm detectable by standard hematoxylin and eosin (H+E) staining or immunohistochemistry (IHC) using mainly broad-spectrum cytokeratins or by other special methods, for example, flow cytometry or molecular testing, are referred to as isolated tumor cells (ITC). The finding of ITC does not adversely affect patient survival time and is defined as pN0 with information about their occurrence by marking as "i" or "mol" depending on the method of detection (pN0[i+], pN0[mol+]) [16, 22].

The neoplastic infiltration of the mediastinal lymph node capsule found in microscopic examination indicates a non-radical surgical procedure (pR1). The continuity of the capsule is not always trackable, depending to a large extent on the method of removing the nodes. While systematic lymphadenectomy allows excision of lymph nodes with a capsule, removal of node fragments (so-called sampling) usually does not allow for capsule assessment. The pathomorphological diagnosis then includes the information that "the evaluation of the node capsule is not possible, and the lymph node was removed in fragments".

Assessment of distant metastases (M)

Distant metastases include lesions other than the primary tumor and mediastinal lymph node lesions within the chest and outside the chest (Tab. 5).

The description of pM disease in the pathomorphological report requires confirmation by microscopic examination.

Evaluation of surgical radicality feature R

The assessment of surgical radicality includes each margin of the performed resection and depends on the type of procedure performed. Most often, the margin consists of the bronchus/bronchi, blood vessels, lung

Table 5. Assessment of metastasis (M disease)

Category	Definition	
MX	Distant metastasis cannot be assessed	
M0	No distant metastasis	
M1	Distant metastasis	
M1a	Nodule(s) in a contralateral lobe Nodule(s) in the ipsilateral pleura or parietal pleura pericardial nodules or pericardium Malignant dissemination or neoplastic pleural or pericardial effusion[1]	Nodule(s) located in the ipsilateral pulmonary and parietal pleura, unrelated to the primary tumor
M1b	Single extrathoracic metastasis in a single organ	• This includes involvement of a single, distant, non-regional node • Metastatic lesion outside the parietal pleura in the chest wall
M1c	Multiple extrathoracic metastases in a single or multiple organs	Metastatic lesion not in contact with the primary tumor, outside the parietal pleura, located in the diaphragm

[1]Pleural or pericardial fluid negative for cancer cells in cytological examination or blood admixture, non-exudative, should be classified as pM0

Table 6. Evaluation of surgical radicality (R feature)

Category	Definition
Rx	Surgical radicality cannot be assessed
R0	No neoplastic infiltration in the dissection margins, radical surgery
R1	Microscopic examination reveals neoplastic infiltration: • positive surgical margin[1] • neoplastic infiltration exceeds the capsule of resected lymph nodes
R1(is)	Carcinoma *in situ* at the surgical margin of the bronchus
R1(cy+)	No cancer infiltration at the surgical margin, cancer cells are present in the pleural or pericardial effusion collected during thoracotomy [pleural lavage cytology (PLC)]
R2	Macroscopic neoplastic infiltration in the dissection margins

[1]Malignant infiltration found in the margins of severed bronchi may occur as:
• infiltration of the bronchial wall;
• infiltration involving the peribronchial tissue (adventitia), also in continuity, spreading from nearby metastatic lymph nodes;
• cancer cells embolism in the lymphatic vessels of the bronchial mucosa

parenchyma, mediastinal lymph nodes, and other elements of additionally removed tissues or organs. Surgical radicality is also specified as the absence of cancer cells in the fluid from the pleural and/or pericardial cavities collected during thoracotomy (pleural lavage cytology, PLC).

Surgical radicality is defined by the R feature (Tab. 6) [2, 22, 24].

The indicators of radical resection include [2, 22]:
- surgical cutoff margins free of neoplastic infiltration (R0);
- removal of the regional lymphatic system involving at least 6 lymph nodes (N1, N2), including lymph nodes of the tracheal bifurcation;
- absence of neoplastic infiltration beyond the lymph node capsule.

The R0(un) feature includes an uncertain cutoff margin (uncertain resection) and applies to:
— estimated number of resected lymph nodes lower than required (< 6);
— detection of cancer metastases in the superior resected mediastinal lymph node.

Pathomorphological diagnosis report

The pathomorphological diagnosis report of surgical material with lung adenocarcinoma should include:
— diagnosis defining the morphological form of cancer, taking into account the percentage of individual tissue components, especially those considered to be less differentiated;
— ICD-O code;
— determination of the degree of cancer histological differentiation (G);
— type of material sent;
— macroscopic description;
— microscopic description, also taking into account prognostic factors: the presence of neoplastic emboli in the lymphatic and hematopoietic system, presence and extent of necrosis, infiltration of nerve fiber bands, stromal immunological reaction, stromal reaction, scar presence, spread through air spaces (STAS);
— assessment of surgical resection margins;
— assessment of margins covering the distance from resection margin to the neoplastic infiltration;

— assessment of the remaining lung parenchyma;
— evaluation of lymph nodes, including possible infiltration of the capsule;
— description of additional tests performed (histo- and immunohistochemical);
— information on qualification for *EGFR* gene mutation testing.

The report should end with the assessment of the pathomorphological stage of the tumor (pTNM) with additional prognostic features pV, pL, pR (pTNLVR) [16, 25]. It is advisable to attach the result of *EGFR* gene mutation testing to the pathomorphological diagnosis report.

Selection of material for the assessment of mutations in the *EGFR* gene

The pathologist qualifies the material for testing using molecular biology methods, selecting the most reliable section containing an adequate number of cancer cells and, if possible, without necrosis and other changes that may adversely affect the test result.

The qualified material with a description of the pathomorphological diagnosis and information including the number of the selected paraffin block, and the adequacy of the material (number of cancer cells, number of cells in relation to other nucleated elements) is transferred to the molecular diagnostics department.

Evaluation of activating mutations in the *EGFR* gene

According to the current recommendations, tests aimed at identifying mutations in the EGFR gene and analyzing PD-L1 protein expression level are the basis for the selection of adjuvant treatment methods in radically operated patients and should be performed in all NSCLC patients [26]. At the same time, there is a need to identify rearrangements in the ALK and RET genes and other rare molecular abnormalities that may have predictive and prognostic significance [27–31].

PD-L1 expression level is determined by immunohistochemistry. However, the identification of the *EGFR* gene variants can be performed using molecular biology techniques by quantitative polymerase chain reaction (qPCR) or next-generation sequencing (NGS). The tests used should detect all mutations that have been reported, with a frequency of at least 1% in NSCLC patients with an *EGFR* gene variant [32].

Tests aimed at detecting deletions in exon 19 and p.L858R point mutations in exon 21 can be performed using the PCR technique [32]. Many commercial tests are now available, and the diagnostic process itself does not require advanced laboratory equipment. The advantage of the PCR test may be the short turnaround time (TAT) and the relatively low cost of the analysis. However, it should be remembered that these tests only detect specific variants in the *EGFR* gene.

According to the current guidelines of the European Society of Medical Oncology (ESMO), NGS should be used routinely in the diagnosis of advanced NSCLC [33]. The method not only allows for the simultaneous analysis of many biomarkers but is also a very effective tool for identifying *EGFR* gene variants. The results of the study conducted by Schrock et al. showed that the use of a specific NGS technique enables the detection of deletions in exon 19 of the *EGFR* gene in tissue material where previous standard diagnostic methods failed to identify these changes [34]. Another study by this group showed a higher efficiency of this technique compared to PCR in identifying not only deletions in exon 19 but also variants in the remaining exons (18, 20, and 21) of the *EGFR* gene [35].

Currently, studies (NCT04302025 and NCT04926831) are ongoing, which focus on identifying genetic variants in genes other than *EGFR* in radically operated patients. In the NCT04302025 study, molecular analyzes are conducted to detect rearrangements of the *ALK*, *NTRK1*, *RET,* and *ROS1* genes and point variants in the V600 codon of the *BRAF* gene [36]. In the latter study, patients were included in the study group based on exon 14 skipping mutation or *MET* gene amplification [37]. The need to identify various genetic variants (point mutations, deletions, insertions, rearrangements, or amplifications) in many genes is another argument for using the NGS method for routine diagnostics of all patients diagnosed with NSCLC. An additional justification is the fact that simultaneous biomarker analysis has been shown to be more effective than sequential testing using single-gene tests [38–41]. Sequential testing has been shown to produce more false positives (3.3%) than simultaneous analysis of several genes (1.4%), as each additional test increases the likelihood of a false positive result. At the same time, it was found that the sequential use of single-gene tests also increases the number of non-diagnostic results (sequential tests — 6.9% *vs.* NGS — 2.7%) [38]. The conducted studies have also shown that diagnostics using sequential tests have a negative impact on TAT or costs [38–40]. In addition, the use of multiple tests also increases the risk of material exhaustion before the end of the diagnostic process in individual patients [35, 38, 40].

Osimertinib in adjuvant treatment after NSCLC radical resection

The value of osimertinib confirmed in patients with advanced NSCLC with the presence of activating mutations in the *EGFR* gene was the justification

Table 7. Characteristics of patients in the ADAURA study (selected features) [7]

Features	Osimertinib [%]	Placebo [%]
Postoperative stage — IB/II/IIIA	32/34/35	32/34/34
Histological type — adenocarcinoma/other	96/4	97/3
Performance status — 0/1	64/36	64/36
EGFR gene mutation — ex19del/eks21sub/T790M	55/45/1	55/45/1
Resection — lobectomy/other types	97/3	94/6
Lymph nodes – N0/N1/N2 disease	41/29/31	42/28/30
Adjuvant chemotherapy — yes/no	60/40	60/40

ex19del — deletion in exon 19 of the *EGFR* gene; ex21sub — substitution in exon 21 of the *EGFR* gene; T790M — replacement of threonine with methionine in exon 20 of the *EGFR* gene

Table 8. Phase III ADAURA study results [7]

Index	Osimertinib	Placebo
Median disease-free survival [months]		
Total patients (stages IB–IIIA)	Not reached	19.6
Patients in stages II and IIIA	Not reached	27.5
Reduction in the risk of death or recurrence [%]		
Total patients (stages IB–IIIA)	80% ($p < 0.0001$)	
Patients in stages II and IIIA	83% ($p < 0.0001$	

for conducting the phase III ADAURA study [7]. The ADAURA study involved 682 patients diagnosed with non-squamous cell lung cancer (adenocarcinoma 96%), who were randomly assigned to receive osimertinib 80 mg daily (n = 339) or placebo (n = 343) for 3 years. The study involved patients after radical resection of the lung parenchyma (pR0 in the postoperative pathomorphological examination), with confirmed an activating mutation in the EGFR gene (only a deletion in exon 19 or a substitution in exon 21). Adjuvant chemotherapy in the ADAURA study was allowed based on individually assessed indications before randomization, but radiotherapy was not allowed. The primary endpoint of the study was to assess disease-free survival in patients with stages IB–IIIA (secondary endpoints: assessment of benefits in individual postoperative stages and the overall population in terms of disease-free and overall survival, impact on quality of life and safety). Selected features of the assessed population are presented in Table 7.

The first analysis of the ADAURA study results showed that endpoints were met – the use of osimertinib in the entire study population allowed for a significant reduction in the risk of death or disease recurrence by 80%. In postoperative stages II-IIIA, the rate was even more favorable and amounted to 83%. In the 2-year follow-up of patients with postoperative stages II–IIIA, 90% of patients receiving adjuvant treatment with osimertinib and 44% of patients receiving placebo were still alive without signs of disease recurrence (other results in Tab. 8) [7].

The cumulative risk of recurrence in the central nervous system (CNS) was significantly lower in the group of patients treated with osimertinib after a 24-month follow-up, 98% of patients receiving osimertinib had no brain metastases compared to 85% of patients in the placebo group (risk reduction by 82%; $p < 0.0001$). Local recurrences were reported in 7% of patients receiving osimertinib and 18% in the placebo group, and distant metastases in 4% and 28% of patients, respectively. Grade 3 or higher adverse reactions occurred in 20% of patients in osimertinib group and 13% in the placebo group. The most common adverse events (all grades) in the osimertinib arm *versus* placebo were diarrhea (46% *vs*. 20%), onychomycosis (25% *vs*. 1%), dry skin (19% *vs*. 6%), and pruritus (19% *vs*. 9%). The rate of treatment discontinuation due to adverse events was 11% and 3%, respectively [7].

Benefits associated with the use of osimertinib in terms of significant prolongation of disease-free survival were also noted in patients who received chemotherapy (84% risk reduction) and those who did not undergo chemotherapy (77% risk reduction) [8].

Longer follow-up of patients in the ADAURA study, presented during the ESMO Congress in 2022, confirmed the above-mentioned observations [8]. Median disease-free survival for patients with stage II and IIIA receiving osimertinib or placebo was 65.8 and 21.9 months, respectively, representing a 77% reduction in the risk of death or relapse. The percentage of patients living without recurrence of the disease reached 70% in the osimertinib group compared to 29% in the placebo group [42].

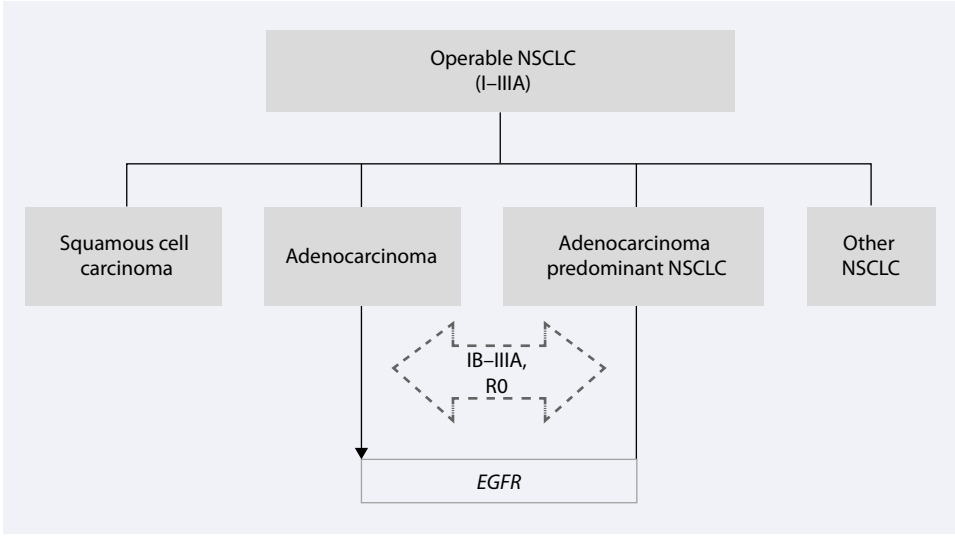

Figure 1. Qualification of patients treated surgically for adjuvant therapy with osimertinib; EGFR — epidermal growth factor receptor; NSCLC — non-small cell lung cancer

The use of osimertinib in the adjuvant treatment after radical resection of the lung parenchyma (R0) is justified in patients with a diagnosis of adenocarcinoma or cancer with a predominance of adenocarcinoma in stages IB, II, and IIIA, with an activating mutation in the *EGFR* gene (only deletion in exon 19 or substitution in exon 21) independently of the expression of the programmed death ligand type 1 (PD-L1). This indication requires *EGFR* gene status testing in each patient with primary lung adenocarcinoma or NSCLC with a predominance of adenocarcinoma component undergoing complete resection (the assessment of PD-L1 status should be a second step after excluding the presence of mutations in the *EGFR* gene).

Patients after incomplete resection (surgical margins with the presence of neoplastic cells R1 or R2) should receive chemotherapy (use of radiotherapy can be considered). In patients with stages II and IIIA after complete resection, apart from osimertinib, adjuvant postoperative chemotherapy should also be used, which should precede osimertinib (except for patients with real and documented contraindications to chemotherapy, which include, for example, kidney failure, neuropathy, and significant hearing impairment). In patients who do not receive adjuvant chemotherapy, the use of osimertinib should be started no later than 10 weeks after lung resection (it is advisable to start treatment as early as possible, provided that the result of *EGFR* gene status is known). In patients receiving adjuvant chemotherapy, osimertinib should be used no later than 26 weeks after surgery. Adjuvant treatment with osimertinib lasts up to 3 years. During the use of osimertinib, control tests should be performed (evaluation of treatment effectiveness and safety) in accordance with the summary of product characteristics (SmPC) and applicable B.6 program. Follow-up examinations after the completion of adjuvant treatment should be conducted in accordance with the currently applicable standard.

Conclusions

New systemic therapies (molecularly targeted drugs and immune checkpoint inhibitors) are increasingly used in the radical management of cancer patients in combination with local treatment. The benefits of combining new drugs with surgery or radiotherapy also apply to NSCLC patients. The results of the ADAURA study, regardless of the lack of final OS results, justified the introduction of osimertinib to the standard of adjuvant postoperative treatment of NSCLC patients. The conditions for optimal use of osimertinib in adjuvant postoperative treatment include appropriate qualification for pulmonary parenchyma resection as well as pathomorphological and molecular diagnostics. Further studies are currently underway, the goals of which include, but are not limited to, identifying the optimal duration of osimertinib treatment, the use of anti-EGFR therapy in patients undergoing resection for very early stage (IA) NSCLC, determining the value of longer use of osimertinib, and detecting resistance mechanisms and methods overcoming lower sensitivity to the drug (Fig. 1).

Article Information and Declarations

Author contributions

R.L.: the concept of the manuscript, development of issues related to the pathomorphological evaluation of the material, development of tables and figures, literature review, and participation in the development of the entire article. M.K.: the concept of the manuscript, development of issues regarding adjuvant treatment with osimertinib, summary, literature review. D.K.: development of issues regarding adjuvant treatment with osimertinib, literature review, and participation in writing the final version of the manuscript. R.K.: concept of the whole manuscript, preparation of the introduction and the final version of the article, tables. T.O.: participation in the preparation of the final version of the manuscript. W.Rz.: substantive elaboration of issues concerning surgical treatment, literature review, tables, and participation in the preparation of the entire manuscript. B.W.: substantive development of issues related to molecular biology, literature review, and participation in the preparation of the manuscript.

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Conflict of interest

R.L.: advisory board or lecture fees from AstraZeneca, Roche, MSD, BMS, Takeda, Amgen, and Boehringer-Ingelheim.
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T.O.: participation in advisory committees or lectures for AstraZeneca, Roche, and Takeda.
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VIA MEDICA

ORIGINAL ARTICLE

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Chemotherapy compliance in elderly patients with solid tumors: a real-world clinical practice data

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ABSTRACT

Introduction. Malignant tumors in elderly people are more than ten times more prevalent than in the younger population. The data on the compliance with chemotherapy in older cancer patients managed outside of clinical trials is scarce.

Material and methods. We retrospectively assessed 181 consecutive cancer patients aged 65 years or more who received systemic chemotherapy. The study aimed to examine chemotherapy compliance in a large series of elderly patients managed in routine clinical practice. We also investigated the ability to complete chemotherapy in relation to selected factors, such as tumor type, treatment setting and line, type of chemotherapy, presence of comorbidities, body mass index (BMI), an expected glomerular filtration rate, hemoglobin level (Hb), a neutrophil-to-lymphocyte ratio, and Eastern Cooperative Oncology Group performance status (PS).

Results. Thirty-three percent of patients did not complete an initially pre-defined chemotherapy plan. The main reasons were disease progression (20%) and unacceptable toxicity (10%). Independent factors related to premature treatment termination included a lower BMI, a lower Hb level, lower PS, and palliative (compared to currative) setting.

Conclusions. In conclusion, premature chemotherapy termination not related to disease progression is relatively rare in elderly patients and may be predicted with routinely used clinical parameters.

Key words: older patients, solid tumor, chemotherapy, real-world data

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Introduction

Older age is the most potent single risk factor for developing a malignant solid tumor. Over 80% of solid tumors are diagnosed in patients over 55 years of age, and 60% in patients over 65 years of age [1]. Malignant solid tumors in patients over 65 years are more than ten times more prevalent than in younger people [2]. Chemotherapy is the principal systemic anticancer treatment modality. Clinical trials indicate that the efficacy of chemotherapy is not related to age, however, treatment-related toxicities are more prevalent in older patients [3–6]. With advancing age, the number of comorbidities and related multiple medications increase. Aging of the society leads to an increasing proportion of older patients, including those with healthy lifestyles and not burdened with significant morbidities. In consequence, the life expectancy in Europe is estimated to exceed 80 years [7]. Physiological changes in the elderly lead to the functional impairment of the digestive tract, cardiovascular system, kidneys, and numerous abnormalities (neurological, emotional and cognitive,

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immunological, and hematological). As a result, older patients are more susceptible to complications of systemic anti-cancer treatments, particularly chemotherapy. Traditionally elderly patients were underrepresented in pivotal clinical trials due to the risks of increased toxicities and related lower compliance rates. This approach has changed since the early 1990s, nevertheless, the proportion of elderly patients in clinical trials has remained lower than in the general cancer patient population [8–10]. Oncogeriatric evaluation tools facilitate a systemic treatment eligibility assessment [11–14] but have not been widely adopted in clinical practice. A real-life data on chemotherapy compliance in elderly patients managed outside of prospective clinical trials and on factors impacting compliance is still relatively scarce.

This study aimed to assess chemotherapy compliance in a large consecutive series of elderly patients routinely managed in a tertiary oncological center. We also investigated the ability to complete chemotherapy in relation to selected factors, such as tumor type, treatment setting, line and type of chemotherapy, presence of comorbidities, body mass index (BMI), an expected glomerular filtration rate (eGFR), hemoglobin concentration (Hb), a neutrophil-to-lymphocyte ratio (NLR), and the Eastern Cooperative Oncology Group (ECOG) performance status (PS).

Material and methods

We retrospectively analyzed a group of 181 consecutive cancer patients 65 years of age or older, who were administered systemic chemotherapy for a year (from January to December 2019) at the Department of Oncology with Daily Unit, Tadeusz Koszarowski Cancer Center in Opole, Poland. Patient and treatment data were extracted from individual patient files. Patients who completed more than one line of treatment in the analyzed period were evaluated only for the initial treatment. The type of solid tumor, treatment setting (curative or palliative), the line of treatment, comorbidities, BMI, an eGFR, an Hb level, and a PS were recorded prior to treatment (Tab. 1). PS was evaluated using ECOG score [15]. Renal function was evaluated using eGFR (according to the Cockroft-Gault formula). The neutrophil-to-lymphocyte ratio (NLR) was calculated based on complete blood count. No primary prophylaxis against neutropenic fever with granulocyte-colony stimulating factor (G-CSF) was instituted and in a couple of cases, G-CSF was used as secondary prevention. Due to the retrospective type of our research no comprehensive geriatric assessment was available for these patients.

This study was approved by the Ethical Committee of the Regional Medical Chamber in Opole. All patient data were anonymized after being extracted from individual patient files, before analysis. The comorbidities were recorded as qualitative variables (0 — no significant comorbidities, 1 — diabetes, diabetes with coexisting cardiovascular disease or other, 2 — cardiovascular disease coexisting with other comorbidities but not with diabetes mellitus, 3 — other significant comorbidities not coexisting with cardiovascular disease or diabetes mellitus). Diabetes mellitus was singled-out as a condition that determines both the renal and microcirculatory statuses, and thus having a much broader systemic effect.

For treatment with curative intent, the number of planned chemotherapy cycles was set in accordance with relevant and current standards of care. The treatment plan for palliative treatment included at least eight chemotherapy cycles given every two weeks, or at least six chemotherapy cycles (four for lung cancer) administered every three weeks. No intended upfront dose reductions were applied. The ability to complete the pre-planned treatment schedule was considered as treatment compliance.

Treatment intent was categorized as follows: 0 — curative treatment, 1 — the first line of palliative treatment, 2 — the second line of palliative treatment, 3 — the third and subsequent lines of palliative treatment. Treatment-related toxicity was assessed in accordance with the Common Terminology Criteria of Adverse Events v 4.0 [16]. The reasons for not completing the treatment plan were classified as follows: 1 — disease progression (PD), 2 — unacceptable toxicity, 3 – health deterioration or other factors not related to cancer progression. Age, sex, type of malignancy, treatment aim (curative or palliative), palliative treatment line (first or later lines), comorbidities, BMI, and an eGFR were included in the analysis.

For continuous variables, the Mann-Whitney-Wilcoxon's test was used, and the qualitative variables were analyzed with Fisher and chi-squared tests. Multivariate analysis was performed using a logistic regression model. The following models were considered: a model with all considered variables, a model with each variable analyzed individually, and a model using the step method selected in the R program in accordance with the Akaike information criterion (AIC). To select the variables appropriately, statistical significance tests based on Wald's statistics were used.

Results

The median patient age was 71 years (range 65–88), and 45 patients (25%) were aged 75 years or older (Tab. 1). The majority of patients presented with a PS 0 or 1. More than 70% of patients were overweight or obese. Due to the small sample size, underweight patients were

Table 1. Patient characteristics

Variables	n = 181	[%]
Age		
Median	71	
Range	65–88	
(65–< 70)	70	38.7
(70–< 75)	66	36.5
(75–< 80)	33	18.2
(≥ 80)	12	6.6
Sex		
Male	94	51.9
Female	87	48.1
Bodyweight		
Median	73.1	
Range	47.0–115.0	
BMI		
Median	27.7	
Range	16.3–40.6	
Underweight or normal (< 25)	53	29.3
Overweight (25–< 30)	67	37.0
≥ 30	61	33.7
ECOG-PS		
0	59	32.6
1	103	56.9
2	19	10.5
eGFR [mL/min]		
Median	83.1	
Range	29.3–162.1	
< 60	24	13.3
60–< 90	83	45.9
≥ 90	74	40.9
Hb level [g/dL]		
Range	8–16.9	
< 10	11	6.1
≥ 10–N	90	49.7
N	69	38.1
> N	11	6.1

Table 1 cont. Patient characteristics

Variables	n = 181	[%]
Comorbidities		
No significant comorbidities	35	19.3
Diabetes or diabetes with coexisting cardiovascular disease or other comorbidities	28	15.5
Cardiovascular disease coexisting with other comorbidity but not with diabetes mellitus	102	56.4
Other significant comorbidities excluding cardiovascular and diabetes mellitus	16	8.8
Cancer type		
Colorectal	81	44.8
Breast	42	23.2
Lung	14	7.7
Gastric	10	5.5
Prostate	10	5.5
Other	24	13.3
Treatment setting		
Curative	47	26.0
Palliative	134	74.0
Line of palliative treatment (n = 134)		
First	70	52.2
Second	44	32.8
Third or higher	20	14.9
Type of chemotherapy		
Single-agent	69	38.1
Combination	112	61.9

BMI — body mass index; ECOG-PS — Eastern Cooperative Oncology Group performance status; eGFR — expected glomerular filtration rate; Hb — hemoglobin concentration; N — normal values range female 12–14 g/dL, male 14–16 g/dL

analyzed together with those with normal weight (as any significant skew in distribution was unlikely). The abnormal renal function (eGFR < 60 mL/min) was diagnosed in 13% of patients. Nearly half of patients presented with anemia, including 6% with a Hb level of 10 g/dL or less. The median NLR in the whole study cohort was 2.6, and 87% of patients had leucocyte and neutrophil levels within reference ranges. The most common malignancies were colorectal and breast cancers (45% and 23%, respectively). Three-fourth of the patients were treated with palliative intent, and the remaining patients received adjuvant treatment. Among those treated in the palliative setting, 52% received first-line treatment, 33% second-line, and 15% third- or subsequent lines. In all patients, chemotherapy was initiated at standard doses, according to the body surface area.

Treatment was not completed as planned in thirty--three percent of patients (Tab. 2). The most common reason was disease progression (20%), followed by unacceptable toxicity (10%). Major toxicities leading to premature treatment termination included dehydration and dyselectrolytemia related to uncontrollable diarrhea, oral cavity mucositis restricting adequate nutrition, and hematotoxicity. Grade 4 adverse events occurred in 13% of patients. There were no treatment-related deaths. Five patients (2.8%) stopped therapy prematurely due to a significant deterioration of overall health status not accompanied by apparent treatment-related adverse events or progression. Two of these patients presented with persistent significant fatigue, depression, and lack of appetite. Three patients did not show up for their scheduled visits, two necessitated in-patient treatment and one was lost to follow-up. In the univariate analysis, factors associated with premature treatment termination included a lower body mass and lower BMI, a lower eGFR, a lower Hb level, and an increasing chemotherapy line (Tab. 3).

Patients who completed the treatment schedule had a significantly higher BMI, a higher Hb level (> 9.8 g/dL except for one patient), and a higher eGFR (Fig. 1). None of the four underweight patients was able to complete the scheduled treatment (two due to PD, and another two due to treatment-related toxicities).

Table 2. Reasons for treatment non-completion and severity of adverse events

Variables	n = 181	[%]
Treatment		
Completed	121	66.9
Not completed	60	33.2
Reasons for treatment non-completion (n = 60)		
Progression of disease	37	20.4
Unacceptable toxicity	18	9.9
General health status deterioration	5	2.8
Adverse events severity (n = 174)		
1	69	38.1
2	44	24.3
3	37	20.4
4	24	13.3

We also conducted a univariate analysis of quantitative variables of more than two categories and those that differed significantly between the study subgroups that were able and were unable to complete the planned treatment schedule (Fig. 2). The treatment schedule was more often completed in a curative compared to a palliative setting (94% and 58%, respectively) and in those with a good ECOG-PS at baseline (Tab. 3, Fig. 2).

The stepwise multivariate analysis of risk factors for not completing treatment included BMI, an ECOG-PS, an Hb level, an eGFR, and a chemotherapy line. Body mass was not considered due to its close correlation with BMI.

The PS, the Hb level, and treatment line were statistically significant at the 90% confidence level, therefore, they were included in the final model. In addition, in accordance with the AIC, despite the lack of significance in the model using all variables, the BMI was also included, as it showed significance in the univariate model and the model selected by the step method. The coefficients obtained in the model define the influence of selected variables on the chance of implementing the treatment plan. A higher BMI and a higher Hb level were positive predictors of treatment completion, i.e. an increase in BMI by one unit and the Hb level by

Table 3. Completion of planned treatment according to clinical factors

Variable	Treatment not completed n = 60	Treatment completed n = 121	p value
Sex			
Female	30 (34%)	57 (66%)	0.8347
Male	30 (32%)	64 (68%)	
Age [years]	72 (65–83)	70 (65–88)	0.0512
Bodyweight [kg]	69 (47–102)	76 (47.7–115)	0.0007
BMI [kg/m^2]	27.0 (16.3–37.4)	29.1 (20.7–40.6)	0.0073
ECOG-PS			
0	10 (17%)	49 (83%)	0.0001
1	37 (36%)	66 (64%)	
2	13 (68%)	6 (32%)	
eGFR	73.1 (35.6–162.1)	87.5 (29.3–139.9)	0.0015
Hb level [g/dL]	12 (8.5–16.9)	13 (8–16.6)	0.0004
Treatment setting			
Curative	3 (6%)	44 (94%)	0.00001
Palliative	57 (42%)	77 (58%)	
Type of chemotherapy			
Single-agent	27 (39%)	42(61%)	0.2383
Combination	33 (29%)	79 (71%)	
Absolute lymphocyte counts	1.76 (0.7–4.4)	1.89 (0.88–5.93)	0.4704
Percentage of lymphocytes	23 (7–50.9)	27 (9–53)	0.0106

BMI — body mass index; ECOG-PS — Eastern Cooperative Oncology Group performance status; eGFR — expected glomerular filtration rate; Hb — hemoglobin concentration

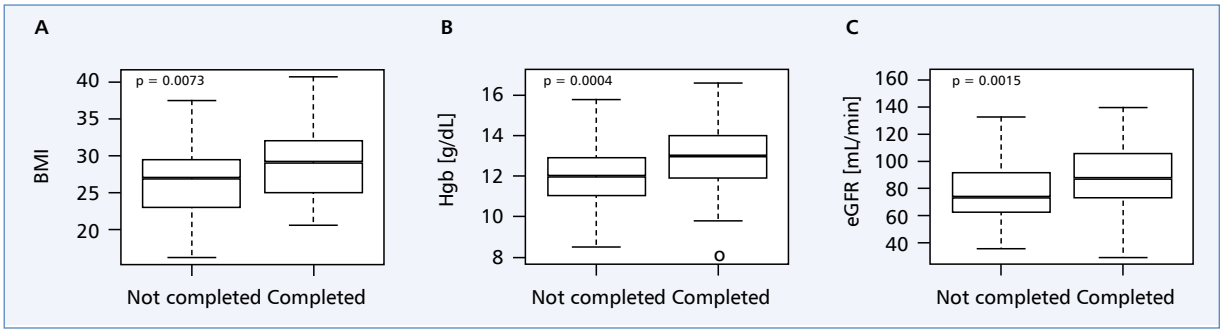

Figure 1. Treatment plan completion by body mass index (**A**), the hemoglobin concentration level (**B**), and the expected glomerular filtration rate (**C**); BMI — body mass index; eGFR — expected glomerular filtration rate

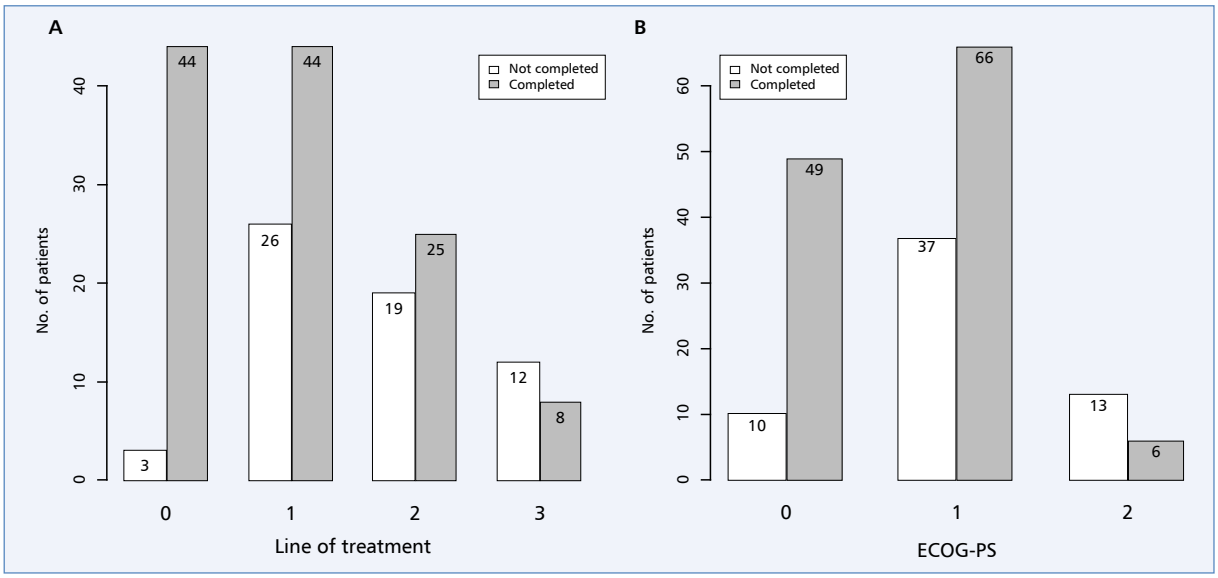

Figure 2. Treatment plan completion by line of treatment (**A**: 0 — currative treatment; 1, 2 i 3 — lines of paliative treatment) and by the Eastern Cooperative Oncology Group performance status (**B**)

1.0 g/dL increased the chance of treatment completion by 9% and 36%, respectively. In turn, increasing the PS and line of chemotherapy by one decreased the odds of treatment completion by 56% and 58%, respectively.

We also evaluated the occurrence of treatment--related toxicities in relation to all studied variables. Due to their small number, patients were divided into none/mild (CTC grade 0–2) and severe (CTC grade 3–4) adverse events groups. Severe adverse events occurred almost twice more often in PS 2 patients (58%) compared to those with PS 1 and PS 0 (31% and 31%, respectively; Tab. 4).

Severe adverse events were more frequent in patients with gastric (70%) and prostate cancers (60%) than in those with colorectal (23%), breast (29%), and lung cancers (36%). Severe adverse events were more frequent in patients with the eGFR <60 ml/min (50.0%) compared to those with the eGFR between 60 and < 90 mL/min and 90 mL/min or more (37% and 24 %, respectively).

Discussion

Many studies show that chemotherapy in elderly patients is equally effective, but sometimes more toxic. Every 5 years after the age of 65, the patient's chance of undergoing planned oncological treatment is significantly reduced.

Adherence to systemic therapies in elderly patients has been a matter of several studies, but factors influencing the ability to complete treatment have been analyzed only occasionally. For example, in a systematic review of the literature including 18 studies, the treatment adherence rate varied from 52% to 100%, but only one qualitative study asked older adults about reasons

Table 4. Treatment toxicity according to selected variables

Variable	CTC G 0–2	CTC G 3–4	Total
ECOG-PS			
0	41 (69%)	18 (31%)	59
1	71 (69%)	32 (31%)	103
2	8 (42%)	11 (58%)	19
Cancer type			
Colorectal cancer	62 (77%)	19 (23%)	81
Breast cancer	30 (71%)	12 (29%)	42
Lung cancer	9 (64%)	5 (36%)	14
Gastric cancer	3 (30%)	7 (70%)	10
Prostate cancer	4 (40%)	6 (60%)	10
Other	12 (50%)	12 (50%)	24
eGFR [mL/min]			
< 60	12 (50%)	12 (50%)	24
61–90	52 (63%)	31 (37%)	83
> 90	56 (76%)	18 (24%)	74

ECOG-PS — Eastern Cooperative Oncology Group performance status; eGFR — expected glomerular filtration rate

for non-adherence [17]. In consequence, factors influencing treatment compliance in elderly patients across particular studies remain inconsistent. Controversial factors include patient age of 75 years or more, comorbidities, marital status, the need for hospitalization, general health condition, and communication abilities, to mention only a few. Most data hitherto have been collected within clinical trials, where the study population may be more motivated to complete the treatment compared with the general population, and our study is one of the few addressing this question in the real-world setting.

Inadequate knowledge on factors influencing chemotherapy compliance may result from different methods of data collection (administrative databases, clinical databases, or chart reviews) and a lack of relevant standardized guidance. For example, a review of 115 phase III trials in breast cancer demonstrated a large variability of reported outcomes, including relative dose intensity, number of cycles, dose modification, and early treatment discontinuation [18].

The prognostic value of age of cancer patients treated by chemotherapy has been a matter of many studies. The systematic review of 708 published papers on the effectiveness and safety of chemotherapy in older patients with colon cancer showed inconclusive data, with studies demonstrating better and worse outcomes in elderly populations [19]. However, grade 3 and 4 treatment-related toxicity in this study was related to age. Similarly, a multicenter review of 895 unresectable pancreatic cancer patients demonstrated no significant difference in survival of younger *vs.* older (> 65 years) patient treated by chemotherapy (333 *vs.* 274 days, respectively $p = 0.09$), and these results remained similar even when the age cut-off for older patients was increased to 70, 75, and 80 years [20].

In our study similarly to other series, BMI was found to significantly impact treatment compliance [4, 21]. In almost half of the patients, the baseline Hb level was below the normal value, including 5.5% of patients with Hb levels below 10 g/dL. As expected, a low Hb level was related to the inability to complete the planned treatment.

Recently, the prognostic value of NLR in cancer and other disorders, such as cardiovascular and infectious diseases, has been addressed [22]. Most studies show a higher NLR value in cancer (3.0) than in inflammatory diseases (1.97–2.5) [23–26]. We have not demonstrated any significant relationship between NRL and the ability to complete scheduled treatment. However, the majority of older patients in this series presented with normal levels of both lymphocytes and neutrophils.

