

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

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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 re-

sults 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 TKI-EGFR in patients with advanced NSCLC naturally raised the question of the possibility of using

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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 subscapular 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 I.

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.

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.

Table I. 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 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 ($\geq 50\%$) and meeting the eligibility criteria for treatment with osimertinib (disease stage IB–IIIA, radical surgery RO), *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 [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 2021 World 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. II). 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. III);
- 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 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);
 - 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 IV.

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 radica-

Table II. 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

Table III. 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 <i>in situ</i>	
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: <ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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

Table IV. 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: <ul style="list-style-type: none"> • 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)

lity 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. V).

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 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. VI) [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;

Table V. 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	<ul style="list-style-type: none"> • 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

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

Table VI. 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: <ul style="list-style-type: none"> • positive surgical margin¹ • neoplastic infiltration exceeds the capsule of resected lymph nodes
R1(is)	carcinoma <i>in situ</i> 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

¹Malignant 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

- 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 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 VII.

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. VIII) [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 pa-

Table VII. 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
<i>EGFR</i> 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 VIII. 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% ($p < 0.0001$)	

tients living without recurrence of the disease reached 70% in the osimertinib group compared to 29% in the placebo group [42].

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,