We are aware of the limitations of this study, mainly due to its retrospective nature and patient heterogeneity. Additionally, the analysis of treatment compliance was based only on the ability to complete the planned number of cycles and did not include relative treatment intensity. Nevertheless, this data shed some light on chemotherapy compliance in elderly patients managed in routine practice. Notably, although around one-third of patients were unable to complete planned therapy, in two-thirds of instances treatment interruption was due to disease progression. Hence, age should not be considered a negative selection factor for chemotherapy if not accompanied by other adverse variables. Questionnaires such as Activities of a Daily Living, which assess the ability of a patient to independently care for basic needs like eating, washing, moving around, or the questionnaire called Instrumental Activities of Daily Living, evaluating patients ability to manage finances, do shopping, use a bus, phone, and take medications were

shown to be useful in the assessment of the functional status [27]. In our series of factors related to premature treatment, termination included routinely measured parameters, such as BMI, the PS, or the Hb level. The answer to the question of whether these predictors may be used instead of geriatric assessment scales remains to be established.

Conclusions

— A limited body of knowledge exists in fulfillment of chemotherapy, in elderly patients with solid tumors, outside of clinical trials. Thus, real-world data needs to be explored.
— We demonstrated the feasibility to predict chemotherapy failure in older patients using routinely measured parameters, such as BMI, eGFR, or hemoglobin concentration.
— We have shown that in the palliative setting, the ability to complete the therapy was impaired more often by the disease progression than treatment-related toxicities.
— Thus, our findings may be important in daily practice.

Article Information and Declarations

Conflict of interest

The authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

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Efficacy of pemetrexed plus a platinum rechallenge in the treatment of pleural mesothelioma

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ABSTRACT

Introduction. Pemetrexed-based rechallenge therapies can be used as an option in the treatment of pleural mesothelioma. We aimed to investigate the efficacy of pemetrexed-based rechallenge in mesothelioma.

Material and methods. A total of 132 patients who received chemotherapy for unresectable or metastatic pleural mesothelioma in the Medical Oncology Clinic of Dicle University Medical Faculty between 2005 and 2020 were included in our study. Pemetrexed plus platinum rechallenge treatments were compared with other chemotherapy regimens in terms of survival.

Results. In our study, 31 (23.4%) of a total of 132 patients received rechallenge pemetrexed plus platinum treatment. There was no statistically significant difference between median progression-free survival of patients who received pemetrexed plus cisplatin or gemcitabine plus cisplatin in the first-line therapy [5 months *vs.* 8 months (HR = 1.43; 95% CI 0.59–3.45; p = 0.376)]. In the second-line treatment, patients who received rechallenge pemetrexed plus platinum therapy had statistically significantly higher median PFS than those who received gemcitabine plus platinum [6 months *vs.* 4 months (HR = 0.46; 95% CI 0.22–0.94; p = 0.011)] due to a previous good response. In the second-line treatment, median overall survival was 15 months with gemcitabine plus platinum and 29 months with pemetrexed plus platinum rechallenge (p = 0.007).

Conclusions. This study demonstrated that the pemetrexed plus platinum regimen was more effective than gemcitabine plus platinum in the second-line treatment in terms of both progression-free and overall survival in patients who had previously benefited from pemetrexed-based chemotherapy and had not progressed up to 6 months after first-line treatment.

Key words: pleural mesothelioma, pemetrexed, rechallenge

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Introduction

Mesothelioma is a rare tumor arising from serous structures such as the pleura, pericardium, peritoneum, and tunica vaginalis. Mesothelioma is caused by asbestos exposure [1], and it is observed more frequently in Diyarbakır province and its surroundings compared to other regions of Türkiye due to natural asbestos exposure [2]. Pleural mesothelioma accounts for 80% of all mesotheliomas [3]. Currently, platinum-based chemotherapy and immunotherapy treatments are the standard first-line treatment options for advanced mesothelioma [4]. Phase III prospective randomized trials have shown that cisplatin and antifolate combination therapy is superior to single-agent cisplatin in the first-line treatment of advanced pleural mesothelioma. Early studies have historically shown that adding raltitrexed to cisplatin contributed an overall survival (OS) benefit

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of 2.6 months [5]. On the other hand, Vogelzang et al. [6] reported a 2.8-month OS benefit with the addition of pemetrexed to cisplatin compared to cisplatin alone. The addition of bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF), to combination chemotherapies in the first-line treatment has shown an OS advantage [hazard ratio (HR) = 0.77 (0.62–0.95); p = 0.0167] [7]. Second-line treatment of patients with mesothelioma with the use of pemetrexed and cisplatin provides better response and disease control rates and longer OS than cisplatin in pemetrexed naive patients [8]. Clinical studies are showing the benefit of vinorelbine and gemcitabine in patients progressing after pemetrexed-based chemotherapy administered in the first-line treatment [9, 10]. Rechallenge therapy with pemetrexed in subsequent steps is a strategy that can be used for patients who previously had a good response with pemetrexed [11].

In recent years, immune-checkpoint inhibitors have become a treatment option in addition to platinum-based therapies in pleural mesothelioma [12]. However, there are problems with access to immunotherapy in developing countries due to drug costs. Therefore, chemotherapy rechallenge therapies are used as an alternative treatment strategy. In this study, we aimed to investigate treatment efficacy of in patients who were followed up for pleural mesothelioma in our center and received pemetrexed-based rechallenge therapy in their next-line treatment.

Material and methods

A total of 132 patients who received chemotherapy for unresectable or metastatic pleural mesothelioma in the Medical Oncology Clinic of Dicle University Medical Faculty between 2005 and 2020 were included in our study. We analyzed retrospectively clinicopathologic characteristics [age, sex, smoking, Eastern Cooperative Oncology Group (ECOG) performance status, stage at presentation, and histologic subtype], treatment modalities (surgery, radiotherapy, chemotherapy), treatment responses, and survival times based on the hospital archive system. The postoperative period, first and second-line treatments, and treatment responses were evaluated. Survival rates were compared between the pemetrexed plus platinum rechallenge treatment and other chemotherapy regimens after the pemetrexed plus cisplatin treatment in the postoperative period or first-line treatment.

Patient characteristics

All patients included in the study had histopathologically confirmed mesothelioma diagnoses. Patients whose cancers were resectable at the time of diagnosis underwent pleurectomy/decortication or extrapleural pneumonectomy. In patients who underwent complete resection, pemetrexed plus platinum ± radiotherapy was given postoperatively.

Some of the patients who had received postoperative chemotherapy with pemetrexed plus platinum and who developed relapse 6 months after the end of treatment were given pemetrexed plus platinum rechallenge first-line treatment. Other patients who had postoperative treatment received first-line gemcitabine plus platinum treatment because they relapsed earlier than after 6 months. The number of patients who received immunotherapy or bevacizumab plus chemotherapy was low, and they were not included in the study.

In unresectable or relapsed patients, some of the patients who received pemetrexed plus platinum treatment in the first-line treatment and achieved at least partial response and in whom no progression was observed 6 or more months after the end of treatment were given rechallenge pemetrexed plus platinum treatment in the second-line treatment. Others received second-line gemcitabine plus platinum treatment.

Treatments and definitions

Disease staging was performed according to the American Joint Committee on Cancer (AJCC) classification (version 8 — 2017). The performance status of patients at the beginning of treatment was determined according to ECOG criteria.

Pemetrexed plus platinum regimen — pemetrexed 500 mg/m^2 (day 1) plus cisplatin 75 mg/m^2 or carboplatin AUC 5 (day 1) — was used every 3 weeks (vitamin B12 and folic acid prophylaxis were routinely administered). The gemcitabine plus platinum regimen included gemcitabine 1000 mg/m^2 (days 1 and 8) plus cisplatin 75 mg/m^2 or carboplatin AUC 5 (day 1) every 3 weeks. Postoperative treatment was administered for 6 cycles. In the first- and second-line treatment, chemotherapy was completed in 6 cycles in patients who did not show progression in the first 3 cycles.

Tumor response evaluation was performed every 3 months by computed tomography (CT) or positron emission tomography (PET) according to the RECIST v 1.1 criteria. Progression-free survival (PFS) was calculated as the time from treatment initiation to progression, and OS was calculated as the time from metastatic disease diagnosis to death.

Statistical analysis

PASW Statistics for Windows, Version 18.0. (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were used to evaluate parameter frequency and patient characteristics,

Student's t-test was used for parametric tests with normal distribution, and the Mann-Whitney U test was used for the analysis of non-parametric variables and parametric variables without normal distribution. Kaplan-Meier survival analysis was used for survival analysis, based on log-rank p value. Cox regression analysis was used for univariate and multivariate analysis of survival times. The enter method was used for univariate analysis, and the backward stepwise likelihood ratio method was used for multivariate analysis. The confidence interval (CI) of 95% and two-way p significance value < 0.05 were accepted.

Results

A total of 132 patients, 58 (43.9%) females and 74 (56.1%) males, were included in our study. The median age at diagnosis was 57 (32–78) years. The majority — 83.2% (n = 111) of patients were ECOG 0–1 at diagnosis, and most of them (68.9%) were diagnosed with stage III–IV disease. The most commonly diagnosed was the epithelioid subtype with a rate of 73.6% (n = 84). Almost one-third [33.3% (n = 44)] of patients had undergone surgery. In total, 29 (22%) patients received postoperative pemetrexed plus cisplatin regimen. A total of 55 (41.7%) patients underwent radiotherapy for postoperative, palliative, or drain areas. In the first-line treatment, 71.2% (n = 94) patients received pemetrexed plus cisplatin, 22.7% (n = 30) patients received gemcitabine plus cisplatin, and 6.1% of patients received other treatment regimens. There were 49 (37.1%) patients on second-line treatment. As a second-line treatment regimen, 30.6% (n = 15) of patients received pemetrexed plus platinum, and 69.4% (n = 34) of patients received gemcitabine plus platinum. In total, 31 (23.4%) patients received rechallenge pemetrexed plus platinum treatment. Of the patients who underwent rechallenge treatment, 16 (51.6%) received the same treatment in the postoperative setting and were, therefore, rechallenged in the first-line setting. The remaining 15 (48.4%) patients had received pemetrexed plus platinum in the first-line treatment and were rechallenged with pemetrexed plus platinum in the second-line treatment due to good response during initial chemotherapy. The clinicopathologic features of the patients are presented in Table 1.

When patient characteristics were compared between the groups, patients who received and did not receive postoperative pemetrexed plus cisplatin had similar characteristics in terms of age, sex, smoking, performance status, and histologic type. Again, when the patients who received pemetrexed plus platinum rechallenge in the second step were compared with those who received gemcitabine plus platinum, no statistically significant difference was observed between the clinicopathologic features in both groups (Tab. 2).

Table 1. Characteristics of patients

	n = 132 (%)
Age (median, range)	57 (32–78)
Sex	
Female	58 (43.9)
Male	74 (56.1)
Smoking	
Yes	50 (37.9)
No	59 (44.7)
Unknown	23 (17.4)
ECOG performance status	
0–1	111(83.2)
≥ 2	21 (16.8)
Initial stage	
I–II	41 (31.1)
III–IV	91 (68.9)
Histologic subtypes	
Epithelioid	84 (73.6)
Non-epithelioid	22(16.7)
Unknown	26 (19.7)
Surgery	
P/D	39 (29.5)
EPP	5 (3.8)
No	90 (66.7)
Adjuvant chemotherapy	
Yes	29 (22)
No	103 (78)
Radiation therapy	
Yes	55 (41.7)
No	77 (58.3)
First-line treatment options	
Pemetrexed + cisplatin	94 (71.2)
Gemcitabine + cisplatin	30 (22.7)
Others	8 (6.1)
Second-line treatment options (n = 49)	
Pemetrexed + platin	15 (30.6)
Gemcitabine + platin	34 (69.4)
Pemetrexed re-challenge (n = 31)	
In the first line	16 (51.6)
In the second line	15 (48.4)

ECOG — Eastern Cooperative Oncology Group; EPP — extrapleural pneumonectomy; P/D — pleurectomy/decortication

Table 2. Comparison of patients according to the treatments they receive in the first and second line

All patients (n = 132)	Previously received postoperative treatment (n = 29)	Previously not received postoperative treatment (n = 103)	p value
Age (mean, std dev.)	55.3 (± 11.4)	56.6 (± 10.4)	0.55*
Sex			0.91**
Female	13 (44.8)	45 (43.7)	
Male	16 (55.2)	58 (56.3)	
Smoking (n = 109)			0.10**
Yes	13 (61.9)	37 (42)	
No	8 (38.1)	51 (58)	
ECOG performance status			0.37**
0–1	26 (89.7)	85 (82.5)	
≥ 2	3 (10.3)	18 (17.5)	
Histologic subtypes (n = 106)			0.07**
Epithelioid	23 (92)	61 (75.3)	
Non-epithelioid	2 (8)	20 (24.7)	
Second line (n = 49)	**Pemetrexed + platin rechallenge n = 15 (%)**	**Gemcitabine + platin n = 34 (%)**	**p value**
Age (mean, std dev.)	54.6 (± 9.02)	53.6 (± 10.3)	0.75*
Sex			0.07**
Female	9 (60)	11 (32.4)	
Male	6 (40)	23 (67.6)	
Smoking (n = 41)			0.32**
Yes	6 (42.9)	16 (59.3)	
No	8 (57.1)	11 (40.7)	
ECOG performance status			0.41***
0	14 (93.3)	28 (82.4)	
≥ 1	1 (6.7)	6 (17.6)	
Histologic subtypes (n = 46)			0.41***
Epithelioid	13 (92.9)	26 (81.3)	
Non-epithelioid	1 (7.1)	6 (18.8)	
Initial stage			0.78**
I–II	5 (33.3)	10 (29.4)	
III–IV	10 (66.7)	24 (70.6)	
Primary surgery			0.93**
Yes	9 (60)	20 (58.8)	
No	6 (40)	14 (41.2)	
Radiation therapy			0.83**
Yes	7 (46.7)	17 (50)	
No	8 (53.3)	17 (50)	

ECOG — Eastern Cooperative Oncology Group; *Student's t-test; **Chi-square test; ***Fisher's exact test

There was no statistically significant difference between median PFS of patients who received rechallenge pemetrexed plus platinum in the first-line therapy and patients who received gemcitabine plus platinum in the first-line therapy [5 months *vs.* 8 months (HR = 1.43; 95% CI 0.59–3.45; p = 0.376)] (Fig. 1). In the second-line treatment, patients who received rechallenge pemetrexed plus

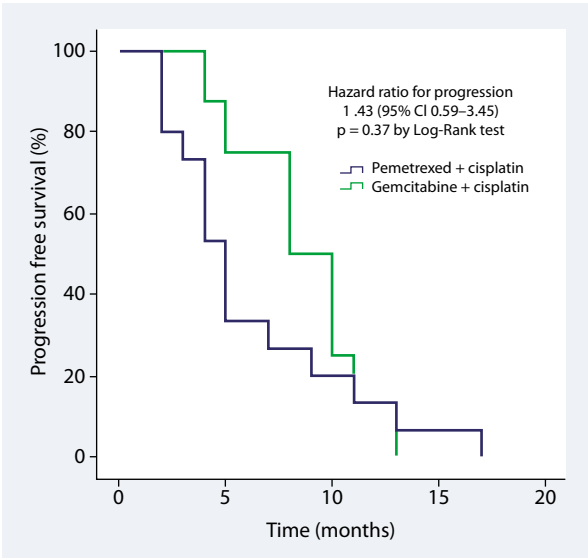

Figure 1. Comparison of progression-free survival results of rechallenge pemetrexed plus cisplatin and gemcitabine plus cisplatin treatments in first-line treatment in patients who developed relapse after adjuvant chemotherapy; CI — confidence interval

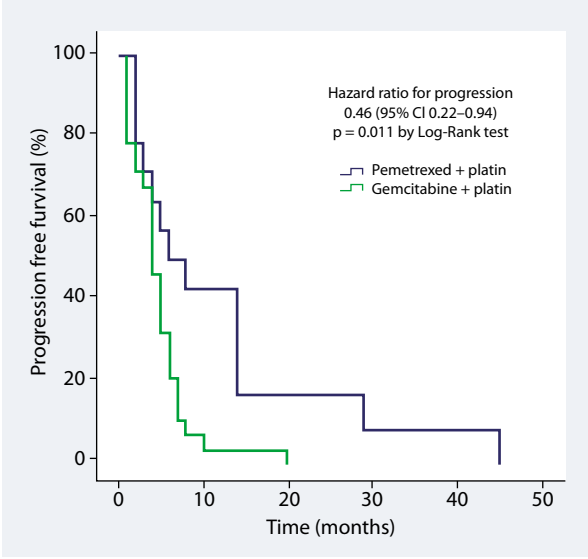

Figure 2. Comparison of progression-free survival results of rechallenge pemetrexed plus platinum and gemcitabine plus platinum treatments in second-line treatment; CI — confidence interval

platinum therapy had statistically significantly higher median PFS than those who received gemcitabine plus platinum [6 months *vs.* 4 months (HR = 0.46; 95% CI 0.22–0.94; p = 0.011)] (Fig. 2). However, patients who received rechallenge pemetrexed plus platinum therapy in the first-line treatment had lower median PFS than patients who received front-line pemetrexed plus platinum therapy [5 months *vs*. 8 months (HR = 1.89; 95% CI 1.01–3.34; p = 0.019)] (Fig. 3). Median OS in chemotherapy-naive patients on first-line treatment was 14 months with pemetrexed plus cisplatin, 12 months with gemcitabine plus cisplatin, and 7 months with pemetrexed plus platinum rechallenge. No statistically significant difference was observed between the groups. In the second-line treatment, median OS was 15 months with gemcitabine plus platinum and 29 months with pemetrexed plus platinum rechallenge (p = 0.007). Objective response rates and other details are given in Table 3.

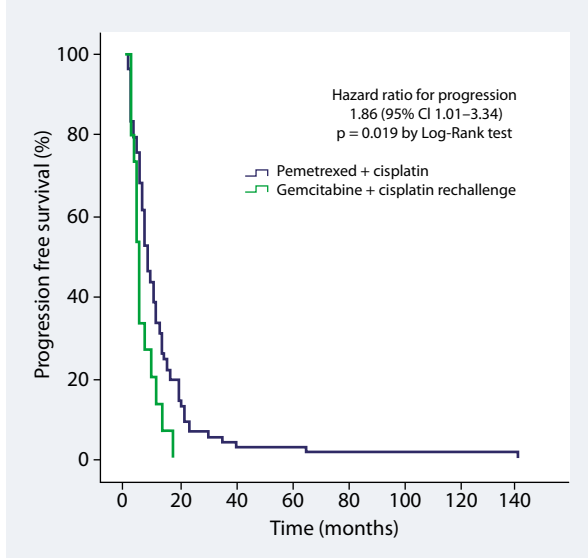

Figure 3. Comparison of progression-free survival results of rechallenge pemetrexed plus cisplatin as first-line treatment in patients with relapse after ajuvant therapy and upfront pemetrexed plus cisplatin treatments in unresectable patients who have not received any previous treatment; CI — confidence interval

When evaluated together with other potential prognostic factors in multivariate analysis, there was no statistically significant difference between median PFS of patients who received pemetrexed plus platinum in the postoperative treatment and during the first-line treatment and median PFS of patients who received gemcitabine plus platinum (HR = 2.06; 95% CI 0.59–7.14; p = 0.25) (Tab. 4). In the second-line setting, median PFS was significantly higher in the rechallenge pemetrexed plus platinum arm than in the gemcitabine plus platinum arm, independently of other prognostic factors (HR = 0.39; 95% CI 0.18–0.85; p = 0.018) (Tab. 5).

In subgroup analysis, when rechallenge pemetrexed plus platinum treatment was compared with gemcitabine plus platinum treatment in terms of PFS, rechallenge pemetrexed plus platinum treatment had higher PFS than gemcitabine plus platinum treatment in patients with good response to pemetrexed plus platinum and a history of radiotherapy (Fig. 4, Tab. S1 — supplementary).

Table 3. Comparison of rechallenge pemetrexed treatment with other treatment arms

	n	ORR [%]	mPFS [mo]	p value*	HR	95% CI	mOS [mo]	p value*
First-line (patients received pemetrexed) n = 94				**0.019**				0.097
Pemetrexed + cisplatin (Chemonaive)	78	36.4	8		reference		14	
Pemetrexed + cisplatin (Re-Ch.)	16	31.3	5		1.89	1.01–3.34	7	
First-line (previously received postoperative treatment P + C) n=24				0.376				0.85
Gemcitabine + cisplatin	8	37.5	8		reference		12	
Pemetrexed + cisplatin (Re-Ch.)	16	31.3	5		1.43	0.59–3.45	7	
Second-line n = 49				**0.018**				**0.007**
Gemcitabine + platin	34	11.7	4		reference		15	
Pemetrexed + platin (Re-Ch.)	15	20	6		0.46	0.22–0.94	29	

CI — confidence interval; HR — hazard ratio; mo — months; mPFS — median progression-free survival; ORR — objective response rate; *log-rank P

Table 4. Univariate and multivariate analysis of first-line progression-free survival outcomes in patients who previously received postoperative pemetrexed plus cisplatin

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age	0.99	0.97–1.01	0.68			
Sex (female*/male)	1.57	1.09–2.27	0.015			
ECOG PS (0–1*/> 2)	1.09	0.67–1.77	0.71			
Histological subtypes (epithelioid*/others)	1.92	1.17-3.13	0.009			
Smoking (no*/yes)	1.66	1.11–2.49	0.014			
Radiation therapy (no*/yes)	0.97	0.68–1.40	0.89	0.43	0.12-1.52	0.19
Chemotherapy regimen (Gem + P*/Pem + P Rch)	1.43	0.59-3.45	0.42	2.06	0.59–7.14	0.25

CI — confidence interval; ECOG PS — Eastern Cooperative Oncology Group performance status; Gem + P — gemcitabine plus platin; HR — hazard ratio; mo — months; Pem + P Rch — pemetrexed plus platin rechallenge; *Reference category

Table 5. Univariate and multivariate analysis of progression-free survival in second-line therapy in patients who had previously used pemetrexed plus cisplatin

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age	1.02	0.99–1.05	0.14	1.01	0.98–1.05	0.31
Sex (female*/male)	1.22	0.66–2.22	0.51			
ECOG PS (0–1*/> 2)	1.17	0.51–2.64	0.70			
Histological subtypes (epitheloid*/others)	0.98	0.40–2.38	0.97			
Smoking (no*/yes)	1.08	0.56–2.09	0.80			
Radiation therapy (no*/yes)	0.73	0.40–1.31	0.29			
Surgery (no*/yes)	1.14	0.63–2.06	0.66	1.55	0.79–3.00	0.19
Chemotherapy regimen (Gem + P*/Pem + P Rch)	0.42	0.20–0.88	0.02	0.39	0.18–0.85	0.018

CI — confidence interval; ECOG PS — Eastern Cooperative Oncology Group performance status; Gem + P — gemcitabine plus platin; HR — hazard ratio; mo — months; Pem + P Rch — pemetrexed plus platin rechallenge; *Reference category

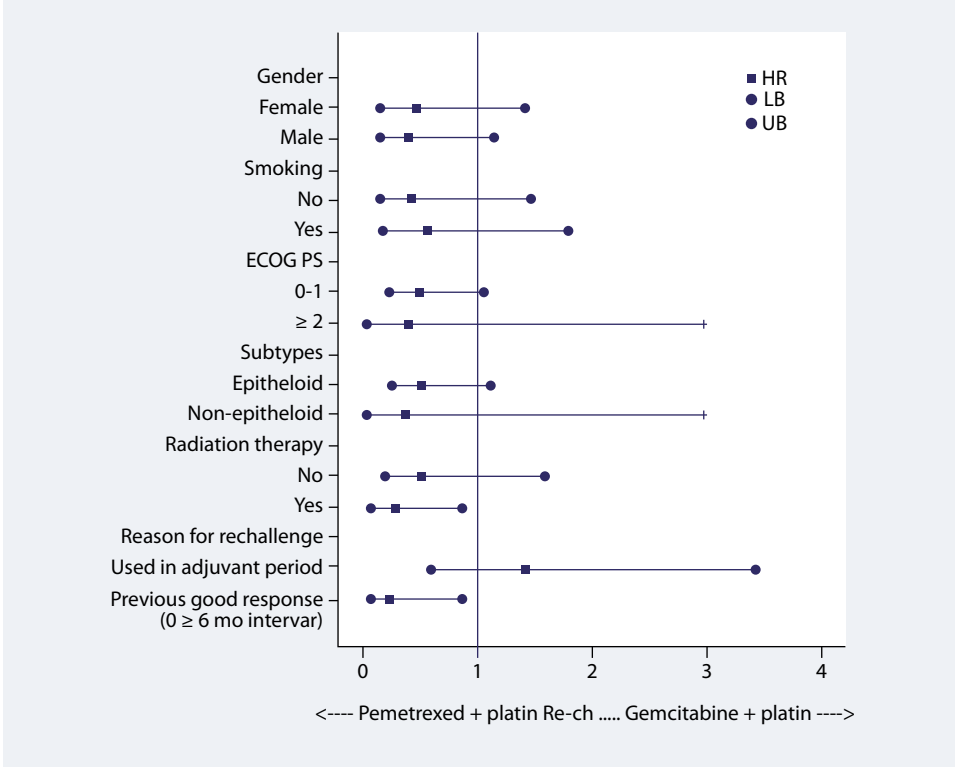

Figure 4. The subgroup analysis for patients who previously received pemetrexed plus platinum in adjuvant treatment included first- and second-line treatments. All other subgroup analysis results were for second-line treatment only; ECOG PS — Eastern Cooperative Oncology Group performance status; HR — hazard ratio; LB — lower bounder; UB — upper bounder

While rechallenge with pemetrexed plus platinum had better results in almost all subgroups, the benefit was greater with rechallenge treatment, especially in patients with a good response to previous pemetrexed plus platinum treatment and a history of radiotherapy.

Discussion

Although pleural mesothelioma is a rare disease, it has a very poor prognosis [13]. Most patients present with unresectable disease. In these patients, pemetrexed plus cisplatin treatment is mostly used in the first-line treatment in regions where access to immunotherapy is problematic [6]. In our study, the majority of patients (66.7%) presented with unresectable disease. The number of patients who underwent surgery for pleural mesothelioma and subsequently developed relapsed metastatic disease was 44 (33.3%). Very few patients with mesothelioma are suitable for surgery. The majority of these patients relapse after surgery. Therefore, pemetrexed and cisplatin combination therapy, which is effective in first-line treatment, may be used in postoperative treatment [14]. In our study, 29 (22%) of the operated patients had received pemetrexed plus cisplatin as adjuvant treatment.

In our study, 94 patients received pemetrexed plus cisplatin combination therapy as first-line treatment. Of these patients, 16 (17%) had previously received pemetrexed plus platinum in the postoperative setting. Rechallenge pemetrexed plus platinum treatment resulted in an objective response rate (ORR) of 31.3% and median PFS of 5 months, while in patients who received no prior treatment, the ORR was 36.1% and median PFS was 8 months. Median PFS was longer in patients who received no prior treatment (HR = 1.89; 95% CI 1.01–3.34; p = 0.019). For those who received pemetrexed-based therapy postoperatively, gemcitabine-based therapies had similar PFS outcomes to rechallenge pemetrexed-based therapy in first-line treatment (HR =1.43; 95% CI 0.59–3.45; p = 0.37). Taylor et al. [15] compared single-agent pemetrexed therapy in chemotherapy-naive patients with patients who had previously received pemetrexed-based therapy and had achieved benefits. In their study, time to progression in chemotherapy-naive patients was 6 months and the ORR reached 10.5%, while time to progression was 4.9 months and the ORR was 12.1% in patients who had received previous treatment [15]. Jänne et al. [16] compared a pemetrexed single agent with pemetrexed plus cisplatin combination therapy in the treatment of previously treated malignant mesothelioma in a phase

III study. In their results, the ORR was found to be 5.5% with single-agent pemetrexed and 32.5% in the combination arm [16]. In our study, median PFS was 5 months and the ORR was 31.3% with first-line pemetrexed platinum rechallenge therapy. Our response rates were similar to the literature. However, in patients who had received pemetrexed plus cisplatin in the postoperative setting, the use of pemetrexed-based combination therapy in first-line treatment was not superior to the use of gemcitabine plus platinum. The addition of bevacizumab to pemetrexed plus cisplatin treatment in first-line treatment improved PFS [7]. Patients receiving bevacizumab were not included in our study. In addition, recent studies with immunotherapy combination have shown that nivolumab plus ipilimumab treatment is effective in the first-line treatment of malignant mesothelioma [17]. In our country, very few patients received immunotherapy because of the problem of access. Therefore, patients receiving immunotherapy were excluded from the study. In countries where access to immunotherapy is problematic, rechallenge therapy remains an important treatment option.

Patients who have not progressed under pemetrexed treatment in first-line treatment have the potential to benefit from pemetrexed treatment in second-line treatment [18]. However, especially as it is understood from retrospective studies, patients in whom the time from the end of first-line treatment to progression is longer than 6 months are more likely to benefit from pemetrexed treatment [19, 20].

In patients who had received platinum in first-line treatment, re-adding platinum in the second-line treatment increased both the disease control rate (70.6% *vs*. 44.6%) and median PFS duration (6.6 months *vs*. 2.5 months). Zucali et al. [21] found that pemetrexed rechallenge therapy in second-line treatment reduced the risk of progression, especially in patients < 65 years of age and time to progression ≥ 12 months. Bearz et al. [19] reported median PFS of 4 months with rechallenge pemetrexed single-agent and 5.7 months with pemetrexed plus platinum in second-line treatment. In another study, Ceresoli et al. [20] found a 19% ORR with pemetrexed single agent and a 48% ORR with platinum combination.

Studies on second-line treatment in mesothelioma have reported median PFS of 3–6 months and OS of 10–12 months with other chemotherapy regimens [22–28]. Second-line immunotherapy produced median PFS of 2.8–6.2 months with tremelimumab and 4 months with avelumab, while the ORRs were found to be 20% with pembrolizumab and 13.2% with nivolumab [29–33]. In our study, the ORR were observed in 15 of 49 patients (30.6%) who received rechallenge pemetrexed plus platinum. The remaining 34 (69.4%) patients were treated with gemcitabine plus platinum. Although both treatment arms had clinicopathologic similarities (Tab. 2), patients who received a rechallenge had a better clinical course compared to the other arm. In the arm receiving rechallenge pemetrexed plus platinum, the ORR was 20%, median PFS was 6 months and median OS was 29 months. In the gemcitabine plus platinum arm, the ORR was 11.7%, median PFS was 4 months, and median OS was 15 months. Both ORR, median PFS, and median OS values were higher in the rechallenge arm (HR for PFS = 0.46; 95% CI 0.22–0.94; p = 0.018), (log-rank p = 0.007 for OS). We found that pemetrexed plus platinum combination therapy may be an effective treatment option for second-line treatment in patients with time to progression ≥ 6 months for whom this therapy has shown efficacy after first-line treatment. In our study, when evaluated together with other potential prognostic factors in multivariate analysis, the use of rechallenge pemetrexed plus platinum in the second line was the only independent prognostic factor for PFS. In the subgroup analysis performed in patients receiving rechallenge pemetrexed treatment, radiotherapy and benefit from previous pemetrexed treatment (response with previous pemetrexed treatment and time to progression ≥ 6 months) were observed as predictive factors for PFS. Zucali et al. [21] reported that patients aged < 65 years and with time to progression ≥ 12 months achieved better PFS than rechallenge treatment patients. However, many retrospective data have reported that if time to progression is ≥ 6 months, the potential to benefit from rechallenge treatment may be high [19, 20].

The limitations of our study were that it was a single-center retrospective study, the patient groups were heterogeneous, and the number of patients was small. In addition, the group of patients who underwent rechallenge consisted of patients with a better clinical course. This should be taken into account when evaluating the results of the study.

Conclusions

We found that pemetrexed plus cisplatin treatment after postoperative use of the same regimen had similar efficacy to gemcitabine plus cisplatin treatment. In second-line treatment, we found that pemetrexed plus platinum was a more effective therapeutic option than gemcitabine plus platinum in patients who had previously benefited from pemetrexed-based treatment and had not progressed up to 6 months after first-line treatment.

Article Information and Declarations

Data availability statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles defined in the Declaration of Helsinki (permit no: 10/2021).

Author contributions

Z.U.: conception and design of the study, writing of the article.
S.E.: data analysis and interpretation
Z.O.: acquisition of clinical data.
M.A.K.: data analysis and interpretation.
M.K. and Z.K.: acquisition of clinical data.
A.I.: data analysis and interpretation.
All authors have read and approved the final version of this manuscript and have consented for publication.

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Conflict of interest

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Supplementary material

Supplementary Table S1.

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Supplementary material

Table S1. Pemetrexed plus platin *versus* gemcitabine plus platin subgroup analysis results

	HR	CI 95%	p value
Sex			
Male	0.38	0.12–1.15	0.88
Female	0.46	0.15–1.42	0.17
Smoking			
Yes	0.56	0.17–1.78	0.33
No	0.44	0.13–1.47	0.18
ECOG PS			
0–1	0.49	0.23–1.05	0.06
≥ 2	0.02	0.01–104	0.38
Subtypes			
Epithelioid	0.52	0.24–1.13	0.10
Others	0.36	0.01–264	0.46
Radiation therapy			
Yes	0.27	0.08–0.88	0.03
No	0.53	0.17–1.60	0.26
Reason for rechallenge			
Previous good response (≥ 6 mo interval)	0.22	0.06–0.87	0.03
Used in adjuvant period	1.43	0.59–3.45	0.42

CI — confidence interval; ECOG PS — Eastern Cooperative Oncology Group performance status; HR — hazard ratio; mo — months

VIA MEDICA

ORIGINAL ARTICLE

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Pathological complete response and survival of HER2-positive invasive breast cancer following docetaxel, carboplatin, and trastuzumab neoadjuvant therapy: a Vietnamese experience

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ABSTRACT

Introduction. Neoadjuvant chemotherapy for HER2-positive breast cancer consists of a chemotherapy regimen plus trastuzumab with or without pertuzumab. The use of trastuzumab has been shown to improve pathological complete response (pCR), disease-free survival (DFS), and overall survival (OS). Purposes: To evaluate the efficacy and safety of neoadjuvant docetaxel, carboplatin, and trastuzumab (TCH) in the treatment of HER2-positive breast cancer in Vietnamese patients.

Material and methods. This retrospective study reviewed stage II–III HER2-positive breast cancer patients who received neoadjuvant docetaxel, carboplatin, and trastuzumab (TCH) at the Vietnamese National Cancer Hospital. The primary endpoint was the pCR rate which was defined as the absence of invasive tumor in the breast and axillary nodes (ypT0/is, ypN0). The secondary endpoints were DFS, OS, and toxicities.

Results. The complete and partial clinical response of 51 patients were 33.3% and 58.8%, respectively. The pCR rate was 41.2%; there was a significantly higher response in cT1-2 patients compared to cT3-4 ones (61.1% *vs.* 39.3%, p = 0.033). Three-year estimated DFS and OS rates were 81.3% and 93.0%, respectively. Treatment was generally well tolerated. Grade 3/4 neutropenia and anemia were uncommon (21.6% and 7.8%). No symptomatic cardiac dysfunction occurred.

Conclusions. Neoadjuvant TCH, non-anthracycline chemotherapy with single anti-HER2 regimen achieved high efficacy, with a good pCR rate and favorable tolerability in stage II or III HER2-positive breast cancer patients.

Key words: breast cancer, HER2-positive, neoadjuvant chemotherapy, pCR, TCH

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Introduction

Breast cancer (BC) is one of the most common cancers and the leading cause of malignancy-related mortality in women worldwide [1, 2]. In Vietnam, breast cancer is the most common cancer and the fourth leading cause of cancer-related death in women. The International Agency for Research on Cancer reported an estimated 21 555 new cases and 9 345 deaths of breast cancer in Vietnam [2]. Treatment for breast cancer is complex due to its heterogeneity and various molecular subtypes. Among them, newly diagnosed patients in the HER2 overexpression subtype, which was previously considered as an aggressive phenotype with poor prognosis [3–5], accounted for 15–20% of patients.

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Neoadjuvant chemotherapy (NAC) is commonly used for breast cancer patients not only with locally advanced stage but also patients in an early stage, especially with poor prognosis with triple-negative and HER2--positive tumors [6, 7]. In addition to increasing the rate of breast-conserving surgery [8], NAC permits evaluation of the effectiveness of systemic treatments to guide adjuvant treatment [9]. Response to NAC also provides important prognostic information. Patients with pathological complete response (pCR) were reported to have better long-term outcomes [10–12]. NAC for HER2-positive breast cancer consists of chemotherapy and HER2-directed therapy, specifically trastuzumab, with or without pertuzumab. The use of trastuzumab has been shown to improve pCR, disease-free survival, and overall survival [13]. Nevertheless, the addition of trastuzumab to standard therapy may increase toxicity, particularly cardiovascular toxicity [13, 14]. This toxicity is increased when trastuzumab is used concurrently with an anthracycline-containing chemotherapy regimen. Due to concerns about cardiotoxicity, anthracycline-free chemotherapy plus trastuzumab have been explored. The addition of carboplatin and docetaxel to trastuzumab (TCH regimen) was shown to have a synergistic effect in some studies [15–17]. The pCR rates achieved by the TCH regimen in the neoadjuvant setting ranged from 39% to 76% [17–20]. This regimen has less incidence of acute toxicity, cardiotoxicity, and more favorable tolerability. However, most evidence about the efficacy of this regimen was from the adjuvant setting or phase II studies [20–22]. In the GETN(A)-1 trial, a multicenter neoadjuvant study, 70 patients with HER2-positive breast cancer with diagnosed stage II–III received trastuzumab 4 mg/kg (day 1), followed by 2 mg/kg weekly, plus docetaxel 75 mg/m^2 every 3 weeks, and carboplatin (AUC 6) for six cycles before surgery. The pCR rate (ypT0/is ypN0) was 39%, and the objective response rate (ORR) was 95%. Sixty-four percent of the patients had breast conservation and no symptomatic cardiac dysfunction occurred [23]. However, the efficacy and safety of TCH regimens for neoadjuvant therapy have not been evaluated in Vietnamese women with HER2-positive breast cancer. Thus, we conducted this study to evaluate the pCR rates, toxicity profile as well as preliminary results for DFS and OS of the TCH regimen in HER2-positive breast cancer patients with stage II–III in Vietnam.

Material and methods

Study design

In this single-center, retrospective study, 51 HER2--positive breast cancer patients with stage II–III who were treated with a neoadjuvant TCH regimen from January 2015 to December 2021 at the Vietnamese National Cancer Hospital were recruited. The eligible patients need to meet all the following criteria: Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, histopathological diagnosis of invasive breast cancer and immunohistochemical result of HER2-positive, staging II–III (cT1-4, cN0-3, M0), received neoadjuvant therapy with a TCH regimen, a baseline left ventricular ejection fraction (LVEF) of ≥ 50%, adequate hematologic, renal, and hepatic functions. Patients with the following criteria were excluded: bilateral breast cancer or metastatic breast disease; any previous treatment for breast cancer including surgery, radiation, chemotherapy, or endocrine therapy; pre-existing malignancy other than breast cancer; any prior treatment with cytotoxic drugs, in situ carcinoma, another breast malignancy (ex. malignant phyllode tumor). The diagnosis of BC was confirmed by histological evaluation of the biopsy specimens before treatment. An immunohistochemical (IHC) examination was performed before treatment. HER2-positive status was determined by IHC (3+) or IHC (2+) and positive fluorescence in situ hybridization (FISH) using the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) 2018 guidelines. This study was approved by the research committee of the National Cancer Hospital, Vietnam.

Treatment procedures

All patients had their clinical staging evaluated at diagnosis, using the 8th edition of the American Joint Committee on Cancer (AJCC). Physical examination, mammography, and ultrasound were usually performed at baseline and after every three chemotherapy cycles to evaluate clinical response. Treatment includes six cycles of docetaxel 75 mg/m^2, carboplatin AUC6, and trastuzumab 6 mg/kg every 3 weeks (8 mg/kg loading dose). For each cycle, prophylactic G-CSF support was administered on days 2 to 5. Echocardiography to evaluate cardiac function was performed before initiation of therapy, after the third and sixth cycles. Then, LVEF assessments were carried out every 3 months and 1 year after the last cycle of treatment or whenever clinically indicated. After the completion of neoadjuvant chemotherapy, surgery was performed to remove the tumor by conservative surgery or modified radical mastectomy, combined with axillary lymph node dissection within 4–6 weeks after the final dose of chemotherapy. Following surgery, adjuvant endocrine therapy and radiotherapy were administered if indicated. Adjuvant trastuzumab (loading dose 8 mg/kg, followed by 6 mg/kg every 21 days) was continued postoperatively for up to 18 cycles.

Tumor response and toxicity assessment

Clinical response was evaluated by palpation after each treatment cycle and by mammary ultrasound, mammography, or magnetic resonance imaging before surgery, using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Following surgery, tumors were evaluated in their maximum diameter. Tumor and nodal samples were examined with histopathological tests to assess the pathological response. The pCR was defined as the absence of invasive tumor in breast and axillary lymph nodes (ypT0/is ypN0). Disease-free survival (DFS) was defined as the period between the date of surgery and the date of disease relapse (including distant metastases, local and regional recurrence) or death, whichever occurred first. Overall survival (OS) was measured from the date of the diagnosis to death due to any cause. Toxicities after and during six courses of chemotherapy were evaluated according to the Common Terminology Criteria for Adverse Events version 4.0.

Variables of interest

Variables used for analyzing include age (< 50 and ≥ 50 years), performance status (PS) (PS = 0 and PS = 1), menopausal status (premenopausal and postmenopausal), histologic grade (ductal carcinoma and others), clinical tumor stage (T1–T2 and T3–T4), clinical lymph nodal stage (N0–N1 and N2–N3), clinical stage (stage II and stage III), ER/PgR status (positive and negative), and pathological response (pCR and non pCR). All 51 patients were contacted via phone or messages to collect real-time information.

Statistics

All collected data were analyzed and measured by SPSS 20.0 software. Fisher's exact test and Pearson Chi-square test were used to evaluate impact of various factors on the pCR. Disease-free survival and overall survival were estimated by the Kaplan-Meier method. A 2-sided p value of <0.05 was considered statistically significant.

Results

Patient characteristics and treatment

Between February 2015 to December 2021 in the National Cancer Hospital, Vietnam, 51 patients with stage II–III HER2-positive breast cancer were enrolled in the study. All patients received six courses of NAC chemotherapy. Table 1 shows the baseline clinicopathological features of patients. The median age was 46 years old and over half of the women were premenopausal. Considering the cTNM, BCs were staged in

Table 1. Baseline characteristics of 51 patients with HER2+ invasive breast cancer carcinoma

Parameter	n	[%]
Age [years]		
Median (range)	46.0 (26–70 years)	
< 50 years	30	58.5
≥ 50 years	21	41.2
Performance status		
0	42	82.4
1	9	17.6
Menopausal status		
Premenopausal	32	62.7
Postmenopausal	19	37.3
Histology		
Invasive ductal carcinoma, NOS	39	76.5
Invasive lobular carcinoma	9	17.6
Invasive mucinous carcinoma	2	3.9
Invasive carcinoma, unclassified	1	2.0
Histological grade		
1	1	2.0
2	34	66.7
3	16	31.4
Clinical tumor stage		
T1	1	2.0
T2	17	33.3
T3	21	41.2
T4	12	23.5
Clinical nodal stage		
N0	8	15.7
N1	12	23.5
N2	27	52.9
N3	4	7.8
Clinical stage		
II	13	25.5
IIIA	22	43.1
IIIB	12	23.5
IIIC	4	7.8
ER/PgR status		
Negative	13	25.5
Positive	38	74.5
Operation type		
Mastectomy	44	86.3
Breast-conserving surgery	7	13.7
Clinical response		
CR	17	33.3
PR	30	58.8
SD	4	7.8
PD	0	0.0
Pathologic response		
pCR	21	41.2
Non-pCR	30	58.8

CR — complete response; ER — estrogen receptor; NOS — not otherwise specified; pCR — pathologic complete response; PD — progressive disease; PgR — progesterone receptor; PR — partial response; SD — stable response

Table 2. Factors associated with a pathologic complete response (pCR)

Factors	pCR		Analysis		p
	No.	%	OR	95% CI	
Age [years]					
< 50 years	12	40.0	1 (reference)		
≥ 50 years	9	42.9	0.889	0.287–2.756	0.838
Histologic type					
Ductal carcinoma, NOS	17	42.5	1 (reference)		
Other	4	36.4	1.293	0.326–5.137	0.714
Histologic grade					
1 or 2	17	48.6	1 (reference)		
3	4	25.0	2.833	0.763–10.516	0.112
Clinical tumor stage					
T1–T2	11	61.1	1 (reference)		
T3–T4	10	30.3	3.614	1.084–12.046	0.033
Clinical lymph node status					
N0–N1	8	40.0	1 (reference)		
N2–N3	13	41.9	0.923	0.294–2.898	0.891
Clinical stage					
II	6	46.2	1 (reference)		
III	15	39.5	1.314	0.369–4.679	0.673
ER/PgR status					
Negative	8	61.5	1 (reference)		
Positive	13	34.2	3.077	0.836–11.323	0.084

CI — confidence interval; ER — estrogen receptor; NOS — not otherwise specified; OR — overall survival; PgR — progesterone receptor

T3 and N2 which were the most common (43.1 and 52.9%, respectively). Therefore, BCs with stage IIIA (39.2%) and stage IIIB (35.3%) were more common than the other stages. Concerning pathological features, invasive ductal carcinoma, not otherwise specified (NOS) was the most common. Histological grade II was the most common (66.7%). Most patients (74.5%) were positive for hormone receptors (estrogen and/or progesterone receptor; HR) while 25.5% were HR-negative.

Clinical and pathological response

Based on the RECIST criteria, the clinical response and degree were investigated, complete response (CR), partial response (PR), and ORR were 33.3%, 58.8%, and 92.2%, respectively. Seven patients (13.7%) underwent breast-conserving surgery. Pathological complete response was achieved in 41.2% (Tab. 1). Table 2 presents the relationship between clinical and paraclinical features and pCR for HER2-positive BC. Pathological complete response rate was higher in hormone receptor-negative patients compared to hormone receptor-positive patients (61.5% *vs.* 34.2%, p = 0.084). Pretreatment tumor stage was significantly related to response to NAC. Lower cT-stage (cT1-2 *vs.* cT3-4) was a significant predictor of higher pCR rate (p = 0.033), i.e. pCR rates were 61.1% and 30.3% for cT1-T2 stage and cT3-T4 stage, respectively. In addition, the pCR rate was not significantly different irrespective of age groups, clinical lymph node status, histologic grade, and histologic type (p > 0.05).

Long-term outcomes

The median follow-up was 33.0 months. Eight of 51 patients (15.7%) had experienced at least one event. Five patients (9.8%) experienced local relapse (including local lymph node relapses), and 6 patients (11.8%) had metastatic relapses. Estimated 3-year DFS was 81.3% (Fig. 1). Patients who achieved pCR after NAC had better DFS than the ones with residual disease although the difference was not statistically significant (89.7% *vs.* 72.3%, p = 0.220) (Fig. 2). Additionally, DFS was not significantly different with age group, histologic type, histologic grade, clinical tumor stage, clinical lymph node stage, clinical stage and HR status (Tab. 3). Two patients (3.9%) died at 31 months and 34 months. One patient (1.9%) died of lung metastases and the other

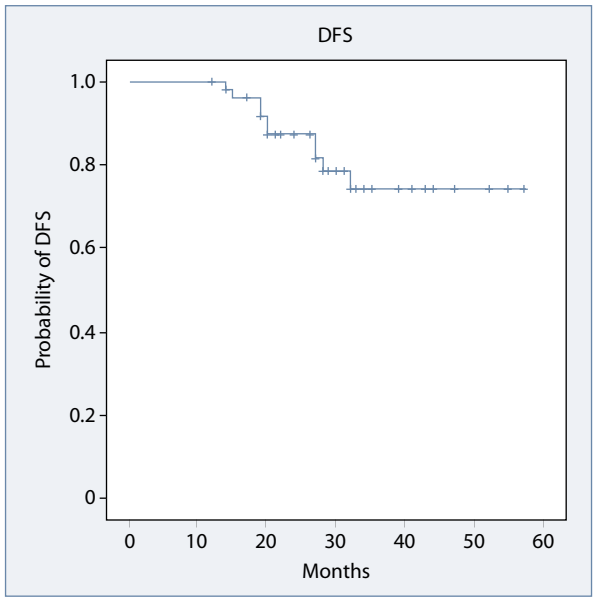

Figure 1. Disease-free survival of HER2+ invasive breast cancers. Kaplan-Meier curve displayed the estimated 3-year disease-free survival (DFS) was 81.3%

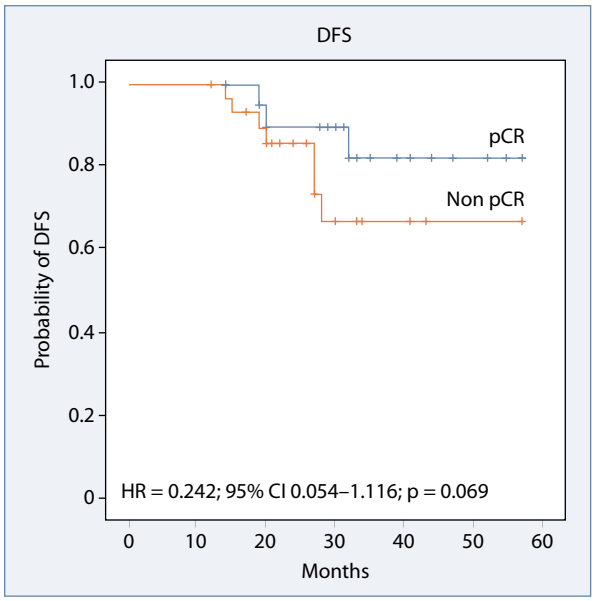

Figure 2. Disease-free survival (DFS) of pathologic response status in HER2+ invasive breast cancers. The Log-rank test displayed that there was not a significant difference between these DFS curves of combination of pCR and non-pCR for infiltrating HER2+ breast cancers; CI — confidence interval; HR — hazard ratio; pCR — pathologic complete response

Table 3. Factors affecting disease-free survival

Factors	No.	HR	95% CI	p
Age [years]				
< 50 years	30	1 (reference)		
≥ 50 years	21	0.331	0.067–1.645	0.177
Histologic type				
Ductal carcinoma, NOS	40	1 (reference)		
Other	11	1.276	0.260–6.262	0.764
Histologic grade				
1 or 2	35	1 (reference)		
3	16	0.287	0.041–2.023	0.210
Clinical tumor stage				
T1–T2	18	1 (reference)		
T3–T4	33	0.716	0.151–3.407	0.675
Clinical lymph node status				
N0–N1	20	1 (reference)		
N2–N3	31	0.913	0.519–153.186	0.132
Clinical stage				
II	13	1 (reference)		
III	38	1.030	0.044–23.930	0.985
ER/PgR status				
Negative	13	1 (reference)		
Positive	38	3.477	0.356–33.962	0.284
Pathologic response				
Non pCR	30	1 (reference)		
pCR	21	0.246	0.054–1.116	0.069

CI — confidence interval; ER — estrogen receptor; HR — hazard ratio; NOS — not otherwise specified; pCR — pathologic complete response; PgR — progesterone receptor

(1.9%) died of bone and brain metastases. Both patients (3.9%) did not achieve pCR after surgery. Estimated 3-year OS was 93.0% (Fig. 3).

Safety and tolerability

Toxicities in our patients are presented in Table 4. Anemia and neutropenia were the most common serious (grade 3/4) adverse events. All patients completed 6 cycles of planned chemotherapy, with 12 patients requiring dose adjustments due to toxicity. None of the patients had LVEF decline or clinical symptoms of heart failure. There were no deaths related to treatment.

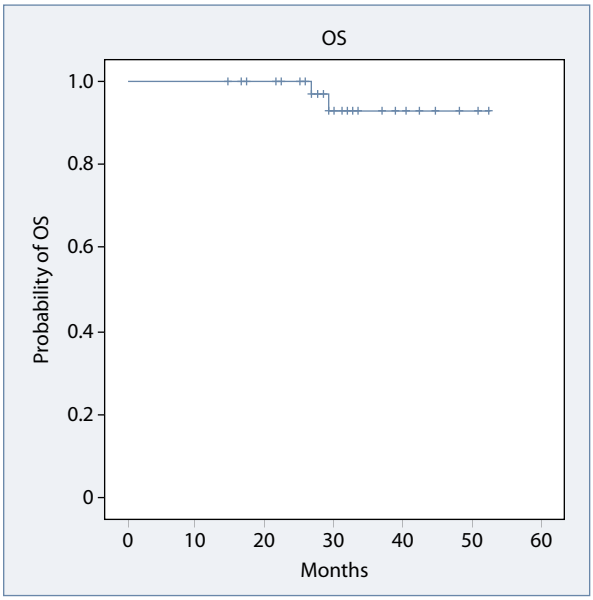

Figure. 3. Overall survival (OS) of HER2+ invasive breast cancers. Kaplan-Meier curve displayed the estimated 3-year OS was observed in 93.0%

Discussion

Breast cancer patients with HER2 overexpression typically demonstrate a poor prognosis due to high malignancy. In the adjuvant setting, this poor prognosis has been significantly improved by anti-HER2 therapy with trastuzumab [24]. In the neoadjuvant setting, the addition of a HER2-targeted therapy to chemotherapy has resulted in an increased rate of pCR and improved DFS and OS [25]. In recent years, the TCH regimen has been increasingly used in some countries but in Vietnam, this regimen has not been widely applied. Our study aimed to evaluate the safety and efficacy of the TCH regimen in a neoadjuvant setting for HER2--positive breast cancer in daily clinical practice. The ORR and pCR were obtained in 92.2% and 41.2% patients, respectively. This result was slightly lower than the results described in previous publications (Tab. 5 [15, 17, 23, 26, 27]). Sugitani et al. [17] reported on 50 HER2-positive patients with stage I–III invasive breast cancer, a pCR of 52% with the TCH regimen. Meanwhile, a retrospective analysis of Echavarria et al. [15] on 84 HER2-positive patients with stage I–III receiving the same regimen demonstrated that clinical characteristics were 2.4%, 65.5%, and 32.1% for

Table 4. Selected adverse events on six-course chemotherapy

Event	All grade		Grade 3/4	
	n1	[%]	n2	[%]
Hematologic toxicities				
Anemia	37	72.5	4	7.8
Neutropenia	23	45.1	11	21.6
Thrombocytopenia	10	19.6	2	3.9
Nonhematologic toxicities				
Infection with neutropenia	0	0	0	0
Infusion reaction	6	11.8	0	0
Mouth ulcer	20	39.2	0	0
Anorexia	39	76.5	1	1.9
Vomiting	10	19.6	0	0
Diarrhea	17	33.3	0	0
Peripheral neuropathy	26	51.0	0	0
Renal toxicity	0	0	0	0
Cardiac toxicity	0	0	0	0
Hepatic dysfunction	18	35.3	0	0

Table 5. Clinical trials and observational analysis on the use of docetaxel–carboplatin–trastuzumab regimen

	n	cCR [%]	PR [%]	ORR [%]	ypT0/isN0 [%]	Design
Sugitani et al. [17]	50	10	56	66	52	Phase II trial
Echavarria et al. [15]	84	34.5	63.1	97.6	47.6	Retrospective analysis
Coudert et al. [23]	70	85	10	95	39	Phase II trial
Kolberg et al. [26]	78				43.6	Retrospective analysis
Bayraktar et al. [27]	65	58.9	19.6	78.5	43.3	Retrospective analysis

cCR — clinical complete response; ORR — objective response rate; PR — partial response; ypT/isN0 — lack of invasive tumor in the breast and axillary lymph nodes

stages I, II, and III, respectively, and the pCR rate was 47.6%. In another study of 39 BC patients who were treated with AC-TH or TCH regimens, Phung et al. [28] showed that clinical complete response (cCR) and pCR rate was obtained in 33.3% and 64.1%, respectively. This observed difference may be due to slightly different patient characteristics. Notably, our cohort had a lower proportion of patients with HR-negative disease (25.5%) as compared to Echavarria et al.'s [15] (45.5%) and Sugitani et al.'s [17] (50.0%) research, which is a subset known to be more sensitive to NAC. Additionally, in our study, another reason is that stage III patients were accounted for a higher percentage than in other studies.

Previous meta-analyses demonstrated lower pCR rates in luminal/HER2 than in non-luminal/HER2 tumors. The KRISTINE trial showed that HR-negative breast cancer patients had up to 19% higher pCR rates than HR-positive patients [29]. This discrepancy might be explained that PIK3CA mutations are associated with reduced rates of pCR to anti-HER2 therapy in HER2--positive/HR-positive tumors [30, 31]. In our study, patients with hormone receptor-negative tumors also had a better response to chemotherapy than the ones with HR-positive tumors (61.5% *vs.* 34.2%). However, we did not observe a statistically significant difference in the pCR rate between two groups, which could be due to our relatively small sample size. Our results show that higher cT-stages have significantly lower pCR rates than lower cT-stages (cT3-4 *vs.* cT1-2; $p < 0.033$). The cT-stage is one of the most important predictors of pCR in breast cancer patients. A study by Caudle et al. [32] on 1 762 patients showed that the tumor stage was a predictive factor of disease progression. Jin et al. [33] also concluded that tumor size was an independent predictor of pathological complete response. The patients with larger tumor sizes were less likely to achieve pCR than those with smaller tumor sizes. The pCR rates for cT1, cT2, cT3, and cT4 were 23.6%, 13.6%, 11.9%, and 10.3%, respectively [33]. Goorts et al. [34] ($n = 2\,366$) showed that for cT1, cT2, cT3, and cT4, pCR rates were 31%, 22%, 18%, and 17%, respectively. Lower cT-stage was a significant predictor of higher pCR rate ($p < 0.001$) [34]. So, clinicians should take cT-stage into account when estimating the likelihood of achieving pCR in an individual patient.

In our study, the median follow-up was 33.0 months. Two patients died at 31 months and 34 months and both of these patients did not achieve pCR after NAC. Estimated 3-year OS and DFS were 93.0% and 81.3%, respectively. In addition, 3-year DFS was better in the pCR group than in the non-pCR group (89.7% *vs.* 72.3%) although there was no significant difference, which may be due to the small study population and inadequate follow-up time. In addition, we found that patients who achieved pCR after NAC had better long-term outcomes despite not achieving statistical significance due to low event data [35].

The TCH regimen was generally well tolerated. Most adverse events were manageable. All the patients were able to complete the planned number of chemotherapy cycles. Anemia (7.8%), grade 3/4 neutropenia (21.6%), and thrombocytopenia (3.9%) were the most common adverse events. No patient experienced febrile neutropenia. In the TRYPHAENA trial, grade 3/4 neutropenia and febrile neutropenia were 46% and 17%, respectively. A study by Sugitani et al. [17] on breast cancer patients treated with a TCH regimen showed grade 3/4 neutropenia (36%), anemia (12%), thrombocytopenia (2%), and febrile neutropenia (6%). Echavarria et al. [15] reported febrile neutropenia and grade 3–4 neutropenia accounting for 6.0% and 16.7% of patients. Patients in our study had a considerably lower rate of adverse events presumably due to prophylactic administration of filgrastim to all patients.

None of the patients developed clinical congestive heart failure during the follow-up period. The safety of this regimen and reduced cardiac complication has also been demonstrated in previous studies in adjuvant and neoadjuvant settings [17, 18]. The BCIRG-006 study found that the anthracycline-free 1-year TCH regimen was associated with a lower risk of asymptomatic LVEF decline (9.4%) and congestive cardiac failure (CCF) (0.4%) compared to doxorubicin, cyclophosphamide, docetaxel, and trastuzumab (18.6% LVEF decline, 2% CCF) or doxorubicin, cyclophosphamide, and docetaxel

(11.2% LVEF decline, 0.7% CCF) [24]. When compared to other regimens that do not contain anthracyclines, the TCH regimen generally has a more favorable safety profile with regard to neutropenia and febrile neutropenia. On the other hand, patients treated with TCH regimens were associated with a significant reduction of cardiotoxicity [17, 24]. A study in Poland on 34 breast cancer patients treated with Neoadjuvant Pertuzumab Plus Trastuzumab in Combination with Docetaxel and Carboplatin regimen confirmed that the regimen is safe and relatively effective. No patients with myocardial dysfunction or a significant decrease in LVEF were observed [36].

Our study has a few limitations. Besides the retrospective nature, the relatively small sample size and short follow-up period may have precluded more significant results regarding predictive factors of pCR, DFS, or OS. Further studies with larger sample sizes may need to be conducted to fulfill these limitations.

Conclusions

In conclusion, neoadjuvant chemotherapy with a TCH regimen showed promising efficacy in HER2--positive breast cancer with high clinical and pathological CR rates while being safe and well-tolerated. This regimen should be used more in the neoadjuvant setting, especially in cases of concern with anthracycline toxicity.

Article Information and Declarations

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics statement

This study was approved by the research committee of Vietnam's National Cancer Hospital under number: 2147/QD-BVK.

Written informed consent was obtained for all patients before enrolling them in the study.

Author contributions

D.T.L.: should be considered the major author. He participated directly in diagnosis, treatment, and follow-up of the patients, performed the literature review, and assisted in drafting parts of the study and formatting the presented material.

K.H.D. and T.A.D.: took part in the diagnostic and treatment consultant and, assisted in the literature review.

L.T.B.: performed patient follow-up, review of patients' records, literature review, and assisted in drafting parts of the study.

C.V.N.: performed diagnostic consultation on the HE stains and immunohistochemical staining, and assisted in the literature review, drafting parts of the study, and formatting the presented material.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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VIA MEDICA

ORIGINAL ARTICLE

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Is the biology of breast cancer different in patients ≥ 80 years old?

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ABSTRACT

Introduction. The highest incidence of cancer occurs in the seventh and eighth decades of life, hence with the lengthening of human life, the number of seniors diagnosed with cancer is increasing. For years, breast cancer has remained the most commonly diagnosed cancer in women in Poland. There is a belief that breast cancer in elderly women has a milder course, grows more slowly, and is biologically less aggressive compared to younger patients.

Material and methods. This study presents characteristics of the biology of 240 breast cancers diagnosed in 232 patients aged ≥ 80 years and compares them with the biology of 295 breast cancers diagnosed in 291 patients in other age groups.

Results. Evaluating breast cancer biology in patients ≥ 80 years of age compared to patients < 80 years of age in our data showed no statistically significant differences.

Conclusions. The belief that breast cancers are less aggressive in the elderly was not confirmed in our study.

Key words: breast cancer at age ≥ 80 years, breast cancer, breast cancer biology

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Introduction

The highest incidence of cancer occurs in the seventh and eighth decades of life due to the relationship between aging and carcinogenesis [1]. As human life expectancy increases, so does the number of elderly people diagnosed with cancer. The prognosis by the Central Statistical Office indicates that in 2030 there will be 2.2 million people in Poland aged ≥ 80 years, while in 2021 there were 1.64 million people in this age group. At the same time, it is known from demographic analyses that the average life expectancy of 80-year-olds in Poland projected for 2020 was about 9 years for a woman and about 7 years for a man [2]. These data indicate that cancer in the elderly is an important and growing social problem.

Breast cancer has remained for years the most frequently diagnosed cancer in women in Poland (Fig. 1 [3]). In 2019, there were more than 19000 new cases of breast cancer in women (22.9% of total cancer incidence), and nearly 7000 women died from the disease (15.1% of cancer deaths). In men, the incidence of breast cancer has remained at a similarly low level for years (about 150 new cases per year) [4]. From the mid-1970s to 2010, breast cancer was the most common malignant cause of death among women in Poland, but mortality from the disease, unlike incidence, remained constant and even showed a slight downward trend in the first decade of the 21st century (Fig. 1). This "divergence" between incidence and mortality trends observed in Poland and other developed countries of the world results from progress in early detection and treatment of this cancer. In recent years, in contrast to most European countries, breast cancer mortality in Poland has been gradually increasing. Data from the National Cancer Registry indicate that this increase has been most related to women over 65 years of age (Fig. 1). Similar observations come from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) registry in the US, where the smallest decrease in breast

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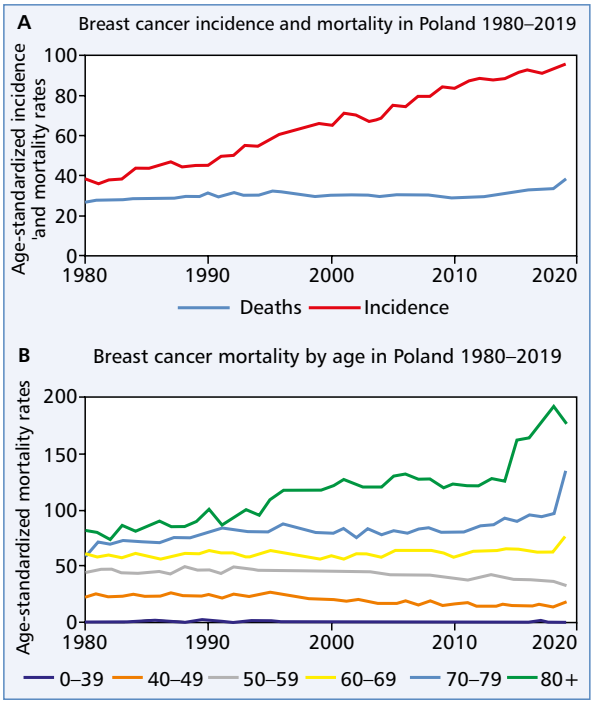

Figure 1A–B. Trends in incidence and mortality of breast cancer in women in Poland from 1980 to 2019 (based on:[3])

cancer mortality was observed in a group of women over 75 years of age [5]. This is all alarming because this group of patients is growing most rapidly.

In 2019, 1921 women aged ≥ 80 were diagnosed with breast cancer in Poland, accounting for 9.8% of the total incidence, and 2054 women died of the disease, accounting for 29.5% of breast cancer deaths in this age group. Similar relationships (3 times higher percentage of deaths than incidence) were observed in the male population, with 23 occurrences at age ≥ 80 (15.4% of total incidence) and 36 deaths (43.3% of breast cancer deaths). It is believed that breast cancers in older women have a milder course, grow more slowly, and are more often of a favorable histopathological type than in younger people [6]. This may suggest potential for a less aggressive treatment in this group of patients. Some investigators believe that the biology of breast cancer is age-dependent [7]. Some retrospective studies suggest that cancers with estrogen receptor (ER) expression are more common in the elderly than in the rest of the patient population, accounting for up to more than 80% of cases in the former [8]. In contrast, HER2-positive [overexpression of human epidermal growth factor receptor type 2 (HER2) or amplification of the encoding this protein *HER2* gene] and triple-negative cancers are relatively less common in the elderly. It has also been shown that poorly differentiated tumors are less common in seniors, and triple-negative tumors have lower Ki67 proliferation index values and are more differentiated than in the younger patient population [9, 10]. Analyses of histologic subtypes indicate that in elderly patients, infiltrating not otherwise specified carcinoma (NOS, formerly called NST — no special type), is the most common diagnosis, but compared to younger patients, other less common subtypes, such as mucinous carcinoma, lobular carcinoma, and intrahepatic papillary carcinoma, are more often to be found [7, 8, 11].

Material and methods

This study aimed to retrospectively analyze the biology of breast cancer in patients aged ≥ 80 years diagnosed at the Breast Cancer Unit (BCU) in Prof. Tadeusz Koszarowski Opole Cancer Center and to compare it with younger patients.

A total of 523 patients were included in the analysis, of whom 232 patients aged ≥ 80 years diagnosed between 2016 and 2020 formed the study group (hereafter referred to as the 80+ group), and 291 patients aged < 80 years diagnosed with breast cancer in 2019 formed the control group (hereafter referred to as the < 80 group). There were 240 breast cancers diagnosed in the study group and 295 in the control group (8 patients in the 80+ group and 4 patients in the < 80 group were diagnosed with synchronous cancers of both breasts). There were 2 males in each group.

Biological characteristics of the disease were assessed and included:

- histologic type (classified as NOS, lobular carcinoma, and other subtypes);
- histologic grade;
- biological subtype — defined based on estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 status — luminal A, luminal B HER2-negative, luminal B HER2-positive, non-luminal HER2-positive, and triple-negative.

The diagnosis of invasive breast cancer in each patient was based on histopathologic examination of material obtained using core needle breast tumor biopsy (most commonly) or surgical excision in cases of extensive infiltration. Almost all examinations were performed in the Department of Pathomorphology of the Opole Cancer Center. Each result included information on the histologic type of the cancer and its grade. The biological subtype of the cancer was determined according to the recommendations of the 2015 and 2017 St. Gallen consensus conferences. Except for one patient, the percentage of cells with ER, PR expression, and the degree of this expression was determined in each case. Any ER or PR response present in ≥ 1% of cancer cells was considered positive. HER2 status was determined by assessing HER2 receptor expression by immunohistochemistry,

and in cases of equivocal results, HER2 gene amplification was additionally assessed by in situ hybridization (ISH). The Ki-67 proliferation index, expressed as a percentage, was assigned to one of two categories — low (values < 20%), or high (values ≥ 20%). Such categorization is in accordance with the Polish Guidelines for Diagnostic and Therapeutic Procedures of Breast Cancer [12]. The St. Gallen recommendations also allow categorization based on the median Ki-67 value, which raises the threshold to 25% in the Department of Pathomorphology of the Opole Cancer Center [13]. The choice of the 20% threshold was dictated by the fact that, generally, this is the accepted threshold in BCU daily practice.

Statistical methods

The statistical package R version 3.3.2 in RStudio version 2022.07.0 was used for calculations. The study used a significance level of $p = 0.05$. The results presented here, including the analysis of the biological characteristics of the cancers, are part of a larger research effort involving many statistical tests. For this reason, Bonferroni's correction was applied to all analyses and a significance level of $p = 0.001$ was assumed for individual tests. The Wilcoxon test, Pearson chi-squared concordance, and Fisher's exact test were used for analysis.

Results

The median age in the study group was 82.7 years (range 80.0–97.0) and 63.6 years (range 27.3–79.6) in the control group. The study group had a significant majority of patients aged from 80 to 84 years and the control group had patients aged from 50 to 69 years, the age for population-based screening (Fig. 2).

In both groups, NOS-type cancer was most commonly diagnosed (75.8% in the 80+ group and 83.4% in the < 80 group). Less common histologic types (including lobular, mucinous, and papillary carcinoma) were diagnosed slightly more frequently in the 80+ group than in the control group (24.2% *vs*. 16.6%, respectively). Poorly differentiated tumors were more common in the < 80 group (32.9% *vs*. 26.7% in patients 80+), but this was not a significant difference either (Tab. 1).

The biology of the tumors was similar in both groups (Tab. 1). ER expression was present with a similar frequency (83.3% in the 80+ group and 84.1% in the < 80 group), as was PR (74.6% and 70.2%, respectively). HER2 positivity was slightly more common in the < 80 group (28.8% *vs*. 17.5% in the 80+ group), but the difference was not significant. The median Ki67 index was 26 in the 80+ group and 25 in the < 80 group (range in both groups 1–100). There were no differences in the percentage of cancers with high

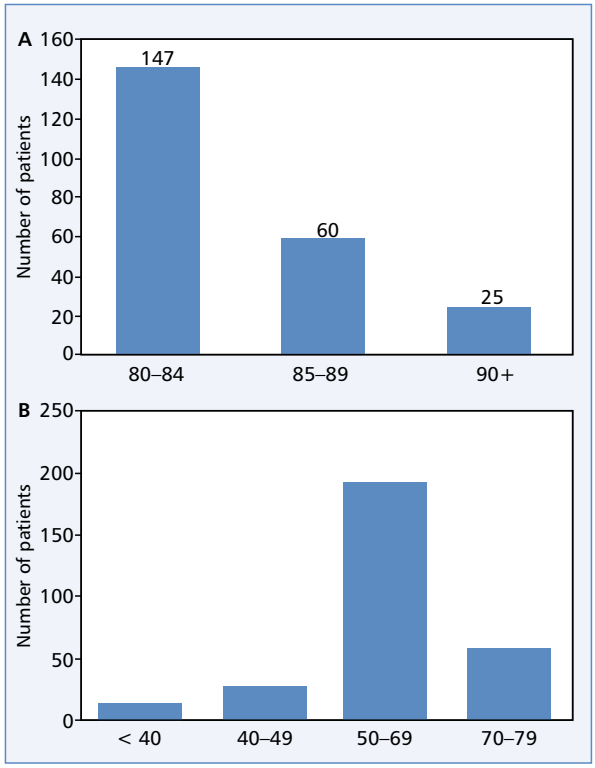

Figure 2. Age distribution of breast cancer patients at the time of diagnosis; **A.** The study group, 80+; **B.** The control group, < 80

(≥ 20%) and low (< 20%) Ki67 in the study groups either. This resulted in a similar distribution of biological subtypes of breast cancer in both groups. Luminal B HER2-negative cancers predominated (42.5% in the 80+ group and 34.9% in the < 80 group), and the largest differences between the groups were in the percentage of luminal B HER2-positive cancers (12.9% and 23.4% of patients, respectively). These differences were not significant.

Cancer focality was assessed by a pathomorphological report and, in patients who did not undergo surgery, based on imaging studies. Multifocal tumors were found more frequently in patients < 80 years (18.4% *vs*. 12% in the 80+ group), but the difference was not significant. In addition, this difference may be due to the higher number of surgical procedures in the control group. In both groups, lobular cancers were more common in the multifocal tumor cohorts; that is 28% in the 80+ group (13.8% in the total group) and 17.7% in the < 80 group (9.8% in the total group).

Discussion

The most commonly diagnosed histologic type of breast cancer, regardless of age, is NOS, but many

Table 1. Characteristics of the tumors

Characteristics	80+ group n = 240 (%)	< 80 group n = 295 (%)	p
Histologic type			
Not otherwise specified carcinoma (NOS)	182 (75.8)	246 (83.4)	0.092
Lobular	33 (13.8)	29 (9.8)	
Other	25 (10.4)	20 (6.8)	
Grading			
G1	50 (20.8)	44 (14.9)	0.114
G2	126 (52.5)	154 (52.2)	
G3	64 (26.7)	97 (32.9)	
Tumor focality			
Unifocal	184 (88.0)	227 (81.6)	0.055
Multifocal	25 (12.0)	51 (18.4)	
Estrogen receptor (ER) status			
ER-negative	39 (16.3)	47 (15.9)	0.998
ER-positive	200 (83.3)	248 (84.1)	
No data	1 (0.4)	0 (0.0)	
Progesterone receptor (PR) status			
PR-negative	60 (25.0)	88 (29.8)	0.264
PR-positive	179 (74.6)	207 (70.2)	
No data	1 (0.4)	0 (0.0)	
HER2			
Negative	196 (81.7)	210 (71.2)	0.004
Positive	42 (17.5)	85 (28.8)	
Unknown	2 (0.8)	0 (0,0)	
Ki67 index [%]			
Median	26	25	0.854
Range	1–100	1–100	
Ki67 by category			
Low (< 20%)	78 (32.8)	102 (34.6)	0.662
High (≥ 20%)	160 (67.2)	193 (65.4)	
No Data	2 (0.8)	0 (0.0)	
St. Gallen* sub-type			
Luminal A	68 (28.3)	76 (25.7)	0.069
Luminal B HER2-negative	102 (42.5)	103 (34.9)	
Luminal B HER2-positive	31 (12.9)	69 (23.4)	
Non-luminal HER2-positive	11 (4.6)	17 (5.8)	
Triple-negative	26 (10.9)	30 (10.2)	
Unknown	2 (0.8)	0 (0.0)	

*In 2 patients diagnosed outside the Opole Oncology Center due to incomplete immunohistochemical examination, the biological subtype of the cancer could not be determined. Due to their poor general condition and their failure to undergo oncological treatment, the re-diagnosis was abandoned

authors emphasize an increase in the proportion of lobular and mucinous carcinomas with patient age [9, 14, 15]. Retrospective studies differ in their assessment of the prevalence of histologic types other than NOS in older patients. In the population we analyzed, NOS was predominant in both 80+ and younger patients, as expected. Although lobular carcinoma was diagnosed slightly more frequently in patients 80+ than in

controls (13.8% *vs*. 9.8%), as were other histologic types (10.3% *vs*. 6.8%), these differences were not significant. The percentage of histologic types other than NOS in patients 80+ reported in the literature ranges from 16% to 31.5% [5, 16, 17]. In our study, this was true for 24% of cancers in the 80+ group, which is consistent with literature data and confirms the increasing prevalence of rarer histologic types of breast cancer with age [18].

Analysis of our data showed no significant differences in the incidence of multifocal tumors between the study and control groups. Such differences between older and younger patients were not shown in Weissenbacher's analysis, although some researchers suggest a higher incidence of multifocal tumors in younger patients, especially those < 40 years of age. [19, 20]. The absence of this difference in our data may be due to the small number of patients < 40 years of age in the study group (14 patients).

Well-differentiated (G1) carcinomas were diagnosed more often in patients 80+ compared to the control group, while poorly differentiated (G3) carcinomas were diagnosed in the < 80 group, but the difference was not significant. This observation is consistent with data reported in the literature [6, 21–23]. However, some investigators have shown significant differences in tumor differentiation, suggesting a more favorable biology of breast cancer in the elderly [24, 25].

Estrogen is known to play an important role in the pathogenesis of breast cancer. During menopause, estrogen levels gradually decline, while adrenal and ovarian androgen levels remain constant or begin to decline slowly, resulting in the relative dominance of androgens. Considering that the hormonal balance in older women is different from that of premenopausal ones, the biology of 80+ breast cancer is even more interesting. The expression of hormone receptors in the tumor, including ER alpha (ER-a) and beta (ER-b), PR, and androgen receptor (AR), indicates the type of sex hormones on which the tumor is dependent. However, the pattern of expression of these receptors in relation to menopausal status or age is still controversial.

In our study, we did not observe differences in ER and PR expression or median Ki67, and in the analysis of HER2 status, the differences were not significant, which is consistent with the results of other authors [15, 16, 21, 23, 24, 26]. This resulted in a similar distribution of biological subtypes (according to St. Gallen) in the 80+ and < 80 groups, which is also consistent with data in the literature [22]. In both study groups, the majority of HER2-positive cancer patients showed ER expression (74% in the 80+ group and 80% in the < 80 group). It would be interesting to evaluate the AR expression in the study population; unfortunately, it is not a routine practice [27].

Breast cancers in patients aged ≥ 80 years evaluated in our study were characterized by different combinations of biological and pathomorphological features, with no significant differences compared to younger patients. Therefore, assessment of prognosis and therapeutic management in older patients should be individualized and take into account the biology of the disease, rather than generalized rules based on the age of patients, as suggested by other authors [28].

Conclusions

The results of our evaluation of breast cancer biology in patients ≥ 80 years of age compared with patients < 80 years of age showed no statistically significant differences. The belief that breast cancer is less aggressive in the elderly than in the general population was not confirmed in our study.

Article Information and Declarations

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None.

Author contributions
J.H.-K.: should be considered the major author, author of the concept, methods, research, data analysis, manuscript preparation; P.Z.: data collection, data analysis, manuscript preparation; A.S. statistical analysis; B.R.: should be considered the senior author, author of the concept, methods, research, data analysis, manuscript preparation.

Conflict of interest
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VIA MEDICA

REVIEW ARTICLE

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Diagnosis and treatment of rhabdomyosarcomas

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ABSTRACT

Rhabdomyosarcoma (RMS) is a soft tissue sarcoma. The primary tumor is most commonly localized in the head and neck, the urogenital system, or the limbs. Classification by the World Health Organization has distinguished four histopathological RMS subtypes: embryonal, alveolar, pleomorphic, and spindle cell/sclerosing. Differential diagnosis of RMS includes melanoma, malignant neoplasm of peripheral nerve sheaths, liposarcoma, and PEComa. Among typical cytogenetic changes in RMS are chromosomal translocations t(2;13)(q35;q14) and t(1;13)(p36;q14). They lead to the formation of fusion genes that have a prognostic value. In the course of RMS, changes may also be present in signaling pathways, including RAS-PI3K, Wnt/β-catenin, receptor tyrosine kinase pathways, and myogenesis regulation. In 30% of patients at the time of diagnosis of RMS, distant metastases are present, most commonly to lungs, lymph nodes, bones, and bone marrow. Treatment of patients with RMS requires a multidisciplinary approach, and steadily perfected diagnostic techniques contribute to the individualization of therapeutic strategies. Optimal treatment of localized RMS is based on surgery combined with radiotherapy and chemotherapy. If distant metastases are present, the basic therapeutic method is multidrug chemotherapy, most frequently based on vincristine, dactinomycin, ifosfamide/cyclophosphamide, and etoposide. Despite intensive treatment, the 5-year survival index for RMS is not greater than 50%. There are still no unequivocal guidelines concerning the treatment in patients with local or distant recurrences.

Key words: rhabdomyosarcoma, sarcoma, soft tissue sarcomas, RMS, RAS, translocation

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Introduction

Sarcomas are a heterogeneous group of malignant neoplasms derived from mesenchymal tissue. The usual classification includes sarcomas derived from bone and soft tissue sarcomas [1]. Rhabdomyosarcoma (RMS) is a soft tissue sarcoma, whose cells differentiate in the direction of striated muscles, which is proved by the expression of skeletal muscle markers [2]. The current World Health Organization (WHO) classification divides RMS into four histological types: alveolar rhabdomyosarcoma (ARMS), embryonic rhabdomyosarcoma (EMRS), pleomorphic rhabdomyosarcoma (PRMS) and spindle cell/sclerosing rhabdomyosarcoma (SCRMS) [3]. In the last several decades, a small improvement in the survival of patients with RMS has

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been observed. This is due to the development of diagnostic methods allowing correct diagnosis of the disease and improving access to multidisciplinary treatment, including modern radiotherapy (RT) techniques. Establishing uniform standards of the procedure that included pediatric treatment protocols in the therapy of adult patients has led to the increase of the 5-year survival rate from 36% to 54% in comparison to treatment with other protocols [4]. Nevertheless, the diversity of anatomical localization of primary RMS tumors, limited methods of treating metastatic disease, and the current lack of targeted therapies make for an unfavorable prognosis in this group of patients. In retrospect, the median overall survival (OS) for RMS patients with distant metastases is from 7 to 22 months [5–7]. Direct toxicity (e.g. cardiotoxicity) and other undesirable effects (e.g. neutropenia) of cytotoxic drugs used in RMS treatment, both in the localized stage and in the disseminated one, remain a large limitation in the choice of optimal treatment [8].

Epidemiology

The incidence of soft tissue sarcomas in Poland is determined to be 4–5 cases per 100 000 persons, which is about 1000 new patients per year [9]. Simultaneously they constitute not more than one percent of all malignant neoplasms in adults [9–11]. Rhabdomyosarcoma is very rare in adults and is responsible for about 3% of soft tissue sarcomas [12]. Epidemiological analysis of 2600 patients with RMS indicated that slightly over 40% of all RMS cases are in adults [13]. These sarcomas occur four times more frequently in the white than in the black population The largest cohort of adults with RMS described so far includes 1071 persons [13]. Moreover, there is a small number of publications from reference centers in Europe, the United States, and Asia, which includes groups from several dozen to several hundred patients. The median age of adults with RMS is very differentiated and varies from 26 to 71.5 years [7, 14].

Risk factors

Based on the available literature two main groups of risk factors for RMS can be distinguished, that is genetic and environmental factors. Persons with some heritable genetic syndromes including Li-Fraumeni [15] or Noonan syndrome [16] are at an increased risk of RMS (Tab. 1). Most patients with RMS do not have any first-degree relatives with neoplastic diseases in their medical history; however, neoplasms among first-degree relatives under 30 years of age are more frequent in RMS patients than in the healthy population. Congenital RMS cases have also been described [17, 18]. Among other factors which increase the risk of RMS are congenital defects [19], prenatal exposure to ionizing radiation [20], cocaine and cannabinoid use by the mothers and fathers [21], or pre-term birth [22].

Table 1. Genetic syndromes predisposing to rhabdomyosarcoma

Genetic syndromes	Responsible genes	References
Beckwith-Wiedemann	*IGF2*, *CDKN1C*, *H19*, *and KCNQ1OT1*	[23]
Costello	*HRAS*	[24]
DICER1	*DICER1*	[25]
Type 1 neurofibromatosis	*NF1*	[19, 26, 27]
Li-Fraumeni	*TP53*	[15]
Noonan	*BRAF*, *KRAS*, *NRAS*, *PTPN11*, *RAF1 and SOS1*	[16]
CMMRD	*MLH1*, *MSH2*, *MSH6*, *PMS2*	[28]
Rubenstein-Taybi	*CREBBP*	[29]
Hereditary retinoblastoma	*RB1*	[30]
Gorlin	*PTCH1*, *PTCH2*, *and SUFU*	[31, 32]
Cardiofaciocutaneous	*BRAF*, *MAP2K1*, *MAP2K2*, *KRAS*	[33]

BRAF — B-Raf proto-oncogene; *CDKN1C* — cyclin dependent kinase inhibitor 1C gene; CMMRD — constitutional mismatch repair deficiency; *CREBBP* — CREB binding protein gene; *DICER1* — dicer 1, ribonuclease III gene; *H19* — H19 imprinted maternally expressed transcript gene; *HRAS* — HRAS proto-oncogene; *IGF2* — insulin-like growth factor 2 gene; *KCNQ1OT1* — KCNQ1 opposite strand/antisense transcript 1 gene; *KRAS* — KRAS proto-oncogene; *MLH1* — mutL homolog 1 gene, *MSH2* — mutS homolog 2 gene; *MSH6* — mutS homolog 6 gene; *NF1* — neurofibromin 1 gene; *NRAS* — NRAS proto-oncogene; *PMS2* — PMS1 homolog 2 gene; *PTCH1* — patched 1 gene; *PTCH2* — patched 2 gene; PTPN1— protein tyrosine phosphatase non-receptor type 1; *RAF1*— Raf-1 proto-oncogene; *RB1* — RB transcriptional corepressor 1 gene; *SOS1* — SOS Ras/Rac guanine nucleotide exchange factor 1 gene; SUFU — suppressor of fused homolog; *TP53* — tumor protein p53 gene

Table 2. Changes in intracellular signal pathways in rhabdomyosarcoma

Signal pathway	Altered genes	Remarks	References
RAS-PI3K	*NRAS, KRAS, HRAS PTPN11, NF1, BRAF, PIK3CA*	Over 80% of RMS tumors show PI3K pathway activation. In 1/3 of FNRMS tumors, RAS pathway perturbations are present. Mutations of the PI3K/AKT signal pathway define an ERMS subgroup with an unfavorable clinical course	[57, 58, 72–75]
RTK	*FGFR2, FGFR4, IGF1R, ERBB2, EPHA3, EFNA1, PDGFRA*	*FGFR4* mutations occur in about 7% of FNRMS *IGFR1* overexpression is observed in FPRMS *PDGFRA* gene overexpression is characteristic of FPRMS tumors	[46, 76–80]
Oncogenesis suppression pathways	*PTEN, TP53, MDM2, CDKN2A, CDKN1C*	*PTEN* gene mutation occurs in FNRMS tumors *TP53* gene mutation occurs in about 12% of FNRMS tumors	[36, 81–83]
Wnt/β-catenin	*CTNNB1*	*CTNNB1* gene mutation (encoding β-catenin) is common in FNRMS tumors	[83]
Sonic Hedgehog signal pathway	*GLI1*	In ERMS an excess of genetic material from the 12q13 may be present, where the transcription factor GLI1, which is frequently overexpressed, is located	[57]
Pathways of regulating epigenetics and myogenesis	*MYOD1, BCOR, ARID1A*	*MYOD1* mutations are characteristic of a particularly aggressive form of FNRMS	[57, 64, 84]

ARID1A — AT-rich interaction domain 1A gene; *BCOR* — BCL6 corepressor gene; *BRAF* — B-Raf proto-oncogene; *CDK2NA* — cyclin dependent kinase inhibitor 2A gene; *CDKN1C* — cyclin-dependent kinase inhibitor 1C gene; *CTNNB1* — catenin beta 1 gene; *EFNA1* — ephrin A1 gene; *EPHA3* — EPH receptor 3 gene; *ERBB2* — Erb-B2 receptor tyrosine kinase 2 gene; *FGFR2* — fibroblast growth factor receptor 2 gene; *FGFR4* — fibroblast growth factor receptor 4 gene; *GLI1* — GLI family Zinc Finger 1 gene; *HRAS* — HRAS proto-oncogene; *IGF1R* — insulin-like growth factor 1 gene; *KRAS* — KRAS proto-oncogene; *MDM2* — MDM2 proto-oncogene; *MYOD1* — myogenic differentiation 1 gene; *NF1* — neurofibromin 1 gene; *NRAS* — NRAS proto-oncogene; *PDGFRA* — platelet derived growth factor receptor alpha gene; *PIK3CA* — phosphatidylinositol-4,5-biphosphonate 3-kinase catalytic subunit alpha oncogene; *PTEN* — phosphatase and tensin homolog gene; PTPN11 — protein tyrosine phosphatase non-receptor type 11; RTK — receptor tyrosine kinases; *TP53* — tumor protein p53 gen

Pathogenesis

Even though RMS cells differentiate in the direction of myoblasts, it is not clear if they develop from the same cell lines from which striated muscle differentiates. Considering the anatomical variety of this neoplasm and the range of its oncogenic lesions, it can be assumed that they attain the myoblast phenotype by induction of the expression of genes characteristic of skeletal muscle [34]. Moreover, it has been shown that RMS can form as a result of oncogene expression both in skeletal myoblast cell lines [35, 36] and in non-myogenic cell lines [37]. Rhabdomyosarcomas may be derived from tissues such as skin, fat, or nerves [38]. The pathogenesis of RMS is based both on genetic material mutations (see subchapter 5) and the resulting perturbations of signal transduction pathways regulating cell function (Tab. 2). A high tumor mutational burden defined as the sum of somatic mutations of the genetic material of the tumor correlates with a poorer prognosis for RMS patients [39].

Cytogenetic aberrations

Alveolar RMS

The most characteristic chromosomal translocations present in ARMS are t(2;13)(q35;q14) and t(1;13)(p36;q14), leading to the formation of fusion genes *PAX3-FOXO1* and *PAX7-FOXO1* [40]. The former of these translocations are more common (55% *vs.* 23%). Their presence is associated with a poorer clinical prognosis [41]. The protein products of these genes are transcription factors. Their expression is stronger in comparison to the products of corresponding genes which did not undergo fusion (wild-type) [42, 43]. Additionally, the products of fusion genes are stabilized at the post-translational stage by the phosphorylation of the chimeric protein, which decreases their intracellular degradation [44]. Transcription factor PAX3--FOXO1 increases the expression of the following genes: *ALK* receptor tyrosine kinase gene, encoding the anaplastic lymphoma kinase, *FGFR4*, fibroblast growth factor receptor 4 gene, protooncogene *MYCN*, *MYOD1* myogenic differentiation 1 gene, and *MYOG* (myogenin gene) [34]. In about 80% of ARMS, strong cytoplasmic ALK expression has been observed, most commonly associated with the amplification of this gene. In single cases, the presence of mutations has also been observed (substitution or the loss of a whole exon) [45]. Moreover, PAX3-FOXO1 interacts with proteins participating in modulating chromatin activity including BRD4, (bromodomain-containing protein 4) and CHD4 (chromodomain helix DNA-binding protein 4) [46, 47]. A role in the development and invasiveness of RMS is also played by excessive activation of the *MET*

Table 3. Molecular analyses to identify the PAX/FOXO1 fusion

Alveolar rhabdomyosarcoma	*PAX3-FOXO1*	t(2;13)(Q35;Q14)
	PAX7-FOXO1	t(1;13)(p36;q14)
	PAX3-FOXO4	t(X;2)(q13;q36)
	PAX3-NCOA1	t(2;2)(p23;q36)
	PAX3-NCOA2	t(2;8)(q36;q13)
	FOXO1-FGFR1	t(8;13;9)(p11;q14;q32)

protooncogene, which takes place probably as a result of the activity of the fusion protein [48]. Amplification of 13q31 with the MIR17HG region (encoding miR-17--92, which also undergoes amplification in other neoplasms), occurring mainly in ARMS with the PAX7--FOXO1 fusion, is probably associated with a poorer clinical course of the disease [49]. The 12q13-14, amplification with the CDK4 locus characteristic almost exclusively for ARMS with the PAX3-FOXO1 fusion, has a similar prognostic value [50]. In about 20% of ARMS, the fusion of FOXO1(FKHR) or PAX3 is not present. These tumors, both in the molecular and clinical aspect, resemble ERMS (embryonal RMS) more closely, which indicates the key role of genetic diagnosis and creates a natural division of ARMS into fusion-positive (FPRMS) and fusion-negative (FNRMS) types [38]. For this reason, molecular investigations aimed at identifying PAX/FOXO1 fusions are recommended in all cases of alveolar and embryonal RMS [51] (Tab. 3).

Embryonal ERMS

In as many as 25–50% of ERMS tumors, chromosomal number aberrations are present [52]. They generally concern additional copies of chromosome pairs 2, 7, 8 (even in 70% of cases), 11, 12, 13, and 20 [53]. The loss of chromosome pairs 9 and 10 and 15 is described in 30% of ERMS cases. If gene amplification occurs, it is detected in chromosome regions 12q13-q15, whereas in region11p15.5. homo- or heterozygous deletions are common. Moreover, in this region, the phenomenon of uniparental disomy and gene imprinting may occur [54–56]. Mutations in oncogenes and suppressor genes are more characteristic for ERMS than for ARMS. In both subtypes, changes in cellular signaling associated with receptors for growth factors RAS/PI3K are common through somatic mutations (more common in ERMS) or changes in the expression of key genes for this pathway (through specific fusions in ARMS) (Tab. 2, 4) [57].

Pleomorphic RMS

RMS pathogenesis at the molecular level is poorly characterized. A complex karyotype is most commonly present in PRMS with numerous structural and numerical aberrations but without specific changes, which has been confirmed by molecular analyses [53, 58]. A common gene mutation in PRMS is probably a mutation of the *TP53* gene, especially in tumors appearing at a young age [59].

Spindle cell/sclerosing RMS

Spindle cell/sclerosing rhabdomyosarcoma is the most recent RMS to be distinguished in the histopathological classification. Within this subtype successive ones are distinguished with characteristic molecular changes and clinical appearance [60]. The first group are variants with rearrangement of the *VGLL2*/*NCOA2* genes, which occur in children under the age of 5 years or as congenital neoplasms [61] (Tab. 5).

They are characterized by a good prognosis with a tendency to local recurrence [62]. Other possible gene fusions occurring in RMS with this clinical presentation are *SRF*/*NCOA2, TEAD1*/*NCOA2*, *VGLL2*/*CITED2* [60]. A separate group is spindle cell/sclerosing RMS with a somatic activating mutation of the *MYOD1* gene at position Lys122, occurring both in children and adults [63]. The mutation may be homo- or heterozygous and the mutated gene interacts with the *MYC* oncogene [64]. Tumors of this type are characterized by a poor prognosis, especially in children and adolescents [63]. In adolescents the mutation *MyoD1* p.Leu122Arg — associated with a very poor prognosis — is characteristic. The third group are patients with spindle cell RMS without molecular changes and the fourth patients with a diagnosis of spindle cell RMS developing in bones with *EWSR1*/*FUS-TFCP2* or *MEIS1-NCOA2* translocations. In adults the presence of bone tumors is correlated with very poor prognosis and is characteristic for spindle cell/sclerosing RMS with the fusion of *MEIS1*/*NOAC2* or *EWSR1*/ /*FUS-TFCP2* genes, and so far it is known only from the description of several dozen cases [65–70]. The remaining cases of spindle cell RMS, which do not have the alterations described above, occur most commonly around the area of the testes or within the abdominal cavity [71].

Clinical picture

Rhabdomyosarcoma may occur in almost any anatomical localization, most commonly in the extremities (approx. 25%), head and neck region (approx. 20%), and urogenital tract (approx. 20%) [13]. Localization within the head and neck also encompasses the area of the eye socket [85] and the parameningeal area including the nasal cavity [86], sinuses [87–89], the nasopharyngeal cavity [90], and the subtemporal fossa. It may develop within the parotid glands [91], the thyroid

Table 4. Histopathological differential diagnosis of Rhabdomyosarcoma (RMS)

Neoplasm	Morphological characteristics	Immunohistochemical markers						Other information
		Keratin	Desmin	Protein S100	Myogen /MyoD1	HMB-45/Melan A	SMA	
ERMS	Ovoid and star-shaped cells loosely placed in myxoid stroma, less commonly morphology of small, round cells, presence of cells with the character of immature rhabdomyoblasts	+/–	+	–/+	+	–	+/–	Lack of characteristic cytogenetic marker, in most cases loss of heterozygosity at locus 11p15 [136]
ARMS	Small, round, and monomorphic myoblasts separated by empty oval or elongated spaces (similar to lung tissue structure)	+/–	+	–/+	+	–	+/–	70–90% of cases show translocations: t(2;13)(q35;q14) or t(1;13)(p36;q14) [137], FISH with set of *FOXO1* (*FKHR*) and *PAX3* probes used in diagnosis
Ewing sarcoma	Visible areas composed of small monomorphic cells; Homer-Wright rosettes present	+/–	–	–/+	–	–	–	Positive staining for FLI-1 in about 80% of cases [138], Positive staining for CD99
Melanoma	Pleomorphic, epithelial, or fusiform cells with poor cohesion, melanin, presence of distinct nucleoli	–	–	+	–	+	–	BRAF mutations in approx. 50% of patients
MPNST	Various numbers of cells within neoplastic lesions, cells in bundles, coils, or "herringbone pattern"	–	–	+/–	–	–	–	In over 80% of cases neurofibromin 1 (*NF1*) gene mutations present [139], loss of nuclear expression of H3K27me3/INI1
PEComa	Perivascular proliferation of epithelial and fusiform cells with light, acidophilic cytoplasm with granulocytes; nucleoli visible	–	+/–	–	–	+	+	In about 80% of cases deletions and/or loss of heterozygosity (LOH) in the 16p13.3 region [140, 141]
DFSP	Large density of cell arrangement in histological appearance with poorly visible borders of neoplastic lesions, radial arrangement of cells with fusiform morphology	–	–	–	–	–	–	Characteristic translocation t(17;22)(q22;q13) [142], CD34 expression [143]

ARMS — alveolar rhabdomyosarcoma; DFSP — dermatofibrosarcoma protuberans; ERMS — aembryonic rhabdomyosarcoma; MPNST — malignant peripheral sheath tumor; PEC-oma — perivascular epithelioid cell tumors; SMA — smooth muscle actin

Table 5. Variants with rearrangement of the *VGLL2/NCOA2* genes occurring in children under the age of 5 years or as congenital neoplasms

Spindle cell/sclerosing rhabdomyosarcoma congenital/infant	*SRF-NCOA2*	t(6;8)(p21;q13)
	TEAD1-NCOA2	t(8;11)(q13;p15)
	VGLL2-NCOA2	t(6;8)(q22;q13)
	VGLL2-CITED2	t(6;6)(q22;q24)

[92], and the oral cavity [93]. RMS within the urogenital tract may occur, among others, in the bladder [94], prostate [95], urethra [96], uterus [97, 98], vulva [99], or scrotum [100]. In adult RMS patients it more commonly develops in an unfavorable anatomical localization, i.e. other than the head and neck (except for parameningeal areas), urogenital tract (except for the bladder and prostate), and the biliary pathways [13, 101, 102]. A few cases of RMS are described in more rare localizations such as the liver [103], breast [104, 105], mediastinum [106], bronchi and lung [107, 108], cardiac muscle [109], pericardium [110], diaphragm [111], retroperitoneal space [112, 113], esophagus [114], stomach [115], or ileum [116].

Primary RMS is, in general, characterized by rapid and aggressive growth with the formation of a pseudobursa. The multiplicity of possible localizations is associated with a differentiated clinical picture. In the initial stages, the course of the disease may be

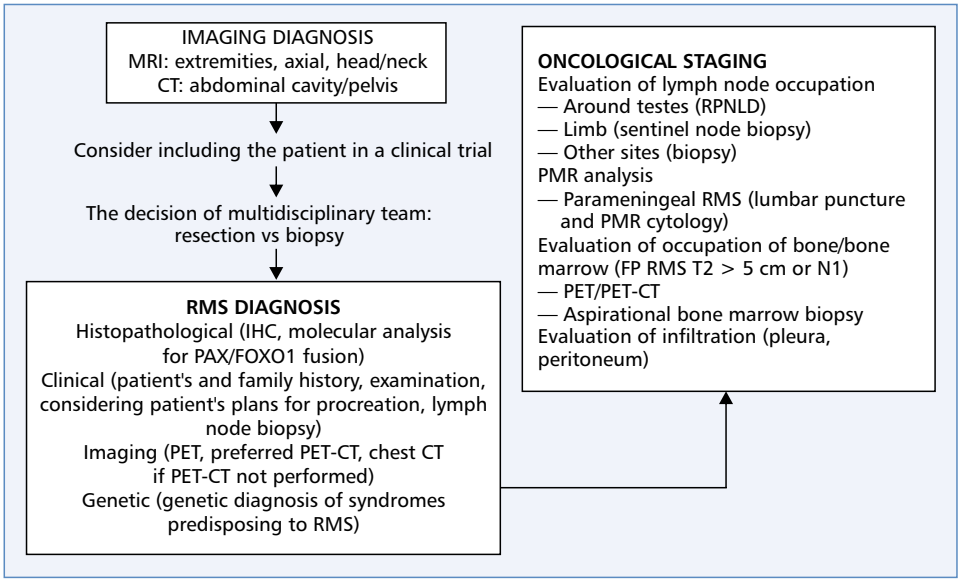

Figure 1. Diagnostic algorithm procedure for rhabdomyosarcoma (RMS). Based on [51]; IHC — immunohistochemistry staining; MRI — magnetic resonance imaging; PET — positron emission tomography; PET-CT — positron emission tomography-computed tomography; PMR — spinocerebral fluid

asymptomatic [38]. The symptoms of focal damage of the nervous system appear in the case of RMS of the perimeningeal area. Within the eye socket, it may cause exophthalmos, perturbations of eyeball mobility, or vision perturbations [117–119]. Localized within the head and neck it may give symptoms of chronic or acute sinusitis, purulent or bloody discharge from the nasal cavity or ear canal, their obturation, or swallowing difficulti es [55, 120]. Because of aggressive growth in a limited anatomical space, cranial nerve paralysis may occur [121]. Rhabdomyosarcoma localized within the urogenital pathways, pelvis minor, or the abdominal cavity may give various symptoms such as chronic abdominal pain [122], bleeding from the birth canal [97], dysuria [123], jaundice [124], intussusception [125], or intestinal obstruction [126]. Edema, often painless, appearing in the case of RMS of the extremities or in the vicinity of the genital organs, may be ascribed to mechanical damage, which delays appropriate diagnosis. There are also descriptions of cases of disseminated RMS, where the first symptoms were perturbations of muscle strength of the extremities or limb paralysis [98]. Metastases may disseminate both through the lymphatic system and blood vessels [127, 128]. The most common localizations of metastases encompass the lungs, lymph nodes, and bone marrow. However, RMS may give metastases to almost all organs. In the literature, there are descriptions of metastatic RMS foci in the breast, peritoneum, pleura, central nervous system, and skin [14, 129, 130]. Bone metastases may manifest as bone pain and hypercalcemia and massive occupation of the bone marrow may cause symptoms typical for leukemia (cytopenia, bleeding, and infections) [45, 131–133]. Epidemiological analysis including 1017 adults with RMS indicated that over 28% of distant metastases were present at the moment of diagnosis, whereas regional dissemination (occupation of regional lymph nodes or primary lesions crossing the boundaries of the primary organ) was present in over 25% of the patients [5].

Diagnostic procedure

Preliminary diagnosis

If RMS is suspected, it is indispensable to carefully plan the whole diagnostic procedure (Fig. 1). The procedure is initiated by coarse needle biopsy with a pathological diagnosis in a sarcoma treatment reference center. Before performing the biopsy, it is recommended to evaluate the progress of the disease by visual imaging [computed tomography (CT) or magnetic resonance imaging (MRI)] of the primary focus and the regional lymph flow to optimally plan the biopsy and eventual further surgical treatment. A significant element of primary diagnosis is also a clinical and radiological evaluation of not only regional but also distant lymph nodes, whose occupation constitutes a generalized neoplastic disease. The criteria of occupation of lymph nodes in RMS have so far not been standardized, and, generally, a node larger than 1 cm in diameter is considered suspicious, regardless of its appearance in radiological imaging [56].

Histopathological diagnosis

Histopathological diagnosis of RMS is difficult, which is confirmed by the statistics of the international Intergroup Rhabdomyosarcoma Study Group (IRSG), according to which every fifth RMS diagnosis was incorrect [101]. In a light or electron microscope, cells can be seen that show differentiation in the direction of skeletal muscle cells — "myoblast-like cells". The next step is to perform immunohistochemical staining for the expression of proteins characteristic for muscle, which include muscle-specific actin and myosin, desmin, myoglobin, the MyoD1 protein, and myogenin [134]. The last two proteins are considered the most important markers of rhabdomyoblastic neoplasm differentiation [52]. Morphologically, RMS myoblasts can have different forms: they may be poorly differentiated (spherical, oval), fusiform, or fully differentiated [52]. Highly differentiated rhabdomyoblasts present as spherical or oval cells containing an acidophilic grainy cytoplasm with an eccentric or central spherical, single or double nucleus [52]. It should be, however, kept in mind that demonstrating rhabdomyoblastic differentiation among the cells of a neoplasm does not by itself determine an RMS diagnosis, as other neoplasms such as mesenchymal chondrosarcomas or sarcomatoid cancers are also characterized by the presence of these cells [135]. Rhabdomyosarcoma embryonal and alveolar belongs to the group of small round blue cell tumors, which are characterized by a low grade of differentiation and morphological similarity (small cell with a large, spherical strongly hyperchromatic nucleus, staining navy blue with hematoxylin) [55]. Other neoplasms that must be considered in the differential diagnosis of RMS are malignant peripheral nerve sheath tumor (MPNST), dermatofibrosarcoma protuberans (DFSP), perivascular epithelioid cell tumors (PEC-oma), melanoma, Ewing sarcoma, ectomesenchymoma, sarcoma-like cancer, including skin and salivary gland cancer, melanoma, liposarcoma, malignant teratoma, anaplastic thyroid cancer and neoplasms derived from nervous tissue. Differential diagnosis is presented in Table 4.

During histopathological diagnosis, the RMS subtype must be established.

Alveolar RMS

Alveolar RMS constitutes about 25% of all RMS diagnoses in adults [13, 144]. In the adult population, it is most common in 10–25 years old but may occur at any age. It is often localized in the soft tissues of the extremities, head and neck, retroperitoneal space, the urinary bladder, or the reproductive organ [145]. It is made up of tightly placed, small, circular, and monomorphic cells separated by empty oval or elongated spaces, which resemble lung alveoli in the histopathological picture. Cell aggregates may also be separated by connective tissue. The cells show a high nucleo-cytoplasmic ratio and are characterized by a high mitotic index. Some ARMS do not have the characteristic segmented location of cells, and their cells are uniformly clustered (the so-called solid form of ARMS), which makes the differential diagnosis with ERMS difficult [146]. Molecular methods including FISH with the FOXO1A set of probes are useful in diagnosing tumors with a solid structure, less characteristic for ARMS (Fig. 2).

Embryonal RMS

According to the available data including the largest group of adult patients with RMS, the frequency of occurrence of this subtype is 20–30% of all histopathological RMS diagnoses [144, 147]. A frequent site for this neoplasm is the head and neck, in particular the eye socket, tissues associated with the meninges, middle ear, nasopharyngeal cavity, and the urogenital system, soft tissues of the extremities, the pelvis, and the retroperitoneal space [148]. It is built of acidophilic primitive ovoid cells, less commonly of round cells resembling immature rhabdomyoblasts. They are loosely disseminated in myxoid stromal tissue [149]. The cells are not distributed in an alveolar fashion characteristic for ARMS [149]. Cellular composition of ERMS reflects embryonal striated muscle development as very poorly differentiated cells up to fully differentiated cells may

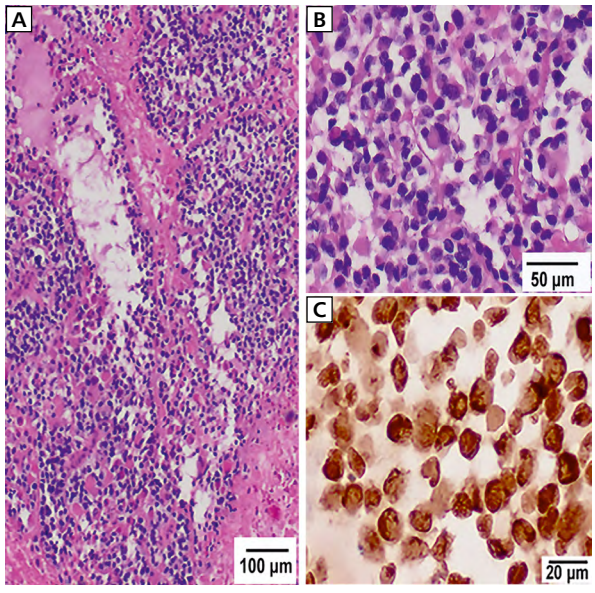

Figure 2. Alveolar rhabdomyosarcoma; **A.** Typical architecture with pseudoalveolar spaces (H&E, 40×); **B.** Neoplastic cells with acidophilic cytoplasm and eccentric cell nucleus — differentiation in the direction of rhabdomyoblasts (H&E, 100×); **C.** In general, strong and diffuse myogenin expression (IHC clone F5D, Dako, 200×)

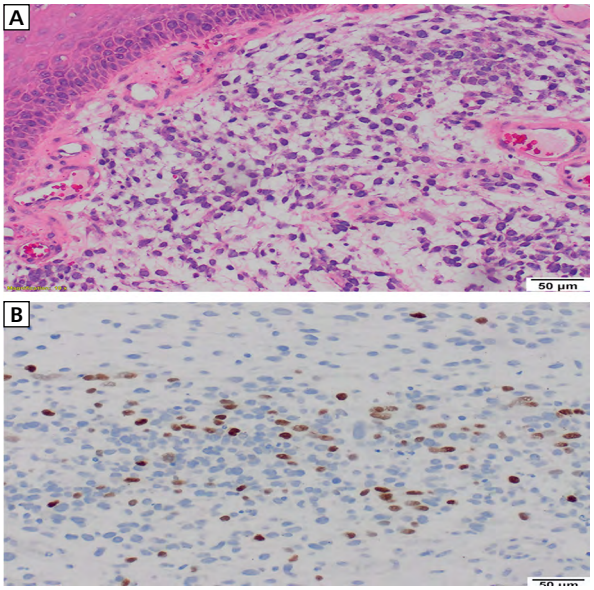

Figure 3. Embryonal Rhabdomyosarcoma of the vaginal wall of a 5-year-old patient (botryoid alveolar subtype); **A.** Primitive, small and ovoid neoplastic cells with poorly expressed differentiation in the direction of rhabdomyoblasts, placed loosely in a myxoid stroma. Supra-epithelial densification of cells visible – the cambial layer H&E, 100×); **B.** Myogenin expression is generally visible only focally (IHC clone F5D, Dako, 200×)

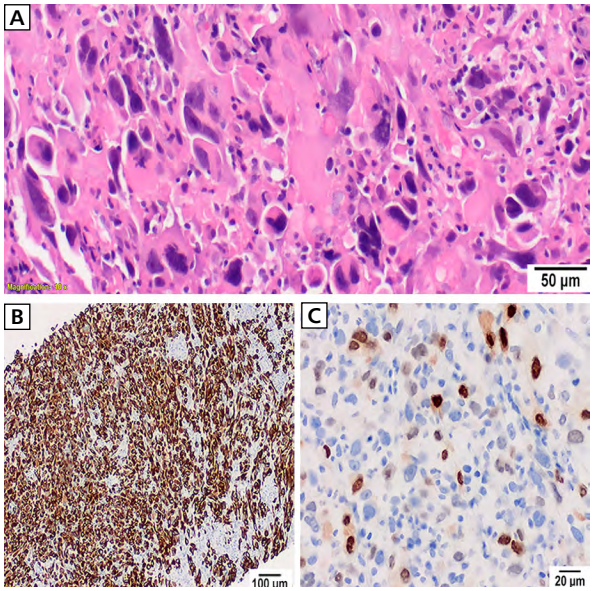

Figure 4. Pleomorphic rhabdomyosarcoma; **A.** Atypical polygonal and fusiform neoplastic cells typical for pleomorphic sarcomas; **B.** Desmin expression is generally multifocal or diffuse, but this marker does not allow for reliable differentiation of RMS from pleomorphic leiomyosarcoma (IHC clone D33, Dako, 40×); **C.** Myogenin is a highly specific marker for RMS, but in pleomorphic RMS, its expression may be poor (IHC F5D, Dako, 200×)

appear [52]. Anaplastic cells with a hyperchromatic enlarged nucleus are present in about 3–13% ERMS, and their presence may correlate with a poorer prognosis for the patients [150, 151]. In the botryoid form of ERMS (botryoid RMS), neoplastic cells form a layer called the cambial layer. This ERMS subtype with a good prognosis is characterized by a linear placement of neoplastic cells and occurs, in general, in the vicinity of mucous membranes, e.g. in the bladder [60]. Botryoid ERMS also often occupies the vagina, biliary pathways, the nasopharynx, and the nasal cavity [152]. In the anaplastic form of ERMS, which is a subtype with a poorer prognosis, the cells have an atypical multiform morphology. Immunohistochemical staining for myogenin indicates a heterogeneous and punctate expression of this protein in ERMS cells, but expression can also be uniform [153] (Fig. 3).

Pleomorphic RMS

Pleomorphic RMS (PRMS)occurs almost exclusively in adults, in particular, after the sixth decade of life, and constitutes even up to 43% of RMS in adults [154, 155]. Its localization is most commonly in soft tissues of the lower extremities (especially the thighs), retroperitoneal space, abdominal cavity, chest, spermatid cord, and the vicinity of the testes [156]. The histopathological picture shows a very low degree of differentiation in the direction of rhabdomyoblasts and requires careful differentiation with undifferentiated pleomorphic sarcoma [38]. Cells that show signs of atypia are pleomorphic; they have shapes from small epithelial to large cells with segmented nuclei and distinct nucleoli. They may be placed in groups, be linear, or be irregularly disseminated [38]. Cellular pleomorphism is diffuse in contrast to disseminated anaplastic cells in ERMS. PRMS is also characterized by poor myogenin expression, however, the largest study including 38 cases indicated that each of the tumors showed positive staining for at least one skeletal muscle marker [60] (Fig. 4).

Spindle cell/sclerosing RMS

In 2013, SCRMS was distinguished as a distinct variant of RMS on the basis of its genetic profile, whereas previously it had been identified as an ERMS subtype [3, 157]. According to WHO, it is the rarest RMS subtype [158]. In the pediatric population, SCRMS is associated with a better prognosis, but in the few descriptions of cases with this neoplasm in adult patients, this no longer holds [159, 160]. Spindle cell/sclerosing rhabdomyosarcoma with the presence of the *NCOA2* and *VGLL2* translocation is correlated with a better prognosis in infants [62]. A poorer clinical prognosis is characteristic of tumors in the parameningeal area and associated with *MYOD1* [62, 161]. In adults, the most common SCRMS localization is the head and neck, less commonly, the extremities and the retroperitoneal space [160].

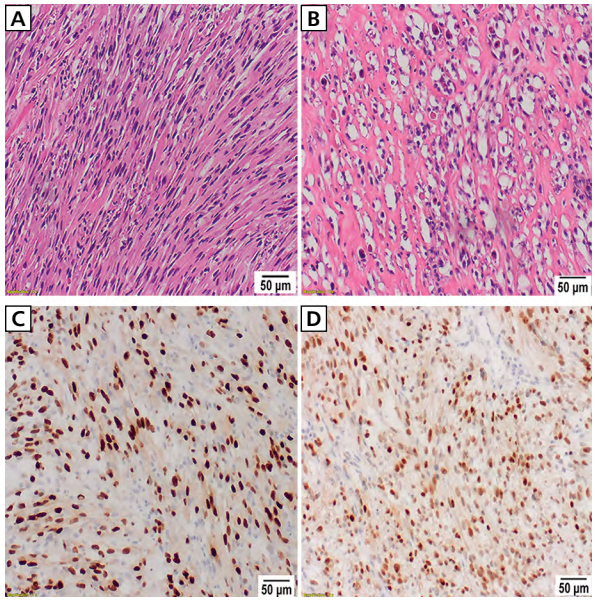

Figure 5. Spindle cell/sclerosing rhabdomyosarcoma; **A.** Fusiform morphology of cells arranged in long bundles (H&E, 100×); **B.** Another part of the same tumor with a more sclerosing and homogeneous stroma and spherical cells — sclerosing variant of RMS (H&E, 100×). Spindle cell/sclerosing rhabdomyosarcoma is the morphological spectrum of the same neoplasm (both photos: H&E, 100×); **C.** Nuclear myogenin expression does not differentiate between particular RMS subtypes (IHC clone F5D, Dako, 100×); **D.** Strong and diffuse MyoD1 expression, often stronger than myogenin expression, is characteristic of SCRMS and is generally a result of the *MYOD1* gene mutation at position L122R (IHC clone 5.8A, Dako, 100×)

Histopathologically, the tumor tissue is composed of fusiform cells arranged in bundles or placed in a swirl. The cells have an elongated and spoke-like nucleus, small nucleoli, and eosinophilic cytoplasm and present varied nuclear atypia, mitotic activity, and pleomorphism [162]. These tumors often contain a rich collagen stroma with disseminated small neoplastic cells, hence the description sclerosing of this RMS variant [163]. Some cases show properties of sclerosing with gaps simulating vessels [158]. Immunohistochemical studies indicate strong positive staining for desmin and MyoD1, local or disseminated myogenin, and no or local immunoreactivity to cytokeratins [164, 165] (Fig. 5).

Extended diagnostic evaluation

After confirming RMS, the next step is to evaluate disease progression and qualification for treatment. The following analyses are recommended: blood morphology with a smear, extended biochemical blood analysis, urine analysis, chest, abdominal and pelvic CT, bone scintigraphy or PET-CT (detecting metastases to lymph nodes and bones), trepanobiopsy of the bone marrow, brain and spine MRI, and cerebrospinal fluid puncture (primary focus within the meninges and if the occupation of meninges is suspected), and sometimes a diagnostic biopsy of suspected lymph nodes [45]. In the case of extremity and trunk RMS, where the percentage of metastases to regional lymph nodes is particularly high, mapping of the lymph system and evaluation of the sentinel lymph node is possible to consider [166, 167].

Bone scintigraphy or PET-CT and two-sided aspirational bone marrow biopsy allow the evaluation of the possible occupation of the bone system and/or bone marrow. In selected cases (tumor < 5 cm, FN-RMS, no evidence of lymph node occupation), it is possible not to evaluate the diagnosis of RMS metastases to bones [51]. Imaging studies allow determining the risk group, which is the basic criterium determining prognosis and treatment intensity (Tab. 6, 7). The TNM RMS system was elaborated by the Intergroup Rhabdomyosarcoma Study Group (IRSG) in 2001 for the pediatric population and young adults [167]. For adult patients both the above-mentioned TNM IRSG system and the TNM classification, according to the American Joint Committee on Cancer for soft tissue sarcomas, can be used [168].

General principles of treating localized disease

Treatment sequence

Rhabdomyosarcomas should be treated in a multidisciplinary fashion in reference centers for pediatric and adult sarcomas. The treatment regimens are based on resection of the primary tumor and eventual metastases to lymph nodes with perioperative radiotherapy or radical radiotherapy when surgical treatment is not possible. Methods of local treatment should be combined with multidrug chemotherapy based on cyclophosphamide or ifosfamide in combination with anthracycline, or dactinomycin and vincristine, or dacarbazine.

Surgery

Surgery is the basic therapeutic option for RMS patients, regardless of the risk group to which they belong. Local treatment must be considered first after diagnosis with the intent of complete resection of the tumor and obtaining microscopically radical surgical margins. Currently, surgery is frequently preceded by chemotherapy and/or radiotherapy [170]. In some cases, there are indications for radical regional lymphadenectomy. If metastases to regional lymph nodes are

Table 6. Evaluation of the degree of RMS progression according to the classification of the Intergroup Rhabdomyosarcoma Study Group TNM

Stage	Localization	T	Size	N	M
1	Favorable	**T1/T2**	a/b	Any	M0
2	Unfavorable	T1/T2	a	N0/Nx	M0
3	Unfavorable	T1/T2	a	N1	M0
			b	Any N	M0
4	Any	T1/T2	a/b	Any	M1

T: locally advanced	**N: lymph nodes**	**M: distant metastases**
T1: locally limited, not infiltrating T2: locally advanced, infiltrating Diameter: a ≤ 5 cm b > 5 cm	N0: not occupied N1: occupied regional lymph nodes (> 1 cm in CT/MRI/18F-FDG) Nx: unknown status	M0: distant metastases absent M1: distant metastases present, spinal cord and the presence of metastatic tumor in the pleura or peritoneum]metastases in extra-regional lymph nodes as well the presence of free tumor cells in the pleural, peritoneal, and cerebral fluid
Good localizations	Eye socket, head, and neck (except for the parameningeal area), urogenital system (except for bladder and prostate)	
Poor localizations	Parameningeal area, limbs, retroperitoneal space, bladder, prostate, biliary pathways*, other	

*As modified by Children's Oncology Group [169]

Table 7. Prognostic RMS evaluation according to the Intergroup Rhabdomyosarcoma Study Group classification

Prognosis (EFS, *event-free survival*)	Stage acc. to IRSG TNM	Clinical group	Localization	Size	FOX01 rearrangement (fusion)	M	N
Excellent (> 85%) Low-risk subgroup A	1	I	Favorable	a/b	Negative	M0	N0
	1	II	Favorable	a/b	Negative	M0	N0
	1	III	Eye socket	a/b	Negative	M0	N0
	2	I	Unfavorable	a	Negative	M0	N0/Nx
	1	II	Favorable	a/b	Negative	M0	N1
Very good (70–85%) Low-risk subgroup B	1	III	Eye socket	a/b	Negative	M0	N1
	1	III	Favorable, except for eye socket	a/b	Negative	M0	N0/N1/Nx
	2	II	Unfavorable	a	Negative	M0	N0/Nx
	3	I/II	Unfavorable	a	Negative	M0	N1
	3	I/II	Unfavorable	b	Negative	M0	N0/N1/Nx
Good (50–70%) Moderate risk subgroup	2	III	Unfavorable	a	Negative	M0	No/Nx
	3	III	Unfavorable	a	Negative	M0	N1
	3	III	Unfavorable	b	Negative	M0	N0/N1/Nx
	1/2/3	I/II/III	Any	a/b	Positive	M0	N0/N1/Nx
	4	IV	Any	a/b	Negative	M1	N0/N1/Nx
Poor (< 30%) High-risk subgroup	4	IV	Any	a/b	Negative	M1	N0/N1/Nx
	4	IV	Any	a/b	Positive	M1	N0/N1/Nx

present, radical RT is used. Taking into consideration the complications of radiotherapy, histological examination of the lymph nodes should be performed to exclude non-neoplastic reactive lymphadenopathy [51]. For localizations in the extremities sparing treatments are preferred [144]. If radical surgery cannot be perfor-

Table 8. Classification of patients to clinical groups taking into consideration the extent of the surgery and the progression of the disease according to the Intergroup Rhabdomyosarcoma Study Group

Clinical group* (CG)	Stage plus result of surgery
I	A — localized disease, no infiltration of surrounding structures and spaces, microscopically radical resection
	B — localized disease, infiltration of surrounding structures and spaces, microscopically radical resection
II	A — localized disease, no infiltration of surrounding structures and spaces, resection microscopically non-radical, macroscopically radical
	B — regional lymph nodes occupied, microscopically radical resection
	C — regional lymph nodes occupied, resection microscopically non-radical, macroscopically radical
III	A — tumor regardless of local stage and regional lymph node occupation, exclusively biopsy
	B — tumor regardless of local stage and regional lymph node occupation, surgery macroscopically non-radical, more than 50% of tumor volume
IV	Any local stage, any surgery result, presence of distant metastases

*As modified by Children's Oncology Group [169]. Clinical group I defines a localized disease, after microscopically radical resection, without involvement of regional lymph nodes (subgroups IA and IB are no longer distinguished). There are also no subgroups in clinical group III

med because of considerable local disease progression, localization, or other contraindications for surgery, qualifying the patient for radical RT should be considered. In the case of ARMS, performing non-radical surgery, decompression treatments, or aggressive mutilating palliative surgery does not improve the prognosis but only delays the moment of initiating systemic treatment (an exception are ERMS metastases to the retroperitoneal space). The scope of the used surgical treatment is important for further planning of radiotherapy. In clinical practice, the classification into clinical groups (CG) according to IRSG is used (Tab. 8).

Radiotherapy

Supplementary radiotherapy is indicated in all patients with RMS stages 1–3 according to IRSG TNM and CG I–III except for ARMS without the FOX01 rearrangement (then a decision should be taken based on risk and benefit analysis). Typically, treatment should be started after the fourth course of chemotherapy. Experience so far suggests that even if cranial nerves are affected or the tumor infiltrates the base of the skull, RT can only be started in week 12 of treatment if it was preceded by a rapid start of neoadjuvant chemotherapy [171]. Radiotherapy should be initiated urgently, regardless of the number of received chemotherapy courses in a situation of vision loss or spinal compression.

Depending on the localization and clinical group (supplementary RT after surgery or radical RT) the used total doses vary in the range of 50–65 Gy. Because of complications after irradiation (particularly visible in children), the aim is to reduce the total dose or to use other methods or RT techniques T [172, 173]. The results of recent IRSG studies indicate that in the pediatric population simultaneously treated with chemotherapy, the RT doses can be decreased to 36–50.4 Gy (28 fractions of 1.8 Gy) without affecting the treatment results. There are no data concerning optimal RT regimens in the adult population. The target volume should be the volume of the primary tumor before chemotherapy and surgery and regional lymph nodes (clinically suspected or in imaging studies). Using modern techniques of RT, proton radiotherapy, or brachytherapy may be associated with the protection of critical organs and contribute to decreasing the percentage of distant treatment complications. RT use in CG II improves local effectiveness from 65% to 83%. The comparison of the results of European (MMT) and American (IRS) studies suggests that in localized forms (low and intermediate-risk group) early use of local treatment using RT may be associated with better local control and OS (84% in IRS-IV studies *vs.* 71% in MMT89 studies, where local treatment, mainly by surgery, was used after obtaining a response to chemotherapy). Also, the percentages of 5-year progression-free survivals were higher in the IRS-IV study, 78% *vs.* 57% in the MMT89 study. A higher number of complications of surgical treatment was, however, observed.

In clinical practice, preferred regimens of irradiation depend on CG:

— CG I: 41.4 Gy in 23 fractions of 1.8 Gy;
— CG II: 45 Gy in 25 fractions of 1.8 Gy;
— CG III in localization other than the eye socket with residual tumor < 5 cm: 50.4 Gy in 25 fractions of 1.8 Gy;
— CG III in localization other than the eye socket with residual tumor > 5 cm: 50.4 Gy in 25 fractions of 1.8 Gy, simultaneous increase of total dose on the residual tumor to 56 Gy in fractions of 2 Gy;
— CG III in eye socket localization: 45 Gy in 25 fractions of 1.8 Gy with simultaneous chemotherapy based on a regimen containing cyclophosphamide;

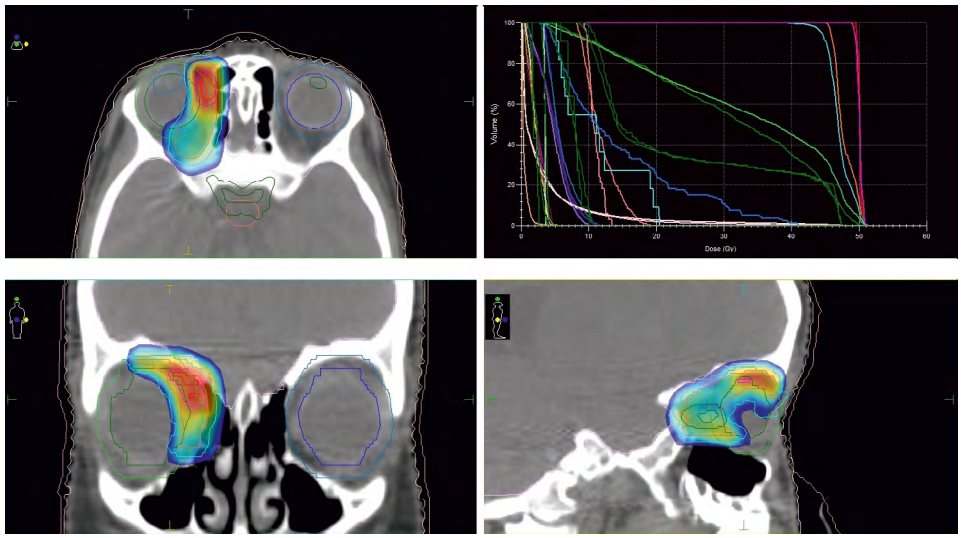

Figure 6. The treatment plan for a 5-year-old boy with a diagnosis of embryonal RMS of the right eye socket (superior medial wall) after chemotherapy. Plan performed by the VMAT SIB technique assumed giving a 45 Gy dose on the area of the primary tumor with the margin (eye socket), with an increase to 50 Gy in the area of the residual tumor in 25 fractionated doses of 1.8 Gy and 2 Gy, respectively

— Parameningeal localization (the nasal cavity, nasopharynx, paranasal sinuses, middle ear, mastoid process, subtemporal fossa, pterygopalatine fossa): the elective volume is the primary tumor, adjacent meninges, and the intracranial area, preferred fractionation scheme is 54–59.4 Gy in 30-33 fractions of 1.8 Gy.

The described regimens apply to the pediatric patient population. For decisions on adult patients concerning fractionation, regimens should be individually taken for each case (Fig. 6, 7).

Chemotherapy

Adding neo- and adjuvant chemotherapy to the treatment of patients without metastases allowed to obtain 60–90% percent of 5-year survival. In patients over 16 years of age and adults, both in multicenter studies and in retrospective analyses, the results of treatment are worse, and the percentage of 5-year survival is in the range of 30–40%. The intensity (2- or 3-drug treatments) and the duration of treatment (6, 12, or 24 months) depend on the risk group (Tab. 6, 7).

In the trials patients both in the disseminated and the localized stage were treated with various chemotherapy regimens, among the most common were doxorubicin monotherapy, doxorubicin plus ifosfamide, doxorubicin plus ifosfamide plus dacarbazine. Some of the patients received regimens in agreement with pediatric standards of RMS treatment, most commonly: ifosfamide plus vincristine plus actinomycin D, ifosfamide plus vincristine plus doxorubicin plus dacarbazine, and ifosfamide plus vincristine plus actinomycin D plus doxorubicin. In over 30% of treated patients, local recurrence of the neoplastic disease was observed, and another 40% developed distant metastases, but both using radiotherapy ($p = 0.011$) and chemotherapy according to pediatric protocols ($p = 0.003$) were associated with statistically better overall survival (OS) in multifactorial analysis. Moreover, using pediatric chemotherapy regimens in treating localized RMS in adults was described in the research of Kojima et al. [174]. This included the following protocols: 1) vincristine 1.5 mg/m^2 (days 1, 8 and 15) plus cyclophosphamide 2.2 mg/ m^2 (day 1) plus actinomycin D 1.5 mg/m^2 (day 1); 2) vincristine plus dactinomycin plus another component chosen among: ifosfamide, etoposide, or doxorubicin. Not only the pediatric chemotherapy regimens but also the whole therapeutic procedure for the adult patient with localized RMS according to pediatric recommendations for the treatment of this disease affects OS and increases the percentage of patients with 5-year local recurrence-free survival (LRFS) [144].

Basic treatment regimens for ERMS and ARMS are presented in Table 8. VAC and VAI/IVA regimens appear to be equivalent. Adding other active drugs to the basic regimen (VAC), such as doxorubicin, etoposide, cisplatin, carboplatin, ifosfamide, or melphalan, according to trial results published so far, did not have a statistically significant effect on OS in patients with RMS in clinical groups III and IV (Tab. 8). Evaluation of the combination of standard therapy VAC/VAI with irinotecan or topotecan is the subject of the ongoing trial

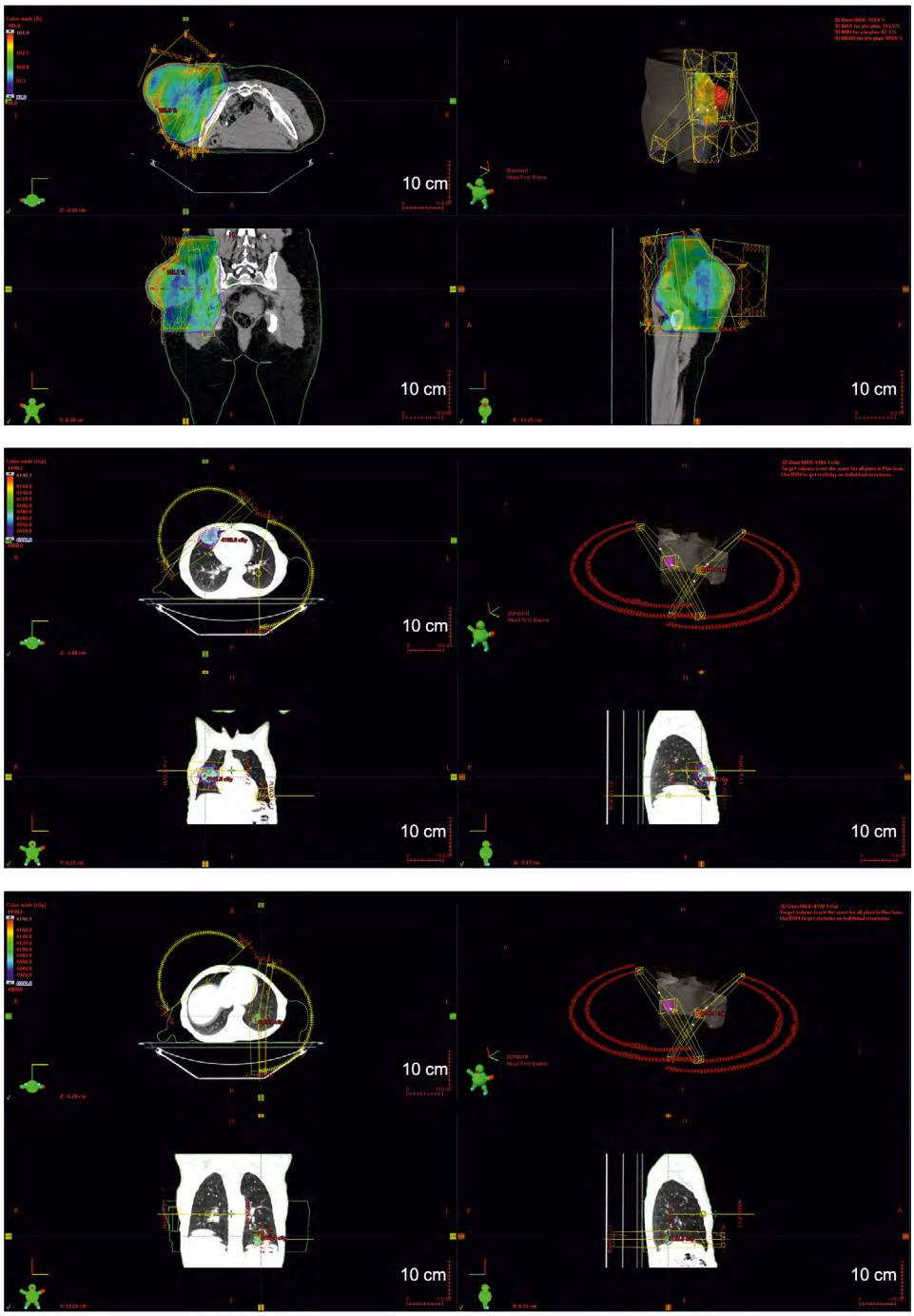

Figure 7. Radiotherapy plans of an adult female patient with pleomorphic rhabdomyosarcoma with a limited number of metastases to the lungs (oligometastatic disease) diagnosed during pregnancy. The patient received chemotherapy based on doxorubicin up to the moment of birth. After the birth of a healthy child, she was qualified for hypofractionated preoperative radiotherapy 5 × 5 Gy (**A**), resection, postoperative chemotherapy, and stereotactic radiotherapy 10 × 4 Gy on the volume of two lung metastases (**B** and **C**)

IRS-V. The results of the European trial with random selection of patients European Paediatric Soft Tissue Sarcoma Study Group (EpSSG) RMS2005 (population < 18 years old) have not been published yet, its aim, among others, was to evaluate in a subgroup of patients with ARMS with the N1 characteristic the effect of adding doxorubicin to standard IVA chemotherapy and the effectiveness of supportive care with vinorelbine and cyclophosphamide (altogether 50 weeks). Based on available data, it is difficult to unequivocally determine

the duration of systemic treatment and indications for supportive treatment in adult patients. In adult patients (with ARMS high-risk group), treatment should last up to 48–52 weeks. In the case of RMS treated in the AI regimen (doxorubicin, ifosfamide plus mesna) or MAID (doxorubicin, ifosfamide plus mesna, dacarbazine), systemic treatment is generally performed until the maximum dose of doxorubicin has been used (if the progression of the disease is not noted previously during the treatment) [14, 175–178]. No clinical trials have evaluated specific chemotherapy regimens of patients with pleomorphic RMS. Most patients receive adjuvant chemotherapy (anthracyclines and alkylating cytostatics) with local tumor treatment by surgery and/or radiotherapy. Because of the differences in the biology and phenotype of this RMS subtype in comparison with ERMS and ARMS, multicomponent pediatric chemotherapy regimens may not be applicable in such cases. On the contrary, in the case of adult patients with RMS other than pleomorphic, the use of regimens described in pediatric guidelines for the treatment of this neoplasm is recommended, and the criteria of age for the inclusion in clinical trials evaluating the effects of treatment with pediatric regimens are often extended from the pediatric population to adults [51, 144] (Tab. 9, Fig. 8).

Observation after treatment

After completion of the treatment, the patient should be observed carefully. The recommended procedure includes the physical examination and imaging studies in the form of CT or MRI of the primary localization and CT of the chest, abdominal cavity, and pelvis using a contrast agent. Medical visits should take place every three months for the first two years, then every six months for the next three years, and subsequently once a year.

General principles of treating disease with distant metastases

In a high percentage of patients, distant metastases are found at the moment of diagnosis, which is linked with a poor prognosis. Treating the patient with distant metastases will include each of the three methods used for localized disease, that is surgery, RT, and chemotherapy. Some authors recommend limiting chemotherapy to VAC or VAC/VI regimens taking into consideration the patient's quality of life and the poor prognosis in this group [51]. Surgery and/or RT of the primary tumor are used for RMS with a limited number of metastases to limit the risk of failure of subsequent therapy. In the case of multiple metastases, the priority is obtaining control of the disease, and if this is successful, local treatment can be considered. In most patients with RMS with numerous metastases, therapeutic procedures are palliative in character. In a retrospective multicenter analysis of RMS patients in stage 4 according to IRSG TNM, among 13 patients included in the trial two underwent resection of the primary tumor, six received palliative RT, and seven palliative chemotherapy [7]. Median OS was 7.1 months. The most common chemotherapy regimens were doxorubicin monotherapy, ifosfamide with doxorubicin, and multicomponent chemotherapy vincristine plus doxorubicin plus cyclophosphamide. Among 14 patients receiving chemotherapy admitted to hospitals with primary disseminated neoplastic disease and progressing to metastatic disease, only in 7 patients a clinical benefit was observed in response to chemotherapy [PR (partial response) or SD (stable disease)]. The only responses after administration of successive lines of chemotherapy in patients with a partial response or stable disease after the first line were observed in patients receiving chemotherapy according to the VAC protocol (vincristine, doxorubicin, cyclophosphamide). Median progression-free survival (PFS) was 2.3 months. Another retrospective study included 4 patients with RMS M1 and palliative chemotherapy was initiated in all patients, and in one of them, treatment was supplemented by palliative radiotherapy [6]. The median overall survival of the treated patients was 21.7 months. In an observational study by Bompas et al. [147] among 46 patients with stage M1 RMS, 19 received surgery, 26 radiotherapy, and 29 received palliative chemotherapy (doxorubicin ± ifosfamide or multidrug therapy based on ifosfamide, vincristine, actinomycin with or without supporting chemotherapy with cyclophosphamide). Complete remission in this group was obtained in only 13 patients (28%). Five-year survival of patients with metastatic disease was 5% (median: 13 months). The results of the clinical trial VIT-0910 indicated that adding temozolomide to the vincristine and irinotecan regimen improves the survival of patients with recurring or resistant RMS [190]. The results of studies in centers treating RMS indicate that the most common chemotherapy regimens used in such patients are multicomponent regimens using combinations such as vincristine, doxorubicin, and cyclophosphamide (VAC), or ifosfamide with doxorubicin or doxorubicin, ifosfamide, dacarbazine, and mesna (MAID) [38]. There are single descriptions of treating RMS patients with a small molecule tyrosine kinase inhibitor pazopanib which suggest that this drug could find application in patients previously treated with standard chemotherapy regimens [191, 192]. Unfortunately, another small-molecule tyrosine kinase inhibitor (crizotinib), which, among others, inhibits ALK kinase, did not have clinically significant activity in monotherapy of ARMS patients [193].

Table 9. Chemotherapy regimens used in rhabdomyosarcoma treatment

Regimen name	Administered drugs	References
Most common regimens of RMS treatment		
VA	Vincristine 1.5 mg/m^2 (max. 2 mg), day 1. Dactinomycin 0.15 mg/kg/d. (max. 0.5 mg/d.), day 1.–5.	[179]
VAC	Vincristine 1.5 mg/m^2 (max. 2 mg), day 1. Dactinomycin 0.15 mg/kg/d. (max. 0.5 mg/d.), day 1.–5. Cyclophosphamide 2.2 g/m^2 plus mesna, day 1.	[179]
VAC	Vincristine 1.4 mg/m^2 (maximum dose 2 mg), day 1, 8, 15 Actinomycin D 1.25 mg/m^2 (maximum dose 2mg), day 1. Cyclophosphamide 1200 mg/m^2, day 1.	[180]
VAC/IE	Vincristine 1.6 mg/m^2, day 1. Actinomycin D 0.45 mg/kg, day 1. Cyclophosphamide 1200 mg/m^2, day 1. Ifosfamide 1800 mg/m^2 plus mesna days 21.–25. Etoposide 100 mg/m^2, day 21. Second line of treatment: cisplatin and etoposide	[88]
VAI	Vincristine 1.5 mg/m^2 (max. 2 mg), day 1. Dactinomycin 0.15 mg/kg/d. (max. 0.5 mg/d.), day 1.–5. Ifosfamide 1.8 g/m^2/d. plus mesna, day 1.–5.	[179]
VIE	Vincristine 1.5 mg/m^2 (max. 2 mg), day 1. Ifosfamide 1.8 g/m^2/d. plus mesna, day 1.–5. Etoposide 100 mg/m^2/d., day 1.–5.	[179]
IVA	Ifosfamide 3 g/m^2/d. plus mesna, day 1.–3. Vincristine 1.5 mg/m^2 (max. 2 mg), day 1. Dactinomycin 1.5 mg/m^2 (max. 2 mg/d.), day 1.	[179]
IVADo	Ifosfamide 3 g/m^2/d. plus mesna, day 1.–2. Vincristine 1.5 mg/m^2 (max. 2 mg), day 1. Dactinomycin 1.5 mg/m^2 (max. 2 mg/d.), day 1. Doxorubicin 30 mg/m^2, 4-hour infusion, day 1.–2.	[179]
VDC	Vincristine 1.5 mg/m^2 (max. 2 mg) Dactinomycin 75 mg/m^2 Dexrazoxane	[51]
IE	Ifosfamide 9 g/m^2 Etoposide 500 mg/m^2	[51]
VI	Vincristine 1.5 mg/m^2 (max. 2 mg) Irinotecan 50 mg/m^2	[51]
VA	Vincristine 1.5 mg/m^2 (max. 2 mg) Dactinomycin 0.045 mg/kg (max. 2.5 mg)	[51]
VAC	Vincristine 1.5 mg/m^2 (max. 2 mg) Dactinomycin 0.045 mg/kg (max. 2.5 mg) Cyclophosphamide 1200 mg/m^2	[51]
Less common regimens of RMS treatment		
–	Vincristine 2 mg Doxorubicin 75–90 mg/m^2 72 h infusion plus dexamethasone (cardioprotection) Ifosfamide 10 g/m^2 divided in boluses for 4–5 days	[38]
–	First line of treatment: Doxorubicin 60 mg/m^2 Cyclophosphamide 600 mg/m^2 Second line of treatment: Cisplatin 75 mg/m^2 Taxol 200 mg/m^2 Next line of treatment: Gemcitabine 1000 mg/m^2 Carboplatin AUC 5	[98]

→

Table 9 cont. Chemotherapy regimens used in rhabdomyosarcoma treatment

Regimen name	Administered drugs	References
–	Preoperative chemotherapy: Doxorubicin (50 mg/m^2) Dacarbazine (1000 mg/m^2) Vincristine (1.4 mg/m^2) Cyclophosphamide (700 mg/m^2) Postoperative: Methotrexate (2 g/m^2)	[181]
–	Cyclophosphamide 1200 mg/m^2 day 1. Vinorelbine 25 mg/m^2 day 1 and 8 Temsirolimus 15 mg/m^2 day 1, 8, and 15 (maximum 12 cycles of treatment).	[182]
–	Cyclophosphamide 250 mg/m^2 day 1.–5. Topotecan 0.75 mg/m^2 day 5.	[183]
–	Cyclophosphamide 25 mg/m^2 each day of the cycle Vinorelbine 25 mg/m^2 on days 1, 8, 15, and 28 of the cycle	[184]
–	Dactinomycin 25 mg/m^2 day 1.–3. Ifosfamide 2500 mg/m^2 day 1.–4.	[185]
–	Dactinomycin 75 mg/m^2 day 1 (every 21 days up to 6 cycles)	[185]
Vinorelbine in monotherapy	Vinorelbine 30 mg/m^2 on days 1 and 8 of the cycle every 3 weeks OR Vinorelbine 33.75 mg/m^2 every week for 6 weeks, then 2 weeks without the drug	[186, 187]
–	Vincristine 1.5 mg/m^2, day 1 and weeks 1, 2, 4 and 5 Irinotecan 50 mg/m^2 for 5 days in week 1 and 4	[188]
GD	Gemcitabine 900 mg/m^2 day 1 and 8 Docetaxel 100 mg/m^2 day 8 In a 21-day cycle	[189]

GD — gemcitabine, docetaxel; IE — ifosfamide and etoposide; IVA — ifosfamide, vincristine, dactinomycin; IVADo — ifosfamide, vincristine, dactinomycin, doxorubicin; RMS — rhabdomyosarcoma; VA — vincristine, dactinomycin; VAC — vincristine, actinomycin, cyclophosphamide; VAI — vincristine, dactinomycin, ifosfamide; VIE — vincristine, ifosfamide, etoposide

Procedure in the case of disease progression during or after treatment

In at least one-third of patients with RMS local or general recurrence will occur [51]. In patients in whom the disease has progressed during the first line of treatment, the prognosis is particularly poor. Patients who completed RMS treatment often do not obtain a full radiological response in layered imaging studies, despite normalization of the PET scan picture, which is probably due to the scarring of the primary tumor site or the differentiation of that tissue. For that reason, the biopsy of the tumor bed after removal of the primary tumor is not recommended except for situations where the primary tumor increases in size or pain occurs [194]. Patients whose PET scan indicates an enhanced signal in the site of the primary tumor pose a particular challenge in respect to the choice of further therapy. Indubitably, they belong to the group of patients with an increased risk of local recurrence and development of distant metastases, and the decision on performing a biopsy of the primary site of the tumor or further surgical resection should be taken after stratification of both risks and potential benefits [195]. A definite suspicion of RMS recurrence requires taking tissue material using a biopsy and histopathological confirmation. Surgical resection of the tumor may be considered if access to the site of recurrence allows this. Radiotherapy is quite often used for the treatment of the primary tumor (if it had not been treated previously) and metastases to the bones and lungs if this is doable. Radiotherapy may be delayed, especially in respect to neoplastic metastases, to evaluate the response to chemotherapy and to avoid myelosuppressive complications due to systemic cytotoxic treatment. Currently, it is particularly important to include patients with RMS recurrence or progression into clinical trials and to use chemotherapeutics with proven activity against RMS. There are no data permitting comparison of the effectiveness of treating

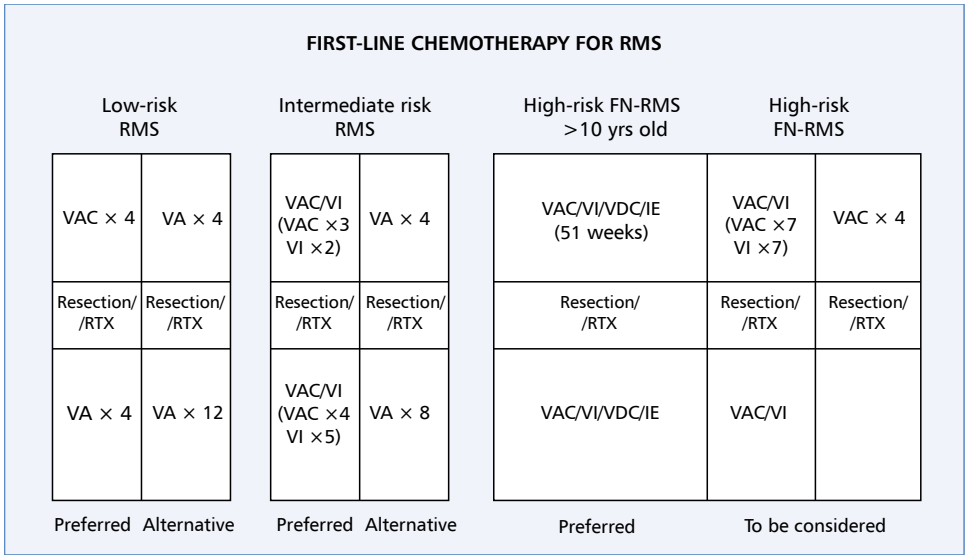

Figure 8. Proposed rhabdomyosarcoma (RMS) treatment regimens in children and young adults. Based on [51]; IE — ifosfamide and etoposide; RTX — radiotherapy; VA — vincristine, actinomycin; VAC — vincristine, actinomycin, cyclophosphamide; VDC — vincristine, doxorubicin, deksrazoksan; VI — vincristine and irinotecan

with specific chemotherapy regimens, thus the decision about the choice of a given type of therapy in patients with RMS recurrence depends on many factors, including among others the first-line treatment protocol, the patient's general status, and the tolerance of earlier therapy. As a rule, second-line chemotherapy is used containing previously mentioned active drugs (platinum derivatives, camptothecin, etoposide, doxorubicin, vinorelbine). In patients with recurrence or primarily generalized RMS, attempts at high-dose chemotherapy have also been made. Phase III trials with a random selection of patients were conducted, in which standard chemotherapy was compared with myeloablative treatment. There are insufficient data to determine the optimum length of chemotherapy duration in the case of recurrence/progression. Patients, in general, receive at least 8 cycles of chemotherapy, if a complete response to therapy and acceptable tolerance of therapy occurs [51]. In the MMT89 and MMT91 trials, a group of 52 patients from a high-risk group who had undergone myeloablative therapy after standard induction chemotherapy were nonrandomly compared with 44 patients treated only with standard inductive and supplementary chemotherapy. The percentages of progression-free survivals were 30% for myeloablative chemotherapy and 19% for standard treatment. In this group of patients, a more significant prognostic factor associated with treatment turned out to be the response to initial inductive chemotherapy. The percentage of OS in patients who were in complete remission after surgery and chemotherapy up to week 18 was 41% in comparison with 14% in patients in whom a complete response had not been obtained ($p = 0.0001$). The available data do not allow a valid evaluation of myeloablative treatment in young adults and suggest the resistance of RMS cells to mega chemotherapy, as most recurrences after treatment occurred in sites previously occupied by the neoplasm [196, 197]. The effectiveness of chemotherapeutics commonly used in treating other types of soft tissue sarcomas in adults such as gemcitabine, docetaxel, or pazopanib, has so far not been sufficiently evaluated in RMS. Moreover, few published data are indicating a good effect of using tyrosine kinase inhibitors in the therapy of patients with RMS. Also, the role of the increasingly popular immunotherapy, including checkpoint inhibitors (antibodies directed against PD-1, PD-L1, or CTLA-1), immunomodulating drugs, or CAR-T cell therapy, has not yet been verified in the context of RMS treatment, but there are single cases of complete response to treatment with drugs from these groups [198].

The algorithm of the procedure to follow if recurrence or progression of rhabdomyosarcoma is suspected is presented in Figure 9 [51].

RMS in adult patients — selected aspects

Due to the rare occurrence of RMS in the adult population, there are no unequivocal guidelines concerning the treatment of this neoplasm. There are no published

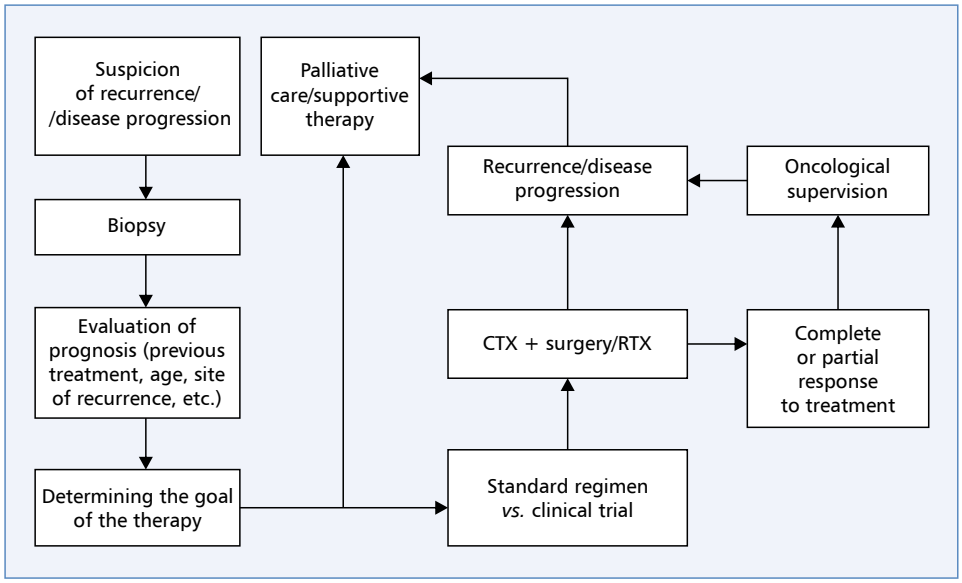

Figure 9. Algorithm of procedure for suspicion of rhabdomyosarcoma recurrence or progression. After [51]; CTX — chemotherapy; RTX — radiotherapy

results of clinical trials with randomization which could be the basis of uniform principles of care for such patients. So far experience in treating adult patients with RMS is based on small groups collected in retrospective analyses [144, 147, 178]. The used treatment regimens differ considerably depending on the center in which the patients are treated and the therapy standards in that center.

In the largest meta-analysis published so far including 533 adult patients with RMS in a localized stage, a large variety of treatment protocols chosen by specialists for patients with RMS was presented [5]. Currently, three main methods are used for the treatment of localized RMS, which include oncological surgery, radiotherapy, and chemotherapy. In the mentioned meta-analysis, the most used methods were surgery plus chemotherapy (27.5%), a combination of the 3 methods (25.1%), and surgery alone (19.0%). The combination of surgery and radiotherapy (1.2%) and radiotherapy and chemotherapy (13.2%) were slightly less common. Monotherapy, that is chemotherapy or radiotherapy, was the least common (3.8%). The median radiation dose used in the case of radiotherapy and surgery was 54 Gy (from 14 to 110 Gy), while when radiotherapy was the only method used the median was slightly higher, namely 56.5 Gy (from 36 to 110 Gy). The most common chemotherapy was as an adjuvant treatment (42.9%) or as primary therapy (15.3%). The protocols were mainly based on cyclophosphamide (22.5%), a combination of cyclophosphamide with anthracycline (21.2%), and a combination of ifosfamide with anthracycline (13.9%). Another study which included 82 patients with locoregionally advanced RMS, had a 5-year overall survival index of 44% [155]. The treatment was most commonly a combination of surgery, radiotherapy, and chemotherapy (30 persons), a combination of radiotherapy and chemotherapy (28 persons), and a combination of radiotherapy and surgery (15 persons). Radiotherapy in the case of a preoperative procedure included irradiation with a median of 50 Gy, whereas postoperative radiotherapy and radiotherapy without surgery had a median of 60 Gy. Chemotherapy was given to 58 patients and included administering doxorubicin or actinomycin D in combination with vincristine or cyclophosphamide. Disease recurrence occurred in 47 patients (57%), most commonly in the form of distant metastases (22 persons), less frequently as a local (11 persons) or loco-regional recurrence (11 persons). In the retrospective study of Noujaim et al. [7] 32 patients with localized RMS were described in whom in 26 cases radical surgery — removal of the primary tumor — was performed. Frequently in as many as 15 patients, postoperative radiotherapy was applied, whereas only 3 persons received chemotherapy or preoperative radiotherapy. Local and distant recurrence was present in 4 and 10 persons, respectively. In a study from another center, including 16 patients with RMS in a localized stage, the most common procedure was radiotherapeutic treatment combined with chemotherapy (11 persons) and chemotherapy alone (2 persons) [6]. Three persons were treated by primary surgery supplemented by chemotherapy or/and radiotherapy. The most frequently chosen chemotherapy regimen (regardless of the stage of the disease) was vincristine, actinomycin D,

cyclophosphamide (VAC) alternating with ifosfamide and etoposide (IE). During radiotherapy in 10 patients, simultaneous chemotherapy was used in the vincristine and cyclophosphamide (VC) regimen. Among patients treated with non-palliative radiotherapy, the median radiation dose was 56 Gy. Among 16 persons treated for primary localized RMS, 4 had local recurrence, whereas metastases or regional dissemination were observed in 6 persons. Treatment with radiotherapy ($p = 0.009$) and chemotherapy lasting longer than 19 weeks ($p = 0.009$), as well as adding a simultaneous regimen of VC chemotherapy to radiotherapy ($p = 0.01$), was associated with a longer OS. In the next group of patients including 111 adults with localized RMS, surgery of the primary tumor was performed in 80% (89 persons), radiotherapy in 73% (81 persons), and chemotherapy was administered to 75% of patients (83 persons) [147]. CT, MRI, and PET-CT can be used to evaluate the periodic effectiveness of the treatment.

Because RMS is rare in adults there is a limited number of papers reporting the results of treating this neoplasm, and their main limitation is their retrospective character (Tab. 10). The lack of unequivocal guidelines concerning the treatment of metastatic disease leads to a large variety of chemotherapy regimens in studies from large centers, which, moreover, differ in their standards of oncological care. Survival of adults with RMS is still much lower than the results obtained in pediatric populations, where 5-year overall survival is OS = 77–87% in children and OS = 20–40% in adults [5, 144, 167, 178]. This fact can probably be explained by several aspects. First, age was shown to be an independent prognostic factor for patients with RMS [200]. A significant difference is the use of lower doses of supportive chemotherapy components in adults in comparison to children, due to the high frequency of serious complications, including bone marrow suppression, infections, and neurotoxic effects, among others [174]. Additionally, the more common histopathological RMS subtypes in adults are pleomorphic and alveolar RMS, they are associated with a poorer prognosis [13]. Five-year overall survival in RMS patients is in the range of 40–50% for localized disease and is from zero to 30% in the case of metastatic disease based on available analyses from large centers [7, 14, 147, 178]. Nevertheless, retrospective studies have shown that initiation of pediatric protocols of localized disease treatment is associated with a better prognosis and obtaining 5-year survivals of patients at the level of 61.5% [144, 147]. The use of radiotherapy was also associated with better survival of the patients both in the local disease stage, as well as for disseminated disease [147, 200, 201]. Including chemotherapy was found to be an independent prognostic factor causing lower mortality due to the progress of the neoplastic disease according to the analysis of patients from a center in Thailand [202]. There are, however, also reports on the lack of improvement of survival of patients subjected to chemotherapy in comparison to a control group [201]. Moreover, tumors smaller than 5 centimeters in size correlate with longer patient survival regardless of other factors [155, 178]. Favorable tumor localization has been distinguished in some elaborations of retrospective studies as an independent factor affecting survival [4]; however, there are also opposite conclusions that multifactorial analysis indicates that this aspect is not statistically significant [5, 203]. In the case of tumors of the urinary tract, localization within the prostate gives a better prognosis as compared to the urinary bladder or kidney RMS [204]. It is worth underlining that obtaining negative surgical margins after tumor resection was distinguished as a prognostic factor in the context of disease recurrence and progression [147, 178]. Surgical resection of the primary tumor remains the standard in localized disease, but it has been shown that this procedure improves survival in disseminated disease in adults [147, 205]. After treatment careful control of recurrences is necessary. Medical visits should take place every 3 months for the first year, every 4–6 months during the second and third year, and subsequently once a year. Among analyses performed during control visits are interview and physical- examination, peripheral blood morphology and biochemistry (parameters of liver and kidney function), imaging studies — CT every 3–6 months for the first 2 years, subsequently once a year for the next 3 years, bone scintigraphy (every 6 months for the first 2 years, subsequently once a year for the next 3 years), the remaining examinations (ultrasound/CT/MRI) of the area of the primary tumor and PET-CT depending on the decision of the multi-specialist team [206].

Selected aspects of pediatric RMS

Rhabdomyosarcoma is the most diagnosed soft tissue sarcoma in children. It constitutes about 5–7% of all pediatric neoplasms and 60% of soft tissue sarcomas. Over one-half of the cases appear in small children aged 2–6 years. In the group of pediatric patients, ERMS (55–70%) and ARMS (25–30%) are the most common [13, 209–211]. The most frequent localization of the disease in children is the head and neck area (eye socket, parameningeal area, soft tissues of the face and neck; about 36%). These are generally cases of ERMS, diagnosed before the age of eight years, rarely metastasizing to regional lymph nodes [166]. The urogenital tract is also a frequent site of RMS occurrence in children (approx. 23%). In respect to prognosis, this localization can be divided into the area of the bladder and prostate, and the area without the bladder and prostate (testes,

Table 10. Studies describing the treatment of adult patients with diagnosed rhabdomyosarcoma

References	Number of patients	Patients' age (median)	mOS		5-OS		Median PFS/DFS/RFS/EFS	
			M(–)	M(+)	M(–)	M(+)	M(–)	M(+)
[7]	45	71.5 (28.4–92.8)	12.8	7.1	–	29%	7.3 (RFS)	2.3 (PFS)
[6]	20	34 (19–79)	53.2	21.7	–	20% (3–year)	19.8 (DFS)	20.4 (DFS)
[147]	157	37 (18–86)	40.0	13.0	43%	5%	9.3 (RFS)	–
[147]	292	55 (18–99)	40.0 (whole cohort)	40.0 (whole cohort)	–	–	–	–
[178]	84	31 (16–76)	35.0	15.0	50%	22%	–	–
[200]	36	29 (21–72)	–	–	–	–	22.4 (PFS)	13.3 (PFS)
[207]	59	56 (38–72)	11.0	9.0	–	–	–	–
[14]	39	26 (16–82)	–	–	44%	0%	–	–
[155]	82 (M–)	27 (17–81)	38	–	44%	–	6.5 (PFS)	–
[144]	171	27 (19–83)			45.7%	4.3%		
[13]	1071	> 19	–	–	47%	–	–	–
[4]	138	28 (16–86)	–	–	45%	approx. 18%	–	–
[180]	8	24 (18–60)	27.3	–	–	–	17.0 (PFS)	–
[203]	66	28 (18–71)	30.0	11.0	36%	11%	17.0 (EFS)	–
[208]	239	19 (10–102)	45.6	16.8	44.1%	18%	22.8 (RFS)	10.8 (PFS)
[203]	66	28 (18–71)	30.0	11.0	35%	11%	–	–

DFS — disease free survival; EFS — event free survival; M(–) — non-metastatic disease; M(+) — metastatic disease; m-OS — median overall survival; OS — overall survival; PFS — progression free survival; RFS — relapse free survival; 5-OS — 5 years overall survival

epididymis, the peritesticular area, penis, vulva, vagina, ovary, uterus) [212]. Vaginal RMS deserves particular attention in this group; it occurs, in general, in small girls and has a very characteristic clinical presentation of botryoid masses "falling out" of the vaginal vestibule, causing bleeding and/ or discharge [124, 203]. Uterine tumors are generally oligosymptomatic and are thus diagnosed in advanced stages [166, 212]. Extremities are a less common localization in children (approx. 20%). The neoplasm, in general, develops in the form of a painless tumor, often giving metastases to regional lymph nodes (50%) [166]. In about 15% of RMS cases in children, at the moment of diagnosis generalized disease is found (stage four of clinical progression according to TNM for RMS) with metastases to the lungs (50%), bone marrow (30–40%), bones (10%) and/or lymph nodes (depending on the localization 5–50%) [166, 212].

Treatment of soft tissue sarcomas in children is based on international protocols of the Cooperative Weichteilsarkom Studiengruppe (CWS) and the European Paediatric Soft Tissue Sarcoma Group (EpSSG), recommended by the Polish Pediatric Solid Tumor Group. The basis for the treatment strategy is appropriate stratification to risk groups based on the following prognostic factors: the disease stage according to the IRSG classification, histological type, age of the patient, size, and localization of the tumor. The risk stratification system is periodically updated. Taking into consideration the most recent data on the prognostic value of the genetic status of ARMS, it should be kept in mind that the current system of stratification will need to be verified in the near future. Currently, this system assumes a division into risk groups. The assignment to a given risk group determines the choice of a specified therapeutic regimen. The American system of risk stratification elaborated by the Children's Oncology Group-Soft-Tissue Sarcoma (COG-STS), differs slightly from the European system created by EpSSG. A detailed description of both systems of risk stratification is presented in Tables 11 and 12.

Recent studies have confirmed the importance of PAX3/7-FOXO1 fusion status as a critical prognostic biomarker following M status [215–217]. Other molecular factors of potential prognostic significance, which have not yet been used in RMS risk stratification, are under investigation. The INternational Soft Tissue SaRcoma ConsorTium will supervise the coordination of further research work and combining clinical and molecular data from different research studies from various medical centers. A modification of the current risk stratification system by including the PAX3/7-FOXO1 fusion status is a subject of several prospective clinical trials [COG

Table 11. Stratification to rhabdomyosarcoma (RMS) risk groups according to Children's Oncology Group-Soft-Tissue Sarcoma. Based on [131, 213, 214]

Risk	Histology	Grade	Clinical group
Low	ERMS	1	I, II, III
	ERMS	2, 3	I, II
Standard	ERMS	2, 3	III
	ARMS	1, 2, 3	I, II, III
High	ERMS	4	IV
	ARMS	4	IV

ARMS — alveolar rhabdomyosarcoma; ERMS — embryonic rhabdomyosarcoma

Table 12. Stratification to risk groups for the localized form of rhabdomyosarcoma (RMS) according to the European Paediatric Soft Tissue Sarcoma Group. Based on [131, 213, 214]

Risk	Histology	Clinical stage	Localization	N Status	Tumor size and patient's age
Low	ERMS	I	All	N0	≤ 5 cm and ≤ 10 years
Standard	ERMS	I	All	N0	> 5 cm or > 10 years
	ERMS	II, III	Favorable	N0	all
	ERMS	II, III	Unfavorable	N0	≤ 5 cm and ≤ 10 years
High	ERMS	II, III	Unfavorable	N0	> 5 cm or > 10 years
	ERMS	II, III	All	N1	All
	ARMS	I, II, III	All	N0	All
Very high	ARMS	II, III	All	N1	All

ARMS — alveolar rhabdomyosarcoma; ERMS — embryonic rhabdomyosarcoma

Table 13. Modified risk stratification system including the PAX3/7-FOXO1 fusion [219]

Risk	IRSG TNM stage	Clinical group	Age	Rearrangement FOXO1 (fusion)
Low	1	I, II, III (only eye socket)	Any	FOXO1–
	2	I, II		
Standard	1	III (without eye socket)	Any	FOXO1–
	1, 2, 3	I, II, III		FOXO1+
	2, 3	III		FOXO1–
	3	I, II		FOXO1–
	4	IV	< 10 years	FOXO1–
High	4	IV	≥ 10 years	FOXO1–
			Any	FOXO1+

(ARST1431) and EpSSG Frontline and Relapsed-Rhabdo-MyoSarcoma (FaR-RMS)] (Tab. 13) [218].

Unfavorable localization encompasses limbs, the parameningeal area, the bladder, and the prostate. The alveolar type of sarcoma, the patient's age > 10 years, and tumor size > 5 cm are also associated with a poorer prognosis [13, 170, 209–211].

Currently, in about 70% of children with locally advanced disease, a permanent cure is obtained after using combined treatment [209, 210]. The optimal time for initiating local therapy is controversial. European protocols recommend surgery and/or radiotherapy after the 3rd cycle, i.e. in week 13 from starting chemotherapy, and for metastatic disease from week 22 [209, 210]. The modality of surgical treatment of RMS is due to the possibility of infiltration of various sites by the tumor, and the course of the disease may be different depending on the tumor localization. Radical surgery is an important prognostic factor, but because of the localization and size of the tumor, it is generally difficult to perform. However,

only in 10% of the patients at the moment of diagnosis, the extent of neoplastic disease allows radical surgical resection [212]. In the remaining patients, the surgical intervention is limited to a biopsy. Analysis of survival did not indicate the superiority of debulking surgery as compared to a biopsy [56]. After performing non-radical tumor resection, a second evaluation by the surgeon is recommended to determine the possibility of radicalization and to consider performing such a procedure before initiating systemic therapy (PRE, pre-treatment re-excision). The premise of the surgical protocol is complete (macroscopic and microscopic) removal of the neoplastic tumor with the margin of the surrounding tissues, without a significant cosmetic effect nor perturbation of function [56, 212, 214]. The surgery before initiating systemic treatment affects risk stratification, allowing classification of the patient to a better group compared to the classification of the primary surgery [220]. The subject of safe tissue margins during primary resection in children remains controversial. Most frequently obtaining a margin of about 0.5 cm is recommended [56]. In the case of locally advanced disease, surgical treatment is only considered after completing induction chemotherapy (DPE, delayed primary excision) when imaging studies show a residual tumor qualifying for radical resection.

Histopathological verification of regional lymph nodes is recommended in patients with the suspected occupation of lymph nodes in a clinical investigation or imaging study and primary RMS localization within the limbs and the peritesticular area (≥ 10 years). This procedure is also recommended in children with ARMS with the PAX/FOXO1 translocation [56]. The recommended method is a biopsy of the sentinel node [56]. Confirmation of regional lymph node involvement is an indication for radiotherapy, as radical lymphadenectomy was not found to improve survival [56].

Rhabdomyosarcoma is a neoplasm with high sensitivity to chemotherapy, therefore, current regimens are based on neoadjuvant chemotherapy. Despite many studies on the intensification of this treatment, the standard in Europe is still the regimen using three drugs: ifosfamide, vincristine, and actinomycin D [209–211]. In turn, COG recommends the VAC regimen composed of vincristine, actinomycin D, and cyclophosphamide. The European group explains the substitution of cyclophosphamide by ifosfamide by a decreased risk of toxicity to the gonads. The treatment of patients qualified to the low-risk group is shorter, and a reduction of the dose of cyclophosphamide is also possible without affecting overall survival. In patients from the moderate risk group, a reduction of the cyclophosphamide dose requires adding the next drug (e.g. irinotecan) to the basic treatment regimen. The greatest challenge is the treatment of patients from the high-risk group and patients with disease recurrence, as for years no improvement of survival indices has been observed. In these groups, attempts are made to introduce new drugs into the treatment regimens now in force and to introduce new treatment methods.

Radiotherapy, except for cases from the low-risk group, is standard supplementary treatment after surgery. However, there are premises for including this treatment before surgery: an easier and more precise definition of the target for irradiation, limiting the volume of normal tissues receiving a high dose, decreasing the risk of secondary tumors (most of the irradiated tissues will be removed), and the radiobiological advantage of irradiating tissues which are better oxygenated. So far little data have been published on this subject. In a group of 17 children with diagnosed RMS of the urogenital tract, Seitz et al. [221] obtained a 5-year EFS of 82%, which is a very promising result. However, the basic advantage of such a treatment sequence is the decrease in the risk of late toxicity. Radiotherapy in the pelvic or parameningeal area, especially in children under 3 years of age, is a treatment associated with a high risk of hindering the development of irradiated tissues and serious toxicity depending on the dose administered to normal organs. The fear of initiating such aggressive treatment in neonates probably contributes to the poorer survival indices in this age group [214]. Hence modern treatment methods utilizing volumetric modulated arc therapy (VMAT) or proton therapy, with a greater possibility of protecting healthy tissues, are currently recommended as the treatment of choice [170, 221, 222]. To further improve the conformality of the distribution and to decrease the dose outside the target volume, the technique of simultaneous irradiation is used, in which different doses are given in different areas of the target volume simultaneously. For a selected group of patients, especially with RMS localized in the organs of the pelvis minor, brachytherapy may be used as part of the procedure together with sparing surgery. This is, however, a form of treatment performed only in a few reference centers [210].

In irradiation of children with an RMS diagnosis a broad range of doses from 36 to 54 Gy is used and, in the case of monotherapy, even up to 59.4 Gy. This depends on the localization and histological type of the sarcoma and, above all, on the extent of the residual disease [209–211]. Research on the use of doses escalated to 59.4 Gy in all patients with tumors exceeding 5 cm in size is ongoing [223].

There are many contradictory data on procedures in metastatic disease. In choosing the optimal type of treatment criteria, identifying 4 prognostic factors may be helpful: age, localization of the primary focus, occupation of the bone marrow, and metastases to at least 3 localizations. Patients with the presence of only one factor attain a 3-year EFS of 44% in comparison to 14% of patients with 2–4 factors [209]. There are no

unequivocal guidelines as to the role of surgery in treating generalized neoplastic disease. The recommended procedure is a biopsy, performed to confirm the presence of a metastatic focus, and surgical resection of the persisting focus after chemotherapy [56, 224].

Despite significant improvements in treating children diagnosed with RMS from the low and standard risk group (3-year EFS > 70%), the effects of treating more advanced diseases are still unsatisfactory, especially in the presence of factors with adverse effects on the prognosis [209–211]. In 20–30% of pediatric patients with localized RMS and 70% with metastatic disease, a recurrence of the disease will occur [225, 226]. Despite gradual improvement in the treatment of such patients, indices of 5-year post-relapse survival (PROS) in this group do not exceed 30% [227].

There are still many questions regarding, among others, the optimal time for introducing radiotherapy, the benefit of escalating the dose, or the role of radiotherapy in persistent disease, especially with a poor prognosis. The possibilities of conventional treatment intensification are limited by complications, and the low survival indices in the group of patients with recurrence and generalized disease indicate that new safer methods of targeted therapy must be sought. Among substances with proven activity against RMS, which have been tested *in vitro* and *in vivo*, are monoclonal antibodies against IGF-1R (cixutumumab and robatumumab), IGF-1R inhibitor (BMS-754807), VEGFR inhibitor (cediranib), RTK inhibitor (sunitinib), AAK inhibitor (alisertib), and mTOR kinase inhibitor (rapamycin). Cixutumumab (IMC-A12), temsirolimus (mTOR kinase inhibitor), and bevacizumab (monoclonal antibody against VEGF) are being tested in clinical trials in combination with chemotherapy in patients with RMS recurrence and generalized neoplastic disease [228–230]. New reports on the efficacy and good tolerance of the combination of vinorelbine with the histone deacetylase inhibitor mocetinostat in RMS is interesting [231].

Conclusion

Most of the data concerning survival and prognostic factors of adults with RMS come from retrospective, single-center analyses including several dozen to several hundred patients (Tab. 10). Despite the development of diagnostic techniques and new technologies in radiotherapeutic treatment, the spectacular improvement in survival attained in the pediatric population has not been possible for adults. This fact indicates that the course of the disease is considerably different in children and teenagers in comparison with adults. In care for the patient with local tumor development, the greatest role is played by oncological surgery combined with radiotherapy and chemotherapy. The evaluation of the effectiveness of these combinations of methods in patients with disseminated RMS is crucial in the context of elaborating an optimal sequence of treatment and chemotherapeutic and radiotherapeutic regimens in adults. The standardization and verification of procedures used in treating patients with RMS metastases are particularly important to prolong and improve their quality of life. Taking into consideration the rarity and the complexity of this disease, patients with RMS should be treated in highly specialized hospital wards with long-term practice in care for persons with soft tissue sarcomas. Cooperation within a multidisciplinary team is crucial. That team should be composed of an oncological surgeon, a clinical oncologist, a radiotherapist, and, depending on the need, physicians of other specializations (e.g. a gynecologist or an ear and throat specialist). Multidirectional treatment and the experience of oncological teams in large specialist centers allow us to obtain the best results of treatment. The need to include adult patients into multicenter clinical trials has been repeatedly stressed, as their results may be the basis for elaborating uniform standards of care for patients with RMS.

Current clinical trials for adult patients with RMS are presented in Table 14.

Table 14. Current clinical trials of adult patients with rhabdomyosarcoma (RMS)

Clinical phase	Intervention	Primary endpoints of the clinical trial	Age of patients recruited for the trial
I/II	AMG479 antibody (Ganitumab) against IGF-1R receptor combined with Src family kinase inhibitor (Dasatinib)	Phase I Determining a safe dose of dasatinib combined with ganitumab in patients with recurrent RMS or resistant-to-treatment embryonal or alveolar RMS Phase II Number of patients with ORR (CR or PR)	> 2 years
III	VAC alternating with vincristine and irinotecan (VI) *vs.* VAC/VI plus temsirolimus	EFS	< 40 years

→

Table 14 cont. Current clinical trials of adult patients with rhabdomyosarcoma (RMS)

Clinical phase	Intervention	Primary endpoints of the clinical trial	Age of patients recruited for the trial
III	VAC *vs.* VAC alternating with vincristine and irinotecan (VI)	EFS RR OS	< 49 years
II	Cabozantinib-s-malate (XL184) — small-molecule tyrosine kinase inhibitor (c-Met, VEGFR2, AXL, RET)	ORR (CR and PR)	2–30 years
I	NK cells from donors without compatibility in HLA system combined with ALT803 (IL-15 analog increasing NK cell cytotoxicity)	Establishing maximum tolerated dose of NK cells in combination with ALT803 administration	18–100 years
II	Vemurafenib (BRAF inhibitor)	ORR (CR and PR)	1–21 years
II	Nab-paclitaxel combined with gemcitabine	RR PFS	12–30 years
I	Vorinostat, vincristine, irinotecan, temozolomide	Establishing maximum tolerated dose of vorinostat (combined with other chemotherapeutics)	1–30 years
I/II	Eribulin and irinotecan	Phase I: Establishing maximum tolerated dose of eribulin combined with irinotecan and establishing the appropriate dose of a combination of drugs for phase II of a clinical trial Phase II: ORR (CR and PR)	5 months– –25 years
I	Mocetinostat combined with vinorelbine	Determining the toxicity of the drug combination Establishing maximum tolerated dose of drug combination	> 13 years
I	Immunotherapy using B7H3 CAR-T cells and B7H3 x CD19 CAR-T cells	Establishing maximum tolerated dose of CAR-T cells Evaluation of immunotherapy toxicity Evaluation of the technology of preparing bispecific B7H3 x CD19 CAR-T cells	< 26 years
II	Regorafenib	PFS	> 5 years
I	Palbociclib combined with temozolomide and irinotecan	Evaluation of toxicity and adverse effects of drug combination RR	2–20 years
I	Lyso-thermosensitive liposomal doxorubicin	Establishing maximum tolerated dose of the drug Evaluation of drug toxicity Evaluation of drug pharmacokinetics	< 30 years
I	Immunotherapy using EGFR806 CAR-T cells	Maximum tolerated dose Evaluation of drug toxicity and adverse effects Evaluation of the yield of the process of obtaining CAR-T cells	< 26 years
I	CLR131	Evaluation of drug toxicity	2–21 years
I/II	Prexasertib combined with irinotecan	Establishing recommended dose of prexasertib combined with irinotecan for phase II trial Evaluation of response to treatment among	> 1 year
I	Olaparib combined with temozolomide or olaparib in combination with temozolomide and irinotecan	Maximum tolerated dose of drug combinations	> 16 years
I/II	Infusion of haploidentical activated NK cells	RR	< 80 years
II	Vincristine and irinotecan or vincristine, irinotecan and temozolomide	PR or CR	6 months– –50 years

CR — complete response; EFS — event free survival; IGF-1R — insulin growth factor 1; NK — natural killers; ORR — objective response rate; OS — overall survival; PR — partial response; RR — response rate; VAC — vincristine, dactinomycin, cyclophosphamide

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Conflict of interest

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VIA MEDICA

REVIEW ARTICLE

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Pancreatic adenocarcinoma — current trends in diagnosis and treatment

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ABSTRACT

Pancreatic cancer, despite significant medical advances, is still a significant clinical problem. This article focuses on discussing risk factors, diagnostic methods, and treatment options. These elements are crucial in making a prompt diagnosis and initiating treatment. On average, a physician in primary care sees a patient with undiagnosed pancreatic cancer once every few years. Knowing the underlying symptoms and referring the patient to an appropriate center can significantly increase survival. Diagnostic methods include physical examination, numerous imaging techniques, and determination of tumor markers in serum. Surgical treatment combined with adjuvant chemotherapy is the only chance of cure for pancreatic cancer patients qualified for surgery. However, most patients experience tumor recurrence. When a tumor recurs, treatment for these patients and patients with unresectable disease is palliative chemotherapy. Numerous studies are currently underway to improve diagnostic and treatment methods.

Key words: chemotherapy, palliative treatment, pancreatic cancer, new treatment trends

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Introduction

Pancreatic cancer is one of the most aggressive cancers with a poor prognosis. Symptoms in patients with pancreatic cancer often appear when the cancer is already advanced. There is no universal screening program to detect pancreatic cancer patients quickly. Only surgical resection offers a chance of a cure; however, the eligible patients are those with cancer localized in the pancreas and patients with resectable tumors and locoregional changes.

Epidemiology

Malignant neoplasm of the pancreas ranks 14th in the classification of tumors due to the incidence of malignant neoplasms [1]. According to a 2018 study, it is the seventh cause of cancer deaths worldwide [2]. The tumor is responsible for more than 200 000 deaths annually worldwide. The 5-year survival rate for people with pancreatic cancer remains at just 6% [3]. Pancreatic cancer is mainly diagnosed in people over the age of 55, and most commonly around the age of 75 [4]. The incidence for both sexes increases with age [5]. Men are more often affected [1]. Studies show that the incidence of pancreatic cancer is higher in developed countries compared to developing countries [6]. There is a steady increase in the incidence, which could make pancreatic cancer the third leading cause of cancer deaths in the European Union [7].

Histological types

The most common histological type of malignant tumor of the pancreas is adenocarcinoma arising from the epithelial cells lining the pancreatic ducts. It ac-

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Table 1. Tumor–node metastasis–metastases (TNM) clinical classification of pancreatic cancer according to the 8th edition of the American Joint Committee on Cancer (AJCC) [8]

T (primary tumor)	
Tx	Primary tumor cannot be evaluated
T0	No evidence of primary tumor
Tis	Pre-invasive cancer (carcinoma in situ; includes PanIN 3 classification)
T1	Tumor size less than 2 cm
T1a	Tumor size less than 0.5 cm
T1b	Tumor size of more than 0.5 cm in diameter, but less than 1 cm
T1c	Tumor size more than 1 cm in diameter, but less than 2 cm
T2	Tumor size more than 2 cm in diameter, but less than 4 cm
T3	Tumor size greater than 4 cm in diameter
T4	Tumor infiltrates the visceral trunk, superior mesenteric artery, and/or common hepatic artery
N (presence of lymph node metastasis)	
Nx	Regional LNs cannot be assessed
N0	No metastasis to regional LNs
N1	Metastasis in 1 to 3 regional LNs
N2	Metastasis in 4 or more regional LNs
M (presence of distant metastases)	
M0	No distant metastases
M1	Distant metastasis

Table 2. Clinical stages of pancreatic cancer according to the 8th edition of the American Joint Committee on Cancer (AJCC) [8]

0	Tis, N0, M0
IA	T1, N0, M0
IB	T2, N0, M0
IIA	T3, N0, M0
IIB	T1, N1, M0 T2, N1, M0 T3, N1, M0
III	T4, any classification N, M0
IV	Any classification T and N, M1

counts for 80% of all tumors and is usually located in the head, less commonly in the body, and most rarely in the tail of the pancreas. Pancreatic ductal adenocarcinomas usually arise from non-invasive precursor lesions of pancreatic intraepithelial neoplasia. Less commonly, carcinomas develop from intraductal papillary mucinous neoplasms or mucinous cystic neoplasms. Other types are lobular carcinoma and pseudopapillary carcinoma. This article focuses mainly on ductal adenocarcinoma because of its prevalence.

Classification

The classification is presented in Tables 1 and 2 [8].

Risk factors

Risk factors for the disease include smoking, chronic pancreatitis, obesity, diabetes mellitus, age over 70, blood type other than 0, alcohol consumption, diet rich in red meat and poor in fresh fruits, vegetables, and folic acid, *Helicobacter pylori* infection, genetic predisposition, exposure to chlorobenzene, nickel, or chromium.

Cigarette smoking is the most important modifiable risk factor for pancreatic cancer. The risk increases with both the duration of smoking and the number of cigarettes smoked. Studies have shown a 74% increased risk in smokers, a 20% increased risk in those who quit smoking compared to non-smokers [9]. It has also been found that at least 10–20 years must pass after smoking cessation for the risk level of the disease to be the same as that of a person who has never smoked [9, 10].

Another risk factor is excessive alcohol consumption. A daily amount of heavy alcohol in excess of 60 grams has been shown to significantly increase the risk of pancreatic cancer [11]. Alcohol is also a major cause of chronic pancreatitis. This condition, through a progressive inflammatory process, leads to fibrosis and loss of acinar and islet cells. Chronic pancreatitis increases the risk of cancer 13-fold.

A diet that includes red meat, animal fats, and processed foods increases the risk of pancreatic cancer. These foods contain carcinogens, nitrites, and N-nitroso compounds for food preservation [12]. Eating fruits and vegetables, including citrus containing antioxidants, reduces the risk of the disease by about 30% [13].

A meta-analysis showed that the risk of pancreatic cancer increases by 10% for every increase in body mass index (BMI) of 5 above normal BMI [14]. Adipose tissue surrounding pancreatic cells has also been shown to promote the formation of pancreatic intraepithelial neoplasia. The increase in obesity in populations of developed countries may be responsible for the increased incidence of this cancer.

Occupational exposure to toxic substances such as nickel, polychlorinated biphenyls, cadmium, arsenic, and pesticides increases the risk of pancreatic cancer by 12% [15–17].

An increased risk has also been observed in patients infected with *Helicobacter pylori* [18] or hepatitis C [19]. Therefore, studies are underway to prove whether *Helicobacter pylori* eradication can help reduce the risk of the disease [20].

In contrast, age, sex, ethnicity, blood group, microbial flora, genetic factors, and family history are among the non-modifiable factors.

Studies have shown that people with a blood type other than 0 are at higher risk of developing pancreatic cancer [21].

According to a study by Stevens and colleagues, people with type I diabetes have a double risk of pancreatic cancer compared to those without the disease [22]. It is important to remember that diabetes, although a risk factor, can also occur as a symptom of pancreatic malignancy. It has been shown that in 1% of patients over the age of 50 who developed diabetes, it was due to concurrent pancreatic cancer.

Familial pancreatic cancer accounts for 5–10% of new cases [23]. Several mutations and associated syndromes are known to predispose to the disease. These include Lynch syndrome (i.e., hereditary non-polyposis colon cancer), Peutz-Jeghers syndrome (caused by a mutation in the *STK11* gene), hereditary chronic pancreatitis syndrome (germline mutation PRSS1), FAMMM (i.e., familial atypical nevus and melanoma syndrome), and mutations in the *BRCA1* or *BRCA2* genes.

Symptoms

The most common symptoms of pancreatic cancer are back pain, shoulder pain, dysphagia, constipation or diarrhea (mostly fatty), lethargy, weight loss (about 10% in 6 months), epigastric pain radiating to the back, and shoulder blade; nausea, vomiting, bloating, newly developed diabetes, pruritus, and jaundice. The first five of the above-mentioned symptoms occur in patients about six months before pancreatic cancer is diagnosed. Less common tumor symptoms include lethargy and newly diagnosed diabetes [24]. Other symptoms that may occur include Courvoisier's sign, palpable tumor in the intra-abdomen, ascites, paraneoplastic syndromes [recurrent thrombosis of superficial (Trousseau syndrome) or deep veins, hyperplasia, dermatomyositis and polymyositis, polyneuropathies, erythema nodosum].

Diagnostics

Diagnostic tests make it possible to classify a patient with pancreatic cancer into one of four categories in terms of the stage of the disease. The following types of tumors are distinguished: resectable, borderline resectable, locally advanced, and metastatic.

To diagnose pancreatic cancer, the following tests are helpful: Ultrasound, abdominal computed tomography (CT), endoscopic retrograde cholangiopancreatography (ERCP; when cholestasis is present, biliary drainage and prosthesis are necessary), magnetic resonance imaging (MRI), endoscopic ultrasound (EUS) with fine-needle biopsy (not performed if the patient is qualified for surgery), Positron emission tomography-computed tomography (PET-CT, to rule out the presence of metastases); determination of tumor markers in serum, mainly CA 19-9.

The most commonly used diagnostic method is abdominal ultrasonography. The sensitivity and specificity of the method depend, among other things, on the experience of the examiner and condition of the patient, his/her preparation for the examination, and range from 75% to 89% and 90% to 99%, respectively [25].

Computed tomography is a method routinely used in diagnosis. When pancreatic cancer is diagnosed, it helps determine whether resection is possible, whether vascular invasion has occurred and its extent, and whether metastasis is present [26].

Endoscopic retrograde cholangiopancreatography is used for drainage and biliary prosthesis. It also allows for the collection of material for histopathological examination. Brush cytology and aspiration cytology performed in this way increase the diagnostic accuracy of pancreatic tumor [27].

Another diagnostic test is MRI, which allows precise imaging of pancreatic lesions without exposing the patient to radiation. Magnetic resonance imaging cholangiopancreatography allows non-invasive evaluation of the pancreatic duct and bile ducts [28]. This method has applications including the presence of intraductal papillary mucinous neoplasm.

Endoscopic ultrasound with fine-needle biopsy is a method with more than 85% diagnostic accuracy for pancreatic cancer. Biopsy material is not necessary for surgical resection of the tumor if there is a reasonable suspicion of cancer. On the other hand, the time required to confirm the diagnosis can significantly delay the initiation of treatment. EUS has higher sensitivity in identifying lesions smaller than 2 cm compared to CT and MRI [29].

Positron emission tomography-computed tomography is a less commonly used diagnostic method. However, combining PET-CT with endoscopic ultrasound increases sensitivity and specificity of the test [30]. This test, although not routinely used, should be considered in patients with suspected adhesions that could not be visualized by other methods.

The CA 19-9 marker is not routinely used to diagnose pancreatic cancer. CA 19-9 [a sialylated Lewis blood group antigen with the sequence NeuNAcα2--3Ga1β1-3Glc (4-Fucα1) NAcβl-3Galβl-4Glc] [31–33] is mainly found in epithelial cells of the pancreatic ducts, biliary tract, gastric and prostate cells. Its levels increase in the presence of ovarian cysts, diverticular intestinal disease, and inflammatory diseases of the pancreas and biliary tract. Increased levels have also been

described in heat stroke, diabetes mellitus, idiopathic pulmonary fibrosis, endometriosis, and thyroiditis. This often results in false positives [34–37]. False-negative results occur in 10% of Caucasians because this population is not capable of producing CA 19-9. About 90% of patients fall into the Lewis (a– b+) or (a+ b–) blood group, in which CA 19-9 testing is possible [38–40]. False-negative results occur in patients with the Lewis (a– b–) blood group because the CA 19-9 antigen is fused to the blood group protein according to the Lewis system. The Lewis antigen of the MUC1 class of proteins is not expressed on the erythrocyte membrane in Lewis blood type-negative patients [41]. The half-life of CA 19-9 is about 1–3 days. The normal result is < 37 U/mL. Changes in the level of this marker are used to monitor treatment of the disease. An increase in the level of the marker may indicate a lack of response to the used treatment or a relapse of the disease [42]. Combining CA 19-9 antigen with CEA antigen increases specificity up to 84% compared to CA 19-9 alone [43]. A biomarker panel consisting of CA125, CA 19-9, and LAMC2 is also recommended, as it has been shown to significantly improve the detection of pancreatic cancer. The combination of these antigens increased sensitivity by 68% up to one year and by 53% up to two years before cancer diagnosis [44]

New diagnostic techniques are being researched, such as confocal laser needle endomicroscopy (which will allow real-time visualization of tissue at the microscopic level in pancreatic cysts during EUS, allowing optical biopsy) and confocal probe-based laser endomicroscopy (which might be used during ERCP for unspecified pancreatobiliary stenosis) [45, 46].

Treatment

Treatment options include surgery, neoadjuvant and adjuvant chemotherapy, chemoradiotherapy, targeted therapy, immunotherapy, and palliative treatment, among others.

A patient's response to treatment depends on many factors, including the biology of the tumor, patient's performance status, and rate of disease progression.

Surgical treatment

Radical surgery is the only method that offers a chance of a complete cure. From 10 to 15% of patients qualify for primary resection. However, the majority of patients who undergo resection experience recurrence. The 5-year survival rate after surgery is 20%. In the remaining 80–85% of patients, the disease is so advanced with generalized metastases that tumor resection is not possible.

Among pancreatic cancers, there are resectable tumors (no infiltration of major venous and arterial structures), borderline resectable tumors (varying degrees of involvement of the superior mesenteric vein or portal vein, coverage of the gastroduodenal artery up to the hepatic artery, and involvement of less than half the circumference of the superior mesenteric artery) [47], and locally advanced tumor. Even for borderline resectable tumors involving the portal vein or mesenteric vein, resection is possible. In cases of arterial involvement, surgical resection is often associated with the histopathological finding of tumor cells at the surgical incision line. Surgical advances and improvements in vein and artery reconstruction techniques have made it possible to operate on tumors that earlier were ineligible for surgical treatment. Whipple method surgery, or pancreatoduodenectomy, with removal of regional lymph nodes, is performed when the tumor is located in the head of the pancreas. The Whipple method includes resection of the pancreatic head, duodenum, proximal part of the jejunum, common bile duct, gallbladder, and part of the stomach. It is possible to later restore the continuity of the gastrointestinal tract by anastomosing the remnants of the pancreas to the stomach or jejunum. In the case of another location of the tumor, i.e. in the body or tail of the pancreas, the tumor undergoes partial resection or the entire pancreas is removed along with the spleen and regional lymph nodes. The goal of the operation is to achieve an R0 resection, as it offers better survival compared to an R1 resection [48]. The main complications that occur after Whipple surgery are leakage from the pancreatic anastomosis and formation of a pancreatic fistula [49].

Another surgical treatment is laparoscopic distal pancreatectomy. This is a minimally invasive technique. It has been shown to be as effective as traditional surgery [50]. It is also possible to use robotic techniques [51]. The success of pancreatoduodenectomy surgery also depends on the experience of the centers where it is performed. The use of adjuvant chemotherapy after surgery has shown a significant improvement in patient survival.

In some patients, biliary drainage is performed when jaundice is present before surgery. The presence of this symptom has been shown to increase the incidence of perioperative infectious complications and affect coagulopathy [52]. Drainage can be performed by the following methods: percutaneous transhepatic cholangiography, and endoscopic retrograde cholangiopancreatography (ERCP).

Adjuvant chemotherapy

The routinely used treatment is adjuvant chemotherapy. There are various treatment regimens. Initially, one of the standard regimens used was gemcitabine in

monotherapy for 6 months [53]. The Charite Onkologie (CONKO)-001 trial compared the use of six cycles of adjuvant gemcitabine treatment in patients with surgically removed pancreatic cancer with observation. (21 postoperative chemotherapy). Mean follow-up time was 136 months. Overall survival (OS) was 22.8 months with gemcitabine *versus* 20.2 months with observation [hazard ratio (HR) = 0.76; 95% confidence interval (CI) 0.61–0.95; p = 0.01]. The 5-year survival rate was 20.7% with gemcitabine and 10.4% with observation, and the 10-year survival rate was 12.2% and 7.7%, respectively [54].

Subsequently, capecitabine was added to the regimen, as it had a beneficial effect in patients undergoing R0 resection. The European Study for Pancreatic Cancer (ESPAC-4) trial compared the use of six cycles of gemcitabine alone (1000 mg/m^2 every week for 3 or 4 weeks) with administration of gemcitabine with orally administered capecitabine (one cycle: 1660 mg/m^2 for 21 days, followed by 7 days off) [55]. The median follow-up period was 43.2 months. OS was 28 months (95% CI 23.5–31.5) with combination therapy and 25.5 months (95% CI 22.7–27.9) with gemcitabine alone (HR = 0.82; p = 0.032). The use of gemcitabine-capecitabine combination therapy increased the 5-year OS rate from 16.3% (for gemcitabine alone) to 28.8% (gemcitabine-capecitabine combination). No significant difference in grade 3/4 toxicity rates was seen between groups. Treatment with capecitabine was associated with a higher incidence of third- or fourth--degree diarrhea (5% of cases *versus* 2% with gemcitabine alone), neutropenia (38% *versus* 24%), and hand-foot syndrome (7% *versus* 0% with gemcitabine alone).

Another treatment option is mFolfirinox (modified folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin). The randomized PRODIGE-24 trial compared, in patients with R0/R1 resection, a treatment regimen of six cycles of gemcitabine [1000 mg/m^2 on days 1, 8, and 15 of the cycle (28 days)] with a regimen of twelve cycles of Folfirinox (oxaliplatin 85 mg/m^2, leucovorin 400 mg/m^2, irinotecan 150 mg/m^2 and 5-FU 2400 mg/m^2 for 46 hours every 2 weeks) [56]. The median follow-up was 33.6 months. Disease-free survival (DFS) was 21.6 months with Folfirinox and 12.8 months with gemcitabine (HR = 0.58; 95% CI 0.46–0.73; p = 0.001). OS was 54.4 months with Folfirinox and 35.0 months with gemcitabine (HR = 0.64; 95% CI 0.48–0.86; p = 0.003). The majority (75.9%) of patients on the Folfirinox regimen experienced grade 3 or 4 toxicities compared to 52.9% of patients on gemcitabine.

A study comparing these two therapies showed that the mFolfirionox regimen had significantly better disease-free survival compared to gemcitabine. However, the administration of mFolfirionox is associated with increased risk of complications. The choice of treatment depends on the patient's postoperative fitness. In fit patients, mFolfirionox therapy is used, while in less fit patients, a regimen with gemcitabine and capecitabine is used [57]. For periampullary localized tumors, a single drug, mainly 5-FU, is used.

Neoadjuvant treatment

Neoadjuvant chemotherapy is used for borderline resectable tumors, i.e. tumors without the presence of distant metastasis or metastasis to regional lymph nodes. These are patients whose infiltration covers less than 180 degrees of the circumference of the superior mesenteric artery or visceral trunk, or those with thrombosis of the superior mesenteric vein and/or the initial segment (less than 2 cm) of the portal vein when vascular reconstruction can be performed.

Neoadjuvant therapy consists of chemotherapy with or without radiation therapy. Retrospective studies from the Surveillance, Epidemiology, and End Results (SEER) and National Cancer databases show that neoadjuvant therapy is recommended in many guidelines for the management of patients with borderline resectable pancreatic cancers [58–60]. The phase III PREOPANC trial divided patients with resectable or borderline resectable pancreatic cancer into groups with diagnostic laparoscopy, neoadjuvant chemoradiotherapy, and surgical resection followed by four cycles of gemcitabine treatment, or with surgery followed by six cycles of gemcitabine treatment. Neoadjuvant treatment consisted of gemcitabine 1000 mg/m^2 on day 1 and day 8 in the first cycle (21 days), gemcitabine 1000 mg/m^2 on day 1, day 8, and day 15 in the second cycle (28 days) with simultaneous application of hypofractionated radiation at a dose of 36 Gy to the tumor and suspicious surrounding lymph nodes, gemcitabine 1000 mg/m2 on day 1 and day 8 in the third cycle (21 days) [61]. The percentage of 5-year OS was 20.5% (95% CI 14.2–29.8) for patients who received neoadjuvant chemotherapy and 6.5% (95% CI 3.1–13.7) for patients with primary surgery (HR = 0.73; 95% CI 0.56–0.96; p = 0.025). Mean OS was 15.7 months in patients in the group receiving neoadjuvant chemotherapy and 14.3 months in patients undergoing surgery. Sixty-one percent of patients treated with neoadjuvant chemotherapy underwent resection. Of these, 41% had negative margins (R0), and 65% of patients had disease without lymph node metastases. In the second group, the resection rate was 72%, resulting in R0 resection in 28% of patients and disease without lymph node metastases in 18% of patients. The optimal neoadjuvant therapy regimen has not been determined. Studies (ALLIANCE, PREOPANC-3, PANACHE-01-PRODIGE, NorPACT-01) evaluating other treatment regimens are ongoing.

Neoadjuvant treatment aims to eliminate micrometastases and shrink the primary tumor to minimize the pos-

sibility of tumor recurrence [62]. Postoperative therapy may be less effective than preoperative therapy due to weaker drug delivery to the tumor locus and low radiation sensitivity caused by reduced oxygenation [63]. Not all patients benefit from preoperative treatment, as some patients have tumors that are not sensitive to chemoradiotherapy. This contributes to delaying surgical treatment or even prevents it. In addition, some patients develop fibrosis within the pancreas under treatment, which can increase the rate of pancreatectomy-related complications [64].

Chemoradiotherapy

Chemoradiotherapy has long been used in locally advanced pancreatic cancer. A study using gemcitabine or 54 Gy chemoradiation with capecitabine in patients with stable disease previously treated with 4 months of gemcitabine chemotherapy, showed no difference in OS between the two groups of patients [65]. Evidence is lacking on whether chemoradiotherapy should be used as an adjunct to chemotherapy [66]. Most available data from randomized clinical trials are insufficient [67–71]. The randomized LAP07 trial divided patients into two groups, the first of which was treated with gemcitabine and the second with gemcitabine with erlotinib for 4 cycles. Patients were then re-divided into a group treated with chemotherapy or chemoradiotherapy (a dose of 54 Gy in 30 daily fractions with capecitabine 800 mg/m^2 twice daily on the days of radiation therapy) [72]. The study was stopped prematurely after the initial analysis. Median follow-up was 36.7 months. Overall survival (from the date of first allocation) was not significantly different between the two groups. Overall survival for chemotherapy was 16.5 months (95% CI 14.5–18.5) and 15.2 months for chemoradiotherapy (95% CI 13.9–17.3; p = 0.83). Overall survival for patients receiving gemcitabine was 13.6 months (95% CI 12.3–15.3) and for patients treated with demcitabine in combination with erlotinib was 11.9 months (95% CI 10.4–13.5; p = 0.09). The ECOG study compared a treatment regimen of gemcitabine alone with treatment with gemcitabine and radiotherapy, followed by gemcitabine alone [73]. The study evaluated survival, which was 9.2 months (95% CI 7.9–11.4) with gemcitabine monotherapy and 11.1 months (95% CI 7.6–15.5) with combination treatment) (p = 0.017). Grade 4 and 5 toxicity was more common with chemoradiotherapy (in 41%) than with chemotherapy (in 9% of cases). Chemoradiotherapy can be used when intensive chemotherapy is not possible.

The use of alternatives to irradiation, such as radiofrequency current ablation, irreversible electroporation, focused high-intensity ultrasound, microwave ablation, and local anti-KRAS therapy (using siG12D-LODER) are also under investigation. These treatments address local lesions and can be performed during laparotomy, percutaneously, or endosonographically [74]

Targeted treatment

Targeted treatment involving the use of monoclonal antibodies or small molecules has very high efficacy in many types of cancer. However, in the case of pancreatic cancer, only erlotinib, a small-molecule EGFR tyrosine kinase inhibitor, has proven effective in treatment [75]. Drugs such as cetuximab, bevacizumab, sorafenib, axitinib, and aflibercept have proven ineffective [76]. A study conducted by the National Cancer Institute of Canada (CAN-NCIC-PA3) compared treatment with gemcitabine alone with a regimen of gemcitabine plus erlotinib (100 mg/d) [77]. It showed that administration of erlotinib with gemcitabine slightly prolonged patient survival compared to gemcitabine monotherapy (HR = 0.81; 95% CI 0.69–0.99; p = 0.038). Median and one-year survival rates were 6.2 months and 23% in patients with the combination treatment and 5.9 months and 17% in patients treated with gemcitabine alone.

Patients who are treated with erlotinib with gemcitabine often develop a skin rash, which is a typical side effect of EGFR inhibition. Its occurrence indicates greater treatment efficacy and increases patient survival. If the rash does not appear until 8 weeks after the start of treatment, it is recommended to discontinue erlotinib, as no beneficial effect on survival length has been observed.

Another combination treatment regimen was a combination of gemcitabine with erlotinib followed by capecitabine therapy compared to a regimen of capecitabine with erlotinib followed by gemcitabine therapy achieving similar treatment efficacy [78]. The combination of the three drugs mentioned above had no effect on patient life expectancy [79]. Research is ongoing into the use of poly(ADP-ribose) polymerase (PARP) inhibitors, such as olaparib, as monotherapy or in combination with chemotherapy in pancreatic cancer patients with germline or somatic mutations in BRCA1, BRCA2, or PALB2 [80]. Olaparib is registered by the US Food and Drug Administration (FDA) for maintenance treatment in adult patients with metastatic pancreatic adenocarcinoma with the presence of a germline mutation in the BRCA gene. This mutation is detected through the use of an FDA-approved test. Its presence allows patients to receive a platinum derivative-based treatment regimen. In patients who do not experience disease progression within 16 weeks of starting the above therapy, further maintenance treatment with olaparib is possible. The POLO multicenter clinical trial showed that progression-free survival (PFS) for patients receiving olaparib averaged 7.4 months (95% CI 4.1–11) compared to 3.8 months (95% CI 3.5–4.9) for patients receiving placebo (HR = 0.53; 95% CI 0.35–0.81;

p = 0.0035). Overall survival time for patients receiving olaparib was 18.9 months (95% CI 14.9–26.2) compared to 18.1 months (95% CI 12.6–26.1) for those receiving placebo (HR = 0.91; 95% CI 0.56–1.46; p = 0.683). The overall response rate (ORR) was 23% for olaparib and 12% for placebo. During the study, olaparib was administered orally at 300 mg twice daily [81]. The following side effects may occur during the use of this drug: nausea, vomiting, abdominal pain, diarrhea, fatigue, headache and dizziness, leukopenia, anemia, thrombocytopenia, and others.

Palliative treatment

Treatment of unresectable metastatic pancreatic adenocarcinoma includes symptomatic treatment and palliative chemotherapy. Chemotherapy for patients with pancreatic cancer with current metastases involves combination therapy with Folfirinox or a regimen with gemcitabine and nab-paclitaxel (that is, albumin-bound paclitaxel). A study comparing the Folfirinox regimen [oxaliplatin, folinic acid (leucovorin), irinotecan, fluorouracil] with gemcitabine monotherapy, showed a better effect of Folfirinox treatment in terms of response and progression-free survival. However, the criteria for patient selection are specific. Therefore, Folfirinox treatment is recommended for patients younger than 75 years, with good performance status and no significant risk of cholestasis or cholangitis. This treatment is associated with increased risk of neutropenic fever, sensory neuropathy, and gastrointestinal toxicity. In a 2011 study, patients were randomly divided into two groups. The first received Folfirinox (oxaliplatin 85 mg/m^2, irinotecan 180 mg/m^2, leucovorin 400 mg/m^2, and 5-fluorouracil 400 mg/m^2 given as a bolus followed by 2400 mg/m^2 as a continuous infusion over 46 hours every 2 weeks), and the second group received gemcitabine (1000 mg/m^2 every week for 7 weeks followed by a week off and for 3 weeks and again a week off) [82]. Overall survival was 11.1 months with Folfirinox and 6.8 months with gemcitabine (HR = 0.57; 95% CI 0.45–0.73; p < 0.001). Progression-free survival was 6.4 months in the Folfirinox group and 3.3 months in the gemcitabine group (HR = 0.47; 95% CI 0.37–0.59; p < 0.001). Another study showed an advantage of gemcitabine treatment in combination with nab-paclitaxel over gemcitabine monotherapy in terms of response and progression-free survival.

The NCT00844649 multicenter trial compared treatment with gemcitabine and nab-paclitaxel with treatment with gemcitabine alone [83]. Overall survival was 8.5 months in the combined gemcitabine and nab-paclitaxel treatment group *versus* 6.7 months with gemcitabine monotherapy (HR = 0.72; 95% CI 0.62–0.83; p < 0.001). Progression-free survival was 5.5 months with combination treatment and 3.7 months with gemcitabine alone (HR = 0.69; 95% CI 0.58–0.82; p < 0.001). The combination of gemcitabine and nab-paclitaxel was associated with greater toxicity than gemcitabine treatment. Grade 3 toxicities occurred in the following proportion of patients treated with the combined regimen: neutropenia in 38%, fatigue in 17%, neuropathy in 17%, and neutropenic fever in 3%. When treated with gemcitabine alone, neutropenia occurred in 27% of patients, fatigue in 1%, neuropathy in 1%, and neutropenic fever in 1%. Several patients who participated in the study were not qualified for treatment with Folfirinox. Therefore, gemcitabine in combination with nab-paclitaxel compared to Folfirinox can be used in a wider group of patients, and side effects that may occur during treatment are easier to manage. Gemcitabine in combination with nab-paclitaxel is preferred in older patients or patients with poorer performance status. It is also possible to use gemcitabine monotherapy in patients in poor general condition.

Nanoliposomal irinotecan with 5-FU, which has been approved by the FDA and the European Medicines Agency, is a possible second-line chemotherapy option. The NAPOLI-1 trial compared nanoliposomal irinotecan monotherapy with treatment with nanoliposomal irinotecan in combination with 5-FU and folinic acid and with treatment with 5-FU and folinic acid [84]. Overall survival was 6.1 months (95% CI 4.8–8.9) for patients treated with nanoliposomal irinotecan with 5-FU and folinic acid and 4.2 months (95% CI 3.6–4.9) for patients treated with 5-FU and folinic acid (p = 0.012). Overall survival for patients treated with nanoliposomal irinotecan alone was 4.9 months (95% CI 4.2–5.6) and 4.2 months (95% CI 3.6–4.9) for patients treated with 5-FU and folinic acid (HR = 0.99; p = 0.94). Nanoliposomal irinotecan in combination with 5-FU and folinic acid was associated with improved OS (HR = 0.58; 95% CI 0.42–0.81). However, combination therapy was associated with more grade 3 and 4 adverse events. Neutropenia occurred in 27% of patients, diarrhea in 13%, vomiting in 11%, and fatigue in 14%. Oxaliplatin and nanoliposomal irinotecan are also used. The American Society of Clinical Oncology Clinical Practice Guidelines for metastatic pancreatic cancer include the use of gemcitabine plus nab-paclitaxel or gemcitabine monotherapy as second-line chemotherapy [85].

The prospective PANCREOX trial evaluated the efficacy of treatment with the FOLFOX regimen (calcium leucovorin, 5-FU, and oxaliplatin) compared to treatment with 5-FU with leucovorin in patients after chemotherapy with gemcitabine [86]. Median follow-up was 8.8 months. Progression-free survival was 3.1 months for FOLFOX and 2.9 months for 5-FU with leucovorin (HR = 1.00; 95% CI 0.66–1.53; p = 0.989).

Grade 3 and 4 toxicity occurred in 63% of patients with FOLFOX and in 11% of patients with 5-FU plus leucovorin. No benefit was found with the addition of oxaliplatin to 5-FU and leucovorin.

Palliative care is also an important part of patient treatment, as it is common for this group of patients to develop obstructive jaundice and duodenal obstruction. These abnormalities require surgical, endoscopic, or radiological interventions. Due to the development of treatment methods, percutaneous biliary drainage has been mainly replaced by endoscopic techniques. A large-diameter metal stent is usually used. This prolongs the patency period of the stent and reduces the incidence of cholangitis [87]. When gastric outlet obstruction occurs, surgical gastrojejunostomy and endoscopic duodenal stents are applicable. The latter method is recommended for patients with short life expectancy and/or poor performance status.

Screening tests

Universal screening for pancreatic cancer in adults is not recommended [88].

The International Cancer of the Pancreas (CAPS) Consortium recommends starting screening of patients in high-risk groups at age 50, with repeat screening every year if pancreatic lesions are not detected [89]. High-risk groups for pancreatic cancer include a family history of pancreatic cancer (at least two first-degree relatives diagnosed with pancreatic cancer), hereditary pancreatic syndromes (Peutz-Jeghers syndrome, familial atypical polycystic melanoma syndrome, hereditary pancreatitis, PALB2 mutation, BRCA2 mutation, Lynch syndrome) [90]. The imaging modalities of choice are EUS and MRI, as they are sensitive and specific enough for small lesions and carry no risk of exposure to ionizing radiation. The ability to detect premalignant and malignant lesions with both methods is about 20%.

Future plans

Immune checkpoint inhibitors are currently used in several types of cancer. However, pancreatic cancer is a poorly immunogenic tumor, and an immunosuppressive environment is created at the site, which is a barrier to effective immunotherapy. Using monoclonal antibodies, inhibition of cytotoxic T-Lymphocyte-associated Antigen 4 (CTLA-4), programmed cell death protein-1 (PD-1), and programmed death ligand-1 (PD-L1) ligand is researched [91]. Research is underway on the use of CTLA-4 or PD1 inhibitors in combination with chemotherapy, radiation, or cytokine antagonists [92]. Anti-CTLA-4, anti-PD-1, or anti-PD-L1 drugs cause T-cell activation [93]. Ipilimumab is a monoclonal anti-CTLA-4 IgG1 antibody that can be used in combination with gemcitabine treatment in patients with pancreatic cancer [94, 95]. The NCT01473940 clinical trial showed that treatment with ipilimumab plus gemcitabine achieves PFS of 2.5 months (95% CI 0.8–4.8) and OS of 8.5 months (95% CI 2.2–10.3) [96, 97]. The most common toxic complications were hematologic manifestations [97]. The NCT01928394 trial is evaluating the efficacy of combining ipilimumab with nivolumab, which is an anti-PD-1 antibody [98]. The NCT02527434 trial of tremelimumab (anti-CTLA-4 IgG2 antibody) used as monotherapy was unsuccessful. Eighteen of 20 patients experienced disease progression. OS was 4 months (95% CI 2.83–5.42) [99]. However, the combination of tremelimumab with gemcitabine in the NCT00556023 trial produced OS of 7.4 months (95% CI 5.8–9.4) [100]. The combination of tremelimumab with durvalumab (anti-PD-L1 antibody) after 5-FU or gemcitabine-based chemotherapy was also studied. With the drug combination, the ORR was 3.1% (95% CI 0.08–16.22) [101]. A small group of patients with microsatellite instabilities in their tumors can be treated with pembrolizumab, as it has been approved by the FDA [102]. Eighty-three percent of pancreatic cancer patients achieved a response to pembrolizumab immunotherapy within a time range of 2.6 months to 9.2 months (assessed using RECIST) [103]. The Keynote-158 trial demonstrated the efficacy of pembrolizumab treatment in dMMR/MSI-H pancreatic cancer. OS was 4.0 months (95% CI 2.1–9.8), and PFS was 2.1 months (95% CI 1.9–3.4) [104]. The NCT02331251 trial evaluated the combination of pembrolizumab with gemcitabine and nab-paclitaxel chemotherapy [105]. Progression-free survival was 9.1 months, and OS was 15.0 months [105, 106]. A dose escalation study of atezolizumab (mAb IgG1 antibody against PD-L1) showed dose tolerance up to 20 mg/kg every 3 weeks [107]. The NCT03829501 study is ongoing [108].

CPI-613 is an inhibitor of two important enzymes of the tricarboxylic acid cycle, pyruvate dehydrogenase and alpha-ketoglutarate. A phase I study of combining CPI-613 with Folfirinox showed a response rate of 61%, prompting continued research into the efficacy of adding this drug to Folfirinox [109]. Losartan, which is among the angiotensin receptor blockers, reduces collagen and hyaluronan production within the stroma of pancreatic cancer, resulting in reduced shear stress and contributing to better drug delivery [110].

There are emerging hopes for techniques to link genetic changes to clinically relevant characteristics such as the pattern of recurrence and response to chemotherapy to create tests used in clinical practice [111]. Another goal is to further improve the identification of specific mutations to individualize therapy [112].

Summary

Pancreatic cancer belongs to a group of cancers with a high mortality rate. It is important to know the risk factors of this cancer and to be aware of modern diagnostic options. Due to the limited possibilities of surgical intervention, other management options for patients with advanced pancreatic cancer are presented.

Article Information and Declarations

Author contributions

A.G.: prepared the first draft of the manuscript, manuscript revision and literature review.
K.K., A.M.: reviewed the literature and translated the manuscript.
K.H.: final preparation of the manuscript and substantive supervision.
All authors approved the final version of the manuscript.

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Conflict of interest

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VIA MEDICA

REVIEW ARTICLE

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Atezolizumab in the treatment of patients with breast cancer

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ABSTRACT

Immunotherapy is a modern method of treatment which is being tested in breast cancer patients. The first approved drug in this group was atezolizumab introduced for the treatment of patients with locally advanced and inoperable or metastatic triple-negative breast cancer (TNBC) with expression of programmed death receptor type 1 (PD-L1) on immunologic cells (IC) of ≥1%, who had not received prior chemotherapy for advanced disease. The results of the registration study IMpassion130 indicated that atezolizumab improved patient outcomes when used in combination with nab-paclitaxel. This article summarizes the most important analyzes of that study. The necessity to use the validated VENTANA SP142 assay to assess PD-L1 expression, which is necessary for the qualification of patients for this therapy, was emphasized. Additionally, the available data on the first results of the studies in patients with early TNBC as well as with human epidermal receptor type 2 (HER2)-positive and estrogen receptor (ER)-positive HER2-negative cancers treated with atezolizumab are discussed.

Key words: atezolizumab, immune therapy, triple-negative breast cancer, VENTANA SP142 assay

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Introduction

In recent years, numerous clinical trials using immunotherapy in patients with various cancers have been conducted, with the results changing the standards of oncology management. Immunotherapy significantly improved treatment outcomes (among others in melanomas, lung cancer, urothelial neoplasms, and squamous cell carcinomas of the head and neck). Immune checkpoint inhibitors have been developed, including antibodies against cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death type 1 (PD-1), and programmed death ligand-1 (PD-L1). In patients with breast cancer, the results of studies with anti-PD-1 (e.g. pembrolizumab) and anti-PD-L1 (e.g. atezolizumab) antibodies are of greater importance [1]. This article summarizes the data on the effectiveness of the first approved immune inhibitor in patients with triple-negative breast cancer (TNBC), such as atezolizumab. The principles of diagnosis and selection of patients for treatment are discussed, and directions of new research on this drug in breast cancer patients are indicated.

First reports

Atezolizumab is a humanized monoclonal IgG1 antibody directed against PD-L1, approved for the treatment of patients with non-small cell and small cell lung cancer, urothelial cancer, hepatocellular carcinoma, and TNBC [2].

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The first reports on the effectiveness of the drug in patients with breast cancer were presented fewer than 10 years ago. A total of 277 patients with advanced solid tumors and hematological malignancies (including 10 patients with breast cancer) participated in the phase I dose-escalation study. Atezolizumab was used as monotherapy. The study aimed to assess treatment safety and determine recommended phase II dose (RP2D). It was highlighted that responders included patients with cancers showing PD-L1 expression, and 1200 mg every 3 weeks was recognized as the recommended dose for monotherapy [3].

Metastatic triple-negative breast cancer

First studies

Another study, designed only for patients with metastatic TNBC, included 116 women, 60% of whom had previously received at least two lines of palliative therapy. Atezolizumab monotherapy was administered intravenously at a dose of 15 mg/kg body weight, 20 mg/kg body weight, or at a fixed dose of 1200 mg. The treatment results were not spectacular. The objective response rate (ORR) in the whole study population was only 10%, the median progression-free survival (PFS) was 1.4 months, and the median overall survival (OS): 8.9 months. However, in patients treated in the first line, the results were better: ORR was 24%, and median OS was 17.6 months. Additionally, significantly better results were found in patients with PD-L1 expression on tumor-infiltrating immune cells (ICs) — median OS of patients across all treatment lines was 10.1 months in patients with PD-L1 expression and 6 months in patients with PD-L1-negative tumors [4].

The above data indicated that immunotherapy alone has some limitations. Studies with chemotherapy-combined treatment were designed, which showed improved treatment outcomes. The GP28328 study included 33 TNBC patients who received atezolizumab (800 mg on days 1 and 15) with nab-paclitaxel (125 mg/m^2 on days 1, 8, and 15 every 28 days). Patients who previously received up to 2 lines of treatment were included in the study. ORR was 39.4%, and clinical benefit was found in 51.5% of patients. The median duration of response was 9.1 months, median PFS was 5.5 months, and median OS was 14.7 months. Adverse events occurred in all patients — the most common were neutropenia (70%), fatigue (67%), alopecia (42%), diarrhea (39%), and peripheral sensory neuropathy (36%). On the other hand, 73% of patients experienced grade 3/4 adverse events (most often neutropenia — 46% and thrombocytopenia — 9%). However, there were no treatment-related deaths [5].

IMpassion130 pivotal study

Earlier results led to designing of a large phase III clinical trial, IMpassion130, which was a pivotal study of atezolizumab in patients with metastatic TNBC. A total of 902 patients with metastatic (90%) or inoperable and locally advanced (10%) TNBC with a very good or good performance status (PS) participated in the study. Perioperative treatment was previously used in 63% of patients. The lungs were the most common location of metastatic lesions. PD-L1 expression was found in 41% of patients. Patients were randomly assigned to the group with either chemotherapy alone or chemotherapy combined with immunotherapy. Atezolizumab was administered at a dose of 840 mg on days 1 and 15, and nab-paclitaxel was administered at the dose of 100 mg/m^2 of body surface area (BSA) on days 1, 8, and 15 every 28 days. The primary endpoints of the study were PFS and OS assessed in the whole study population and in patients with PD-L1 expression but after demonstrating a statistically significant improvement in the overall population. The first results of the study showed a significant PFS improvement in all patients receiving immunotherapy (7.2 *vs.* 5.5 months; $p = 0.0025$), especially in patients with PD-L1 expression (7.5 *vs.* 5 months; $p < 0.0001$). However, the first OS analysis showed no significant differences in the whole study group (21.3 *vs.* 17.6 months; $p = 0.084$), and no statistical evaluation of OS was formally performed in the subgroup of patients with PD-L1 expression. Additional analysis, however, showed a significant clinical improvement in OS in patients with PD-L1-positive tumors (25 *vs.* 15.5 months). ORR was also better in the immunotherapy arm [6]. The results of the study were received with great interest. They identified the TNBC patient population that could benefit most from immunotherapy. In 2021, the final OS results were published. There was no difference in OS in the whole study group (21 *vs.* 18.7 months; $p = 0.078$), while in the additional analysis, the clinical benefit of atezolizumab therapy was again observed in patients with PD-L1 expression (median OS — 25.4 *vs.* 17. 9 months with no statistical significance) [7]. The final results of the study are summarized in Table 1.

Almost all patients treated in the IMpassion130 study experienced side effects. The most common were alopecia, asthenia, nausea, and diarrhea. However, grade 3/4 adverse events were found in 51% of patients in the immunotherapy group and 43% of patients in the control group. In turn, serious adverse events occurred in 24% of patients treated with atezolizumab plus nab-paclitaxel and in 19% of patients receiving chemotherapy alone. The most common grade 3/4 side effects were neutropenia (8% in both groups), peripheral neuropathy (6% in the atezolizumab group *vs.* 3%

Table 1. Summary of the results of the phase III IMpassion130 study — median PFS/OS and ORR (based on [6, 7])

	Atezolizumab + + nab-paclitaxel	Placebo + + nab-paclitaxel	p value, HR
Median PFS Whole study population (months)	7.2	5.5	HR = 0.8; p = 0.002
Median PFS PD-L1+ population (months)	7.5	5.0	HR = 0.62; p < 0.001
Median OS Whole study population (months)	21.0	18.7	HR = 0.86; p = 0.077
Median OS PD-L1+ population (months)	25.4	17.9	HR = 0.67 (95% confidence interval 0.53–0.86)
Objective response rate Whole study population	56%	45.9%	HR = 1.52; p = 0.002
Objective response rate PD-L1+ population	58.9%	42.6%	HR = 1.96; p = 0.002

HR — hazard ratio; ORR — objective response rate; OS — overall survival; PD-L1+ — positive expression of programmed death receptor type 1; PFS — progression-free survival

in the control group), and asthenia (4% *vs.* 3% in the control group). Adverse events leading to discontinuation of at least one study drug were reported in 19% of patients who received combination therapy and 8% of patients in the control group, with neuropathy being the most common. Among the adverse reactions of special interest, a higher incidence of rash (36% *vs.* 26%), hypothyroidism (18% *vs.* 4%) and hyperthyroidism (5% *vs.* 1%), pneumonia (4% *vs.* < 1%) was revealed in patients receiving atezolizumab compared to the control group [7].

The quality of life (QoL) of patients participating in the IMpassion130 study was also assessed. Patients completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) and Breast Cancer Module (EORTC QLQ-BR23). The secondary endpoint in IMpassion130 was time to deterioration in quality of life, which was defined as a reduction in the questionnaire score by more than 10 points from baseline for at least 2 treatment cycles. It was found that the use of atezolizumab did not affect the quality of life in the whole study population and in TNBC patients with PD-L1 expression [7, 8].

The results of the IMpassion130 study were the basis for the registration of atezolizumab for use with nab-paclitaxel in patients with PD-L1-positive advanced TNBC in first-line treatment [2], which is recommended by the European Society for Medical Oncology (EMSO) and the Polish Society of Clinical Oncology (PTOK) [9, 10].

Real-world evidence (RWE) on atezolizumab therapy is currently being collected and requires longer follow-up. The available reports indicate an increasingly frequent PD-L1 expression in patients with metastatic TNBC and thus eligibility for immunotherapy [11]. Based on the analysis of data from the German OPAL registry of breast cancer patients, it was found that the percentage of patients with metastatic TNBC evaluated for PD-L1 expression before first-line palliative therapy increased from 14% in 2018 to 79% in 2020, which translated into using immunotherapy in a greater number of patients [12].

Other studies in metastatic TNBC

The results of the IMpassion131 study, in which paclitaxel (at a dose of 90 mg/m^2 on days 1, 8, and 15 every 28 days) was added to atezolizumab (standard dosing) in one of the study arms were surprising. The primary endpoint of the study was PFS in patients with PD-L1-positive BC and in the whole study population. The secondary endpoint was OS. PD-L1 expression was found in 45% of 651 TNBC patients who participated in the study. The first PFS analysis in patients with PD-L1 expression did not show a significant difference (6 *vs.* 5.7 months; p = 0.20), similarly to the whole study group (5.7 *vs.* 5.6 months; p = 0.86). Furthermore, OS did not differ significantly between the arms, and the obtained results were numerically even worse in the combination therapy arm (22.1 *vs.* 28.3 months in the PD-L1-positive group and 19.2 *vs.* 22.8 months in the whole study population) [13]. The reason for the different results in IMpassion131 has not been clarified and research is ongoing (one of the reasons may be the use of corticosteroids in paclitaxel premedication).

Importantly, a third large clinical trial with atezolizumab (IMpassion132) involving patients with rapid relapse of TNBC may provide new data. The study uses chemotherapy in both arms (capecitabine or carboplatin with gemcitabine) and additionally atezolizumab in the experimental arm [14]. Another

study with atezolizumab in patients with metastatic TNBC was designed, in which other chemotherapy regimens are evaluated (NCT01898117, NCT03164993, NCT03206203, and NCT05266937). In addition, there are studies in which new drugs are added to atezolizumab with chemotherapy (e.g. ipatasertib — NCT04177108 and NCT03800836).

Qualification for treatment

Additional biomarker analyzes were performed as part of the IMpassion130 study. PD-L1 expression was found slightly more frequently when evaluating primary tumor tissues compared to metastatic lesions (44% *vs.* 36%). Interestingly, a positive PD-L1 result was rarely obtained in liver metastases samples (only 13%). In turn, lymph node biopsies were associated with the highest percentage of positive results (51%). As part of additional analyzes, patients were divided into 3 groups: with no PD-L1 expression (IC < 1%; 59% of cancers), and with low (IC ≥1% to < 5%; 27% of cancers) and high PD-L1 expression (IC ≥ 5%; 14% of TNBC). Significantly better treatment results were demonstrated in the groups with low and high PD-L1 expression; however, no significant differences were found between the groups [14]. These observations were the basis for determination of a 1% cut-off point for positive PD-L1 expression in TNBC. ICs include lymphocytes, macrophages, dendritic cells, and granulocytes found in the tumor stroma. On this basis, atezolizumab was approved in August 2019 by the European Medicines Agency (EMA) for the treatment of patients with inoperable and locally advanced or metastatic TNBC with PD-L1 expression on IC cells ≥ 1%, who had not previously received chemotherapy for advanced disease [2]. It should be emphasized that the VENTANA PD-L1 (SP142) test is the only validated method that can be used to assess PD-L1 expression when atezolizumab treatment is planned. As discussed previously, TNBC tissue material obtained during resection or core-needle biopsy from a primary or metastatic tumor can be used to assess PD-L1 expression. On the other hand, cytological samples and decalcified bone tissues are not suitable for this evaluation [15].

An important additional analysis is the evaluation of PD-L1 expression using 3 different antibodies: VENTANA SP142, VENTANA SP263, and DAKO 22C3, performed in 68% of tumors in patients participating in the IMpassion130 study. There were the following percentages of positive results for PD-L1 expression (IC ≥ 1%): 46.4% (SP142), 74.9% (SP263), and 73.1% (22C3). There was a significant difference in the frequency of positive and negative results when comparing the standard test (SP142) with the additional test. The rate of positive results in SP142+ tumors was 69% for SP262 and 22C3. In addition, it was indicated that benefits, in terms of PFS and OS, were primarily observed in patients treated with atezolizumab if tissue PD-L1 expression was detected with the use of SP142. The results of the analysis indicated that it was not possible to replace the validated SP142 test with other antibodies [16].

Similar observations were made in a study aiming to evaluate positive PD-L1 results with various tests. Tissue samples from 447 early TNBCs were assessed. PD-L1 expression (IC ≥ 1%) using the SP142 test was found in 34% of the cases. At the same time, staining with SP263 and 22C3 was performed. In the SP142+ group, double positive results were found in 76% (SP142+/SP263+) and 78% (SP142+/22C3+) cases, respectively, which confirms the discrepancy of the results when using different antibodies [17].

Interesting conclusions also come from the meta-analysis of 20 studies evaluating the rate of PD-L1-positive results in primary tumor and metastatic samples with the use of various tests (most often SP142, n = 8), which confirmed observations from the IMpassion130 study. Positive results were more common in primary tumors (51%) compared to metastases (37%). Considering the TNBC studies in which PD-L1 expression was determined on IC with the SP142 test, a higher percentage of PD-L1-positive results was found in primary tumors (55%) than in metastases (37%). In addition, there was a higher frequency of positive PD-L1 results if lymph nodes or lung metastases were evaluated (lower rate in the case of bone or liver samples). Another analysis compared PD-L1 expression in the material from primary tumors and metastases in the same patients. Discrepancies in the results were found in 39% of cases, with more frequent switching from positive to negative [18].

Additionally, the necessity of proper training of pathologists in the assessment of PD-L1 with SP142 is emphasized, as there is a large discrepancy in the interpretation of results between pathologists, especially in samples from metastases [19].

New directions

Early TNBC

The efficacy of atezolizumab is also assessed in patients with early TNBC. The first NeoTRIP study included 280 patients with stage II-III TNBC (without cT2N0 cases) who were receiving preoperative chemotherapy consisting of nab-paclitaxel (125 mg/m^2) and carboplatin (2 AUC) administered on days 1 and 8 every 21 days. Atezolizumab (1200 mg) was added to the experimental arm. After 8 cycles of therapy, surgery was

performed, and then in both groups, 4 cycles of anthracycline-based chemotherapy were administered. The primary endpoint of the study was event-free survival (EFS) in the whole study population. A secondary endpoint was the pathological complete response (pCR) rate. There was no significant difference in the pCR rate (48.6% in the experimental group *vs.* 44.4% in the control group; p = 0.48). However, it was found that the pCR rate was higher in PD-L1-positive patients in both study arms. The incidence of treatment-related adverse events was similar in both groups except for a significantly higher incidence of serious adverse events in the immunotherapy arm (18% *vs.* 6%) and elevated transaminases with atezolizumab. Data are continuously collected to determine the effect of atezolizumab therapy on EFS [20].

The second large study evaluating the efficacy of atezolizumab in preoperative TNBC therapy was the IMpassion031 phase III study, which included 333 patients with stage II-III breast cancer. Immunotherapy (atezolizumab 840 mg every 2 weeks) was added in the experimental arm to chemotherapy including nab-paclitaxel (125 mg/m^2, 12 infusions weekly). Then the AC regimen (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2, 4 cycles every 2 weeks) with immunotherapy (1200 mg, 11 infusions every 3 weeks) was continued in the atezolizumab arm after surgery. The primary endpoints of the study were pCR in the whole study group and in patients with PDL1 expression. Secondary endpoints included EFS, disease-free survival (DFS), and OS. Patient-reported outcomes (PRO) and safety data were also collected. A significantly higher pCR rate was found in patients receiving chemotherapy in combination with immunotherapy (58% *vs.* 41%; p = 0.0044). In TNBC patients with PD-L1 expression, the pCR rate was numerically increased in the atezolizumab group (69% *vs.* 49%); however, without statistical significance. Data on EFF, DFS, and OS are still being collected. The frequency of grade 3/4 adverse events (AEs) during preoperative treatment was similar in both arms (57% in the atezolizumab group *vs.* 53%), and the most common AEs were neutropenia, febrile neutropenia, leukopenia, anemia, and hypertension. However, treatment-related serious adverse events were observed slightly more often in the immunotherapy group (23% *vs.* 16%). The number of patients who discontinued treatment with atezolizumab or placebo due to adverse events was 21 (13%) and 19 (11%), respectively. The authors of the IMpassion031 study concluded that atezolizumab should be used perioperatively in combination with preoperative chemotherapy in patients with TNBC regardless of PD-L1 expression status [21].

Another large phase III study, the IMpassion030, is currently ongoing to assess the role of atezolizumab added to adjuvant chemotherapy (paclitaxel, followed by doxorubicin/epirubicin plus cyclophosphamide) in patients with stage II-III TNBC [22].

HER2-positive breast cancer

The concept of adding atezolizumab to anti-HER2 treatment is being evaluated in HER2-positive breast cancer patients. The first observations come from the phase II KATE2 study, including 202 patients who previously received trastuzumab and taxoid due to advanced HER2-positive breast cancer. Both arms received trastuzumab emtansine (T-DM1 — 3.6 mg/kg every 3 weeks), and atezolizumab (1200 mg) was added in the experimental arm. The primary endpoint of the study was PFS, and the secondary endpoints were OS, ORR, and duration of response (DoR). First interim analysis indicated no benefit from adding atezolizumab and a higher incidence of side effects, which led to a recommendation to unblind the study. The median PFS was 8.2 months in the atezolizumab group compared to 6.8 months in the control arm (p = 0.38), and the ORR was 45% and 43%, respectively. More grade ≥ 3 adverse events were observed in the immunotherapy group: thrombocytopenia (13% *vs.* 4%), elevated aspartate aminotransferase (8% *vs.* 3%), anemia (5% *vs.* 0). PDL1 expression was found in 42% of HER2-positive cancers. Additional subgroup analyzes showed improved efficacy of combination therapy (median PFS — 8.5 *vs.* 4.1 months, ORR — 54% *vs.* 33%). The authors of the study emphasized that the above analyzes were based on a small group of patients and could only be a hypothesis for further studies on PD-L1 positive HER2-positive cancers [23]. The KATE3 study with a similar design is currently ongoing but includes a population of patients with PD-L1 expression (NCT04740918).

Interesting observations may also come from a large study of atezolizumab used in first-line treatment of metastatic HER2-positive breast cancer in combination with standard therapy (pertuzumab, trastuzumab, and taxoid) (NCT03199885).

The addition of atezolizumab was evaluated in early HER2-positive breast cancer in the IMpassion050 study [24]. Patients with tumors > 2 cm and lymph node metastases (T2-4, N1-3, M0) were randomly assigned to the group with atezolizumab or placebo. Both arms received dose-dense doxorubicin and cyclophosphamide chemotherapy regimen followed by paclitaxel plus pertuzumab and trastuzumab. After surgery, the patients continued treatment with atezolizumab/placebo and anti-HER2 therapy (pertuzumab with trastuzumab or trastuzumab emtansine in the case of residual disease) for a year. Co-primary endpoints of the study were pCR rates in the whole study population and patients with PD-L1 expression. The pCR results in the placebo

Table 2. Summary of data on atezolizumab in patients with triple-negative breast cancer

Humanized monoclonal IgG1 antibody against PD-L1
Dosage: 840 mg intravenously on days 1 and 15 every 28 days
Combination therapy with nab-paclitaxel (100 mg/m^2 on days 1, 8, and 15 of each 28-day cycle)
Eligibility for treatment: VENTANA SP142 test — PD-L1 positive expression on IC cells (≥ 1%)
Improvement in median PFS and OS (PD-L1+ population) and ORR
Maintaining quality of life in patients treated with atezolizumab in combination with chemotherapy
EMA registration: in combination with nab-paclitaxel for the treatment of adult patients with unresectable locally advanced or metastatic triple-negative breast cancer (TNBC) whose tumors have PD-L1 expression ≥ 1% and who have not received prior chemotherapy for metastatic disease

EMA — European Medicines Agency; IC — immune cells; ORR — objective response rate; OS — overall survival; PD-L1 — programmed death receptor type 1; PFS — progression-free survival; TNBC — triple-negative breast cancer

and atezolizumab groups in the whole population were similar and amounted to 62.7% and 62.4%, respectively (p = 0.9551). There was also no significant difference in the subgroup of PD-L1-positive breast cancers: pCR rates in the placebo and atezolizumab arms were 72.5% and 64.2%, respectively (p = 0.1846). Additionally, grade 3/4 adverse events and serious adverse events were more frequent in the atezolizumab group compared to the placebo group. The safety profile of the treatment was consistent with observations from other clinical trials. The results of the study showed that there was no benefit in adding immunotherapy to pre-operative treatment in patients with early HER2-positive breast cancer. Another APTneo clinical trial is being conducted for the same indication, and the role of atezolizumab used perioperatively in combination with anti-HER2 treatment (pertuzumab and trastuzumab) and preoperative chemotherapy (NCT03595592) is also being investigated.

On the other hand, the ASTEFANIA study (NCT04873362) assesses the benefit of adding atezolizumab to T-DM1 as adjuvant treatment in patients with HER2-positive breast cancer with residual disease and high risk of disease recurrence.

ER-positive/HER2-negative breast cancer

There are no data on the efficacy of atezolizumab in patients with ER-positive/HER2-negative breast cancer. The first small studies in combination with hormone therapy (NCT04630210) are being designed.

Summary

Immunotherapy is a modern method of treatment evaluated in patients with breast cancer. The first-in-class approved drug was atezolizumab for the treatment of patients with inoperable and locally advanced or metastatic TNBC with PD-L1 expression on IC cells ≥ 1%, who had not previously received chemotherapy for advanced disease. The results of the pivotal IMpassion130 study indicated that atezolizumab improves outcomes in patients with PD-L1-positive cancers when used in combination with nab-paclitaxel. In qualifying for treatment, it is important to use the validated SP142 test (Tab. 2). In the second study, IMpassion131, no benefit was seen when the drug was used in combination with paclitaxel. More studies are ongoing with other cytotoxic drugs that may change the indications for combination immunotherapy. On the other hand, the first results of studies in early TNBC show a significant improvement in pCR in the whole group of patients. However, the addition of atezolizumab cannot be currently recommended based on the available results of studies in HER2-positive breast cancer. Numerous ongoing clinical trials may change the indications for the use of this drug in patients with breast cancer in the future.

Article Information and Declarations

Conflict of interest

K.P.: honoraria for consultations/lectures/training sessions/clinical trials and fees for scientific congresses: Roche, Novartis, Eli Lilly, Pfizer, MSD, AstraZeneca, Gilead, Teva, Egis.
A.J.G.: honoraria for consultations/lectures/training sessions/clinical trials: AstraZeneca, Novartis, Roche, Gilead, Eli Lilly, Amgen, Pfizer, MSD.
M.K.: honoraria for consultations/lectures/training sessions/clinical trials and fees for scientific congresses: MSD, Bayer, Novartis, Eli Lilly, Pfizer, Roche, Vipharm, Angelini.
W.O.: honoraria for consultations/lectures/training sessions/clinical trials: Roche.

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REVIEW ARTICLE

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Nivolumab in the treatment of thoracic cancer — new possibilities

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ABSTRACT

Immune checkpoint inhibitors have significantly changed the treatment of patients with advanced non-small cell lung cancer in recent years. The value of nivolumab was initially assessed in patients previously treated with systemic therapy. The association of nivolumab with ipilimumab and the interaction of these antibodies on different immune checkpoints have proven effective in solid tumors (melanoma and renal cell carcinoma). The CheckMate-9LA study assessed the value of dual immunotherapy combined with platinum-based chemotherapy in the first-line treatment of advanced non-small cell lung cancer. A clinical benefit – prolonged overall survival in patients receiving combination therapy – was documented. The results of the CheckMate743 trial for patients with pleural mesothelioma provide a basis for changing the current management algorithm for patients with this diagnosis. Patients diagnosed with mesothelioma of a non-epithelioid type particularly benefit from two-drug immunotherapy compared to chemotherapy. Maintaining the safety of treatment using immunotherapy targeting two immune checkpoints remains the challenge.

Key words: immunotherapy, immune checkpoint inhibitors, nivolumab, non-small cell lung cancer, pleural mesothelioma

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Introduction

Immune checkpoint inhibitors (ICIs) have significantly changed the treatment of advanced non-small cell lung cancer (NSCLC) patients in recent years [1]. They can be used in the first-line treatment either alone or in combination with chemotherapy, as well as after failure of the previously performed systemic treatment.

The value of nivolumab, which is a fully human antibody against programmed death receptor (anti-PD-1), was initially assessed in patients after failure of chemotherapy. The results of two pivotal phase III clinical trials (CheckMate-017 and CheckMate-057) led to registering the drug for patients with squamous and non-squamous NSCLC [2, 3]. Combined analysis of long-term results confirmed the significant clinical efficacy of nivolumab compared to docetaxel in terms of overall survival (OS) [4, 5].

Recently, indications for the use of nivolumab in patients with thoracic tumors have been expanded. Based on the results of the CheckMate-9LA study, a regimen consisting of nivolumab (anti-PD-1 antibody) and ipilimumab — an antibody directed against cytotoxic T cell antigen 4 (CTLA-4) — and two cycles of platinum-based chemotherapy in the first line of systemic treatment was registered [6]. The value of combination immunotherapy (nivolumab in combination with ipilimumab) in patients with pleural mesothelioma has also been documented [7].

This article discusses the theoretical basis of combining monoclonal antibodies, nivolumab, and ipilimumab, which inhibit the 2 most important immunological checkpoints (PD-1 and CTLA-4). The most important results of the studies that have become the basis for the registration of nivolumab after failure of chemo-

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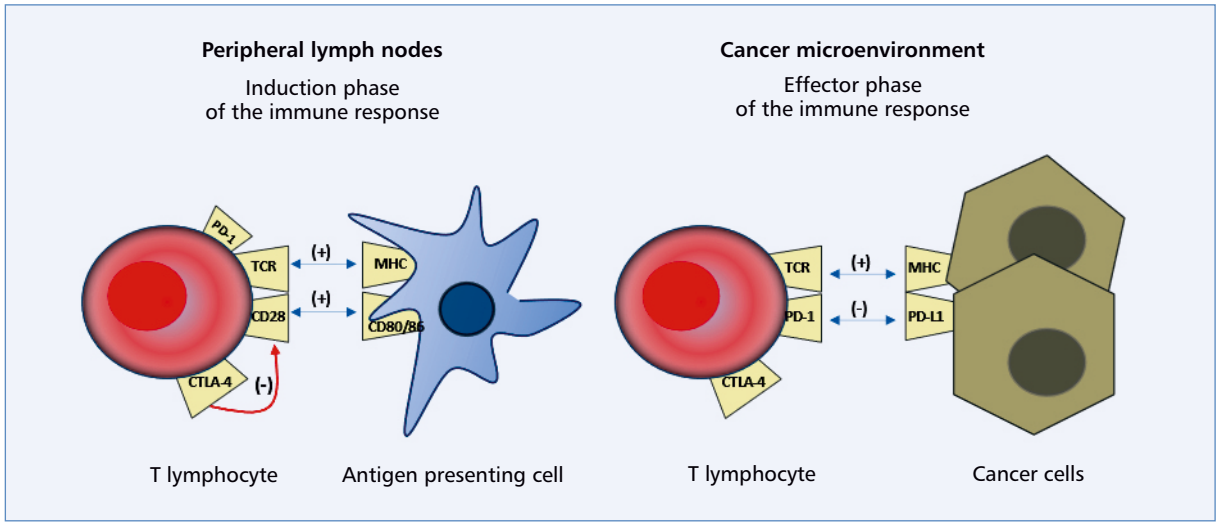

Figure 1. Induction of immune response in lymph nodes and tumor microenvironment (authors' own presentation)

therapy in NSCLC patients are summarized. The efficacy and safety data of immunotherapy with nivolumab and ipilimumab in thoracic cancers in which it has not been used so far (first-line treatment of advanced NSCLC and pleural mesothelioma) are also discussed in more detail.

Immune checkpoint inhibitors: should they be used alone or in combination?

The mechanism of action and clinical efficacy of combined treatment with nivolumab and ipilimumab result from the effect of antibodies on various immune checkpoints. The PD-1 molecule is constitutively expressed on all cells associated with specific immune response (T cells, B cells, and NK). Programmed death receptor ligand 1 (PD-L1) interacts with PD-1 and is present on non-specific immune cells (monocytes, dendritic cells, and tissue macrophages). In inflammatory states and in the environment of pro-inflammatory cytokines, such as tumor necrosis factor alfa (TNF-α) or interleukin 2 (IL-2), the expression of PD-1 and PD-L1 increases, and the interaction of PD-1 with PD-L1 inhibits the activity of PD-1-positive cells (lymphocytes), which is one of many mechanisms suppressing the excessive activity of T lymphocytes and protecting the body against possible autoimmune reactions [8]. PD-L1 can also be found on the surface of cancer cells, which is one of cancer immune escape mechanisms. Nivolumab inhibits PD-1 and reinvigorates T cell activity in cancer, lymph nodes, and tissues. Blocking the function of the PD-1 molecule takes place in the so-called "effector phase" of the immune response when lymphocytes should recognise and destroy tumor cells [8].

Stimulation of the CTLA-4 on the surface of T lymphocytes plays a role in inducing an immune reaction at the stage of antigen presentation (the so-called "early phase" of immune response induction) (Fig. 1). During the stimulation of T lymphocytes in the lymph nodes by antigen-presenting cells (APCs), specific receptor binding between these cells is formed [8, 9]. These interactions are called immunological synapses and involve a T-cell receptor (TCR) on T lymphocytes and major histocompatibility complex (MHC) molecules on APC, CD28 (cluster of differentiation 28) molecules on T lymphocytes and costimulatory molecules CD80 and CD86 on APC, as well as the cytokines network in the microenvironment. These interactions are necessary to stimulate T cells' activity. The CTLA-4 molecule is potent to displace CD28 from binding with CD80 and CD86, thereby disrupting the proper stimulation of T lymphocytes, resulting in inhibition of proliferation and activation of helper T cells and cytotoxic T cells. Moreover, such interaction sheds CD80 and CD86 molecules from the surface of antigen-presenting cells, leading to their inactivation. High CTLA-4 expression on T cells also induces the intracellular protein FoxP3 (forkhead box P3), resulting in turning them into regulatory T cells. The functioning of the CTLA-4 molecule is also one of the mechanisms regulating activity of the immune system [8–10].

The aforementioned interactions indicate a synergistic effect of PD-1 and CTLA-4 blocking, e.g. the combined use of nivolumab and ipilimumab consists in reactivating suppressed helper and cytotoxic T lymphocytes by blocking one of the strongest inhibitory signals (PD-1 and PD-L1 interaction) and restoration of essential (apart from antigen presentation) co-stimulatory signal (binding of CD28 by CD80 and CD86). The use of

Table 1. Roles of CTLA-4 and PD-1 molecules in the immune system and effects of their blocking (based on [8–11] with authors' own modification)

CTLA-4	PD-1
It appears on T lymphocytes during immunological synapse forming in the lymph node Present on activated T cells Ligands include CD80 (B7.1) and CD86 (B7.2) molecules present on many cells in the body	Present on the surface of T lymphocytes (constitutive expression), with increased expression after cell activation Ligands include PD-L1 and PD-L2 molecules, present on the surface of immune cells and cancer cells
Effect of blocking the molecule with an anti-CTLA-4 antibody	**Effect of blocking the molecule with an anti-PD-1 antibody**
Inhibition of T reg activity Increased cytotoxic activity of NK cells Increased phagocytic activity of non-specific immune cells Increased activation and proliferation of cytotoxic T lymphocytes	Increased activation of T lymphocytes not only in the tumor microenvironment but also in peripheral tissues

CTLA-4 — cytotoxic T cell antigen 4; PD-1 — programmed death receptor 1; PD-L1 — programmed death receptor ligand 1; NK — natural killer

ipilimumab additionally reduces the immunosuppressive effect of other cells. The synergistic effect of nivolumab and ipilimumab consists in restoring T lymphocyte activity in the early activation phase and the effector phase of the immune response [8–11]. Characteristics of both molecules' activity are summarized in Table 1.

The interaction of both immune checkpoint inhibitors is also strongly reflected in laboratory testing results. There is a significantly increased percentage of cytotoxic T lymphocytes in the peripheral blood in patients undergoing combined immunotherapy compared to monotherapy with either nivolumab or ipilimumab. High plasma levels of pro-inflammatory cytokines IL-2Rα, IL-1α, and chemokines (e.g. CXCL10) are reported in patients receiving combined immunotherapy, which cannot be obtained with nivolumab or ipilimumab alone. Responders to combined immunotherapy show an increased percentage of Eomes (eomesodermin)+, $CD69^+$, $CD45RO^+$ memory cytotoxic ($CD8^+$) T lymphocytes compared to baseline [9, 11, 12]. Moreover, low expression of other negative immune checkpoints, including T-cell immunoreceptors with Ig and ITIM domains (TIGIT) and lymphocyte-activation gene 3 (LAG3) on T lymphocytes is observed in responders. This phenomenon is not present in patients responding to nivolumab monotherapy. The expression of genes responsible for the immune response profile in peripheral blood leukocytes was also analyzed. In patients undergoing combined therapy, the expression of genes for granzymes A/B, proliferation marker Ki-67, IL-8, and HLA-DR (human leukocyte antigen-DR isotype) was reported, which proves the cytolytic and proliferative activity of cytotoxic T lymphocytes, as well as their potency to infiltrate neoplastic tissue. Patients receiving anti-PD-1 monotherapy have overexpressed genes determining the cytolytic activity of T lymphocytes (genes for granzymes *A*/*B*, *KLRF1*, and *FCRL3*), while patients receiving ipilimumab express genes producing specific cytokines (genes for *Ki-67* and *ICOS*) and related to the ability of T lymphocytes to proliferate. It seems that the gene expression profile after combined immunotherapy ensures both cytolytic and proliferative activity of T lymphocytes [9, 11, 12].

Detailed immunophenotyping of immune cells after combined immunotherapy and monotherapy was performed in animal models [11]. Wei et al. [11] divided the group of tumor-infiltrating cytotoxic T cells into 4 immunophenotypes: T cells with a functionally exhausted phenotype ($PD\text{-}1^{high}$, $LAG3^{++}$, $TIM3^{++}$), terminally differentiated T cells with an activated phenotype ($PD\text{-}1^+$, $LAG3^{int}$, $TIM3^{int}$), T cells in early differentiation stage ($Tbet^{int}$, $CD86^+$, $PD\text{-}1^{+/-}$, $Bcl2^+$), and apoptosis-resistant migrating T cells ($PD\text{-}1^-$, $CD62L^+$, $Bcl2^{++}$). The use of combined immunotherapy significantly increases the percentage of differentiated and activated lymphocytes and significantly reduces the percentage of functionally exhausted lymphocytes compared to nivolumab or ipilimumab alone. However, the type of therapy has no effect on the percentage of the remaining subpopulation of cytotoxic T lymphocytes in the peripheral blood. T helper lymphocytes also include subpopulations of different immunophenotypes: Th1 lymphocytes with an effector phenotype ($PD\text{-}1^+$, $GATA3^+$, $CD44^+$, $CXCR3^{++}$), T lymphocytes with a helper phenotype without chemokine receptors ($CD44^+$, $GATA3^+$, $CD44^+$, $CXCR3^-$), and apoptosis-resistant actively migrating lymphocytes ($PD\text{-}1^-$, $CD62L^+$, $Bcl2^{++}$). Combined immunotherapy significantly increases infiltration by Th1 effector lymphocytes compared to monotherapy with nivolumab or ipilimumab. The immunophenotype of regulatory T lymphocytes enables their division into 3 groups: Treg lymphocytes with a pro-tumor phenotype ($CTLA\text{-}4^{++}$, $FoxP3^+$, $CD25^+$), Treg lymphocytes with an incom-

plete differentiation phenotype (CTLA-4$^+$, FoxP3^{++}, CD25^{++}), and undifferentiated and depleted Treg lymphocytes (CTLA-4$^-$, FoxP3$^{+/-}$, CD25^{++}). Wei et al. [11] found smaller infiltrates by Treg lymphocytes with a pro-tumor immunophenotype in the animal model after using ipilimumab or combined therapy compared to nivolumab alone or untreated models. It was also shown that the percentage of Th1 effector lymphocytes negatively correlated, and the percentage of pro-tumor Treg lymphocytes positively correlated with tumor size [10, 11].

Based on theoretical assumptions, as well as the results of laboratory and clinical tests, other concepts of combining antibodies affecting different immune checkpoints have emerged. Clinical trials are currently ongoing in patients with advanced NSCLC, in which attempts are made to combine classic anti-PD-1 or anti-PD-L1 antibodies with antibodies against inducible T cell co-stimulator (ICOS), LAG-3, T cell immunoglobulin domain and mucin domain-3 (TIM-3), or TIGIT. Patients who did not respond to combined immunotherapy with nivolumab and ipilimumab showed a significantly higher percentage of T cells expressing these molecules. It seems that their presence may play a leading role in inhibiting the activation of T lymphocytes and inducing resistance to existing methods of immunotherapy [8, 10].

Nivolumab's value after chemotherapy failure

The safety and efficacy of nivolumab *versus* docetaxel in patients after chemotherapy failure were assessed in two randomized studies with similar designs. The differentiating factor was the histopathological diagnosis.

The CheckMate-057 study was designed for patients with non-squamous NSCLC. Patients with advanced or recurrent NSCLC and documented disease progression during or after platinum-based chemotherapy were eligible [3]. A total of 582 patients were assigned to two treatment arms: 292 patients to the group receiving nivolumab at a dose of 3 mg/kg every 2 weeks and 290 patients to the group receiving docetaxel at a dose of 75 mg/m^2 every 3 weeks. The primary endpoint was OS, and the secondary endpoints included objective response rate (ORR) and progression-free survival (PFS). The study demonstrated superiority of nivolumab over docetaxel with regard to the assumed endpoints. Median OS was 12.2 and 9.4 months, respectively [hazard ratio (HR) 0.73; 95% confidence interval (CI) 0.59–0.89; p = 0.002]. ORRs were 19% for nivolumab *versus* 12% for docetaxel (p = 0.02). Overall, treatment-related adverse events (AEs) were reported in 69% of patients in the nivolumab group and 88% in the docetaxel group, while clinically significant adverse events (Grades 3–4) were reported in 10% of patients in the nivolumab group and 54% in the docetaxel group [3].

The CheckMate-017 study included 272 patients with advanced or recurrent squamous cell lung cancer — 135 patients were assigned to the nivolumab arm, and 137 patients were assigned to the docetaxel arm [2]. The advantage of nivolumab over docetaxel was confirmed. Median OS was 9.2 and 6.0 months, respectively (HR = 0.59; 95% CI 0.44–0.79; p < 0.001), and ORR was 20% and 9%, respectively (p = 0.008). Treatment-related AEs occurred in 58% of patients in the experimental arm and 86% in the control group. Grade 3 and 4 adverse events were observed in 7% and 55% of patients, respectively. Data regarding nivolumab's value in the second-line treatment are presented in Table 2.

Longer observations confirmed the value of nivolumab [4, 5]. The 4-year OS rates were 14% and 5%, respectively and the 5-year rates — 13.4% and 2.6%, respectively (HR = 0.68; 95% CI 0.59–0.78) [4, 5]. Greater clinical benefit was observed in patients who achieved an objective response to nivolumab treatment. The 4-year OS rate in patients with objective response was 58% in the nivolumab group and 12% in the docetaxel group [4].

Immunochemotherapy in first-line treatment of NSCLC

Immunochemotherapy is now a recognized standard of care in patients diagnosed with advanced NSCLC with PD-L1 expression < 50%, who remain in good general condition and have no significant contraindications to chemotherapy and immunotherapy. In Poland, it is possible to use a regimens based on pembrolizumab, and — from January 1st, 2023 — nivolumab and ipilimumab [13].

CheckMate-9LA — treatment effectiveness

The CheckMate-9LA study was the basis for the registration of a first-line treatment regimen with nivolumab in patients with advanced NSCLC [6]. The study included patients with good performance status (PS) 0–1 according to the Eastern Cooperative Oncology Group (ECOG) score, with no prior systemic treatment due to advanced NSCLC and without molecular disturbances in *EGFR* and *ALK* genes. A total of 719 patients were randomized 1:1 to receive chemotherapy (4 cycles of platinum-based chemotherapy) or immunochemotherapy. The treatment regimen in the experimental arm included 2 cycles of immunochemotherapy (nivolumab 360 mg every 3 weeks plus ipilimumab 1 mg/kg every 6 weeks in combination with platinum-based chemotherapy) followed by immunotherapy (for a total of two years or until the loss of clinical benefit) [6]. The stratification factors included sex, tumor histology, and PD-L1 expression. The primary endpoint was OS, and the secondary endpoint included

Table 2. Efficacy of nivolumab after chemotherapy failure [2, 3]

	CheckMate-017			CheckMate-057		
	Nivolumab	Docetaxel	HR	Nivolumab	Docetaxel	HR
Numer of patients	135	137		292	290	
ORR [%]	20	9	2.6; p = 0.008	19	12	p = 0.02
PFS [months]	3.5	2.8	0.62; p < 0.001	2.3	4.2	0.92; p = 0.39
OS [months]	9.2	6.0	0.59; p < 0.001	12.2	9.4	0.73; p = 0.002
AE (any) [%]	58	86		69	88	
AE (grade 3–4) [%]	7	55		10	54	

AE — adverse event; HR — hazard ratio; ORR — objective response rate; OS — overall survival; PFS — progression-free survival

ORR and PFS measured in an independent review. The advantage of immunochemotherapy in relation to the assumed endpoints was demonstrated in the whole analyzed population. The objective response rates were 38% and 25%, respectively. Median PFS was 6.8 months *versus* 5 months (HR = 0.7; 95% CI 0.57–0.86; p = 0.00012), and median OS was 14.1 months *versus* 10.7 month (HR = 0.69; 95% CI 0.55–0.87; p = 0.00065) [6]. The subgroup analysis showed no benefit of immunochemotherapy in terms of OS in patients over 75 years of age (HR = 1.21; 95% CI 0.69–2.12) and non-smokers (HR = 1.14; 95% CI 0.66–1.97). The predictive value of PD-L1 expression was also assessed with benefits noted in all subgroups (for patients with PD-L1 expression < 1% — HR = 0.62, for patients with PD-L1 expression ≥ 50% — HR = 0.66). Differences in survival parameters determined by the histological tumor type were found. In patients with non-squamous cell carcinoma, median PFS for immunochemotherapy and chemotherapy were 7 and 5.6 months, respectively (HR = 0.74; 95% CI 0.6–0.92), and median OS was 17 and 11.9 months, respectively (HR = 0.69; 95% CI 0.55–0.87). In patients with squamous cell carcinoma, median PFS and OS for immunochemotherapy and chemotherapy were 5.6 and 4.3 months (HR = 0.57; 95% CI 0.42–0.78) and 14.5 and 9.1 months (HR = 0.62; 95% CI 0.45–0.86), respectively [6]. Updated results from the CheckMate-9LA study were also published [14]. With a median follow-up of 30.7 months, the superiority of combined therapy was confirmed. The median OS was 15.8 and 11 months, respectively (HR = 0.72; 95% CI 0.61–0.86). The percentages of patients who were followed up after two years were 38% and 26%, respectively, and the percentages of patients who were free from disease progression were 20% and 8%, respectively [14, 15]. The efficacy data are summarized in Table 3.

During the 2021 World Conference on Lung Cancer, the results of the analysis evaluating the intracranial activity of this therapy regimen were presented [15]. The metastases in the central nervous system (CNS) were found in 51 patients treated with chemoimmunotherapy and in 50 patients receiving chemotherapy alone. The inclusion criterion in that study was the absence of neurological symptoms for 14 days preceding the administration of the first dose of investigational drugs and the completion of local treatment. There is a significant clinical benefit associated with the use of nivolumab and ipilimumab-based immunochemotherapy. Median PFS in this population was 13.5 and 4.6 months respectively (HR = 0.36; 95% CI 0.22–0.60), and the objective response rates were 20% and 10%, respectively. Longer OS was also observed in the group of patients with CNS metastases — median OS in patients with CNS lesions were 19.3 and 6.8 months, respectively (HR = 0.43; 95% CI 0.27–0.67), whereas in patients without CNS metastases they were 15.6 and 12.1 months, respectively (HR = 0.79; 95% CI 0.65–0.95) [15]. The data are summarized in Table 3.

Safety profile of immunochemotherapy

Treatment-related adverse events were observed in 92% of patients in the experimental group and 88% of patients in the control group [6]. Nausea (26%), diarrhea (23%), weakness, pruritus, anemia (21%), skin lesions, and hypothyroidism (19%) were most frequently observed during the administration of immunochemotherapy. In contrast, the most frequent AEs in the chemotherapy group were anemia (38%), nausea (36%), and weakness (18%).

The incidence of clinically significant treatment-related adverse events is summarized in Table 4 [6, 14].

Systemic treatment of patients with pleural mesothelioma

Approximately 360 patients in Poland are diagnosed annually with pleural mesothelioma [17]. Asbestos exposure is the greatest risk factor for cancer development, and the estimated time from exposure to disease onset is usually 30–40 years [18]. The diagnosis of pleural mesothelioma is based on the assessment of pleural specimens obtained during open biopsy or

Table 3. Efficacy of nivolumab in combination with ipilimumab and chemotherapy (based on [14, 16])

	Nivolumab/ipilimumab + + 2 cycles of chemotherapy (n = 361)	4 cycles of chemotherapy (n = 358)	HR
ORR [%]	37.7	25.1	
mPFS	6.8	5.0	0.7
mOS	18	12.6	0.66
mOS			
PD-L1 > 50%	18	12.6	0.66
PD-L1 1–49%	15.4	10.4	0.61
PD-L1 < 1%	17.7	9.8	0.67
mOS			
Squamous cell carcinoma	14.5	9.1	0.62
Non-squamous cell carcinoma	17	11.9	0.69
mOS			
CNS metastases (+)	19.3	6.8	0.43
CNS metastases (–)	15.6	12.1	0.79

CNS — central nervous system; HR — hazard ratio; mOS — median overall survival; mPFS — median progression-free survival; ORR — objective response rate; PD-L1 — programmed death receptor ligand 1

Table 4. Incidence of treatment-related adverse events in the CheckMate-9LA study [6, 14]

Adverse events	Nivolumab + ipilimumab + + chemotherapy (358 patients)		Chemotherapy (349 patients)	
	Any [%]	Grade 3–4 [%]	Any [%]	Grade 3–4 [%]
Any	92	47	88	38
Leading to treatment discontinuation	19	16	7	5
Serious	30	25.4	18	15

videothoracoscopy, including expression of immunohistochemical markers (calretinin, cytokeratin 5/6, WT-1). There are three histological types of malignant pleural mesothelioma (epithelial, sarcomatous, and mixed) with a different clinical course and sensitivity to systemic treatment [19]. The prognosis for pleural mesothelioma is poor, as most patients have advanced inoperable disease at diagnosis. Chemotherapy with cisplatin and pemetrexed is recognized as the standard first-line treatment; the superiority of the doublet regimen over cisplatin was demonstrated — median OS was 12.1 and 9.3 months, respectively (HR = 0.77; p = 0.020), and objective response rates were 41.3% and 16.7%, respectively [20]. Attempts to improve treatment outcomes by combining chemotherapy with anti-angiogenic drugs have failed [21, 22]. The effectiveness of chemotherapy is much lower in patients diagnosed with non-epithelial mesotheliomas — ORR does not exceed 15%, and OS is 4–6 months. Other negative prognostic factors were also determined, and apart from the tumor morphology, they include male sex, elevated lactate dehydrogenase level, weight loss, and thrombocythemia [23].

The chronic inflammatory response to asbestos fibers associated with carcinogenesis of pleural mesothelioma leads to the development of an immunosuppressive tumor microenvironment. Many studies also indicate that mesothelioma tissues are heavily infiltrated by immune cells, which can also be found in pleural effusion [24]. However, according to literature data, the immune system in patients with pleural mesothelioma is very tolerogenic (showing little activity against neoplastic cells) [24, 25]. It has been shown that although the total number of lymphocytes did not change in patients with mesothelioma, the percentages of some T lymphocyte populations (cytotoxic T cells, helper T cells, and NK cells) were significantly reduced [25, 26]. Biopsy studies have shown that despite the high infiltration by macrophages, $CD4^+$, and $CD8^+$ T lymphocytes in some tumors, there were no antigen-presenting cells necessary for antigen recognition and T cell activation. Many studies also show a significant increase in the percentage of regulatory T lymphocytes in the peripheral blood in patients with pleural mesothelioma [25, 26]. The above-mentioned premises theoretically justify the use of combined immunotherapy in mesothelioma [26, 27].

Table 5. Summary of treatment efficacy data [30]

	Nivolumab/Ipilimumab (303 patients)	Chemotherapy (302 patients)
ORR	39.6%	44%
CR	2.6%	–
PR	37%	44%
SD	37%	40.7%
PD	18.2%	4.3%
mPFS [months]	6.8	7.2
	HR = 0.92 (95% CI 0.76–1.11)	
mOS [months]	18.1	14.1
	HR = 0.75 (95% CI 0.63–0.90)	

CI — confidence interval; CR — complete response; HR — hazard ratio; mOS— median overall survival; mPFS — median progression-free survival; ORR — objective response rate; PD — progression disease; PR — partial response; SD — stable disease

In recent years, many studies have been conducted to assess the effectiveness of immune checkpoint inhibitors. Non-randomized studies in patients after chemotherapy failure indicated activity of immunotherapy — the ORR was 8–29%, and median OS was 10–17 months [28, 29].

Nivolumab in combination with ipilimumab in the treatment of patients with pleural mesothelioma

The efficacy and safety of a doublet regimen with dual immune checkpoint blockade were assessed in the CheckMate-743 study [7]. Patients diagnosed with advanced pleural mesothelioma, with good ECOG PS (0–1) and without contraindications for immunotherapy were eligible for treatment. Patients were randomized to receive nivolumab (3 mg/kg every 2 weeks) with ipilimumab (1 mg/kg every 6 weeks) for up to 2 years or to receive 6 cycles of platinum-based chemotherapy with pemetrexed [7]. Initial results and updates after 3 years of follow-up confirm the benefit of immunotherapy [7, 30]. Median OS was 18.1 and 14.1 months, respectively (HR = 0.73; 95% CI 0.61–0.87), and the percentage of patients who remained in follow-up after 3 years was 23% and 15%, respectively. Three years after treatment initiation, 14% of patients who received immunotherapy remained free from disease progression (1% in the chemotherapy arm). Data on survival and treatment response are presented in Table 5.

It should be emphasized that the activity of immunotherapy differs according to histological types of pleural mesothelioma. Patients with a non-epithelial type benefited significantly, as median OS was 18.1 and 8.8 months, respectively (HR = 0.48; 95% CI 0.34–0.69). In the group of patients with epithelial type, the impact on OS was limited, with median OS of 18.2 and 16.7 months, respectively (HR = 0.85; 95% CI 0.69–1.04).

The frequency of treatment-related adverse events (including grade ≥ 3) was similar in both groups (any AE in 80% of patients and grade ≥ 3 AEs in 30% of patients). Diarrhea (21%) and skin lesions (16%) were the most common in the group of patients undergoing immunotherapy, and nausea (37%), anemia (36%), and neutropenia (25%) were the most common in the group of patients receiving chemotherapy. The most common immune-related adverse events (irAEs) were rash (13% of patients), hypothyroidism/thyroiditis (12%), and colitis (9%). The most common grade 3/4 irAEs were hepatitis (5% of patients), colitis (4%), and rash (3%). The frequency of adverse events leading to temporary interruption or permanent discontinuation of treatment is presented in Table 6. In the experimental group, the most common causes of premature treatment discontinuation were colitis and diarrhea (2% of patients each) and anemia in the chemotherapy arm (4% of patients). It was also observed that premature treatment discontinuation due to an adverse event was a favorable prognostic factor in the analyzed group of patients.

Biomarkers assessment in patients diagnosed with pleural mesothelioma

The analysis of prognostic and predictive factors: clinical, morphological, and molecular, is an important part of research evaluating the value of modern anti-cancer therapies.

The CheckMate-743 study analyzed the predictive value of the signature of four genes encoding inflammatory proteins. It has been shown that in the group of patients with higher results receiving immunotherapy, OS was significantly longer than in patients treated with chemotherapy. Median OS was 21.8 and 16.8 months, respectively (HR 0.57; 95% CI 0.40–0.82), and the 3-year survival rates were 35% and 15%, respectively. These findings have not been demonstrated in the group of patients receiving chemotherapy [30].

Table 6. Incidence of treatment-related adverse events in the CheckMate-743 study [30]

Adverse events	Nivolumab + ipilimumab + + chemotherapy (300 patients)		Chemotherapy (284 patients)	
	Any [%]	Grade 3–4 [%]	Any [%]	Grade 3–4 [%]
Any	80	30.7	82	32
Leading to 1 drug discontinuation	22.7	15.3	15.8	7.4
Leading to all drugs discontinuation	17.3	13	1.7	4.6
Serious	21.3	15.7	7.7	6

The predictive value of tumor mutation burden (TMB) in relation to OS was not demonstrated (the analysis was performed in approximately 50% of patients in both groups with available TMB data).

The predictive value of PD-L1 expression was a secondary endpoint in the Check-Mate-743 study. The benefit of immunotherapy was demonstrated in the group of patients with PD-L1 expression ≥ 1% (HR = 0.69; 95% CI 0.55–0.87). In patients with PD-L1 expression < 1%, OS difference was not significant (HR = 0.94; 95% CI 0.62–1.40) [30].

Summary

The value of nivolumab in the second-line treatment of advanced NSCLC has been determined in randomized trials and confirmed in many publications based on real-world data. The synergistic effect of nivolumab and ipilimumab — as a consequence of restoring the activity of T lymphocytes in the early activation phase and in effector phase of immune response — is the basis for studies using both drugs. The results of the studies confirmed the effectiveness of nivolumab in combination with ipilimumab in patients with NSCLC (in combination with chemotherapy) and pleural mesothelioma (immunotherapy alone). The use of immunochemotherapy with dual immune checkpoints blockade allows for improving survival parameters in patients with NSCLC, regardless of histological type and PD-L1 expression level (including patients with PD-L1 expression < 1%). Clinical benefit is also noted in patients with CNS metastases. Age over 75 is probably a negative prognostic factor. Nivolumab in combination with ipilimumab is the first regimen using immune checkpoint inhibitors in pleural mesothelioma that is clinically proven and statistically superior to first-line chemotherapy. Patients with non-epithelial mesothelioma, for whom the systemic treatment methods available so far have shown little efficacy, can particularly benefit.

The use of a doublet immunotherapy regimen (including combination with chemotherapy) is associated with an increased risk of clinically significant adverse effects (including immune-related), which highlights a need for a thorough assessment of indications and contraindications for treatment at the time of patient selection and careful monitoring (especially in the first weeks of treatment).

Article Information and Declarations

Conflict of interest

M.K.-W.: honoraria for lectures from BMS and MSD.
K.W-K.: no conflict to declare.

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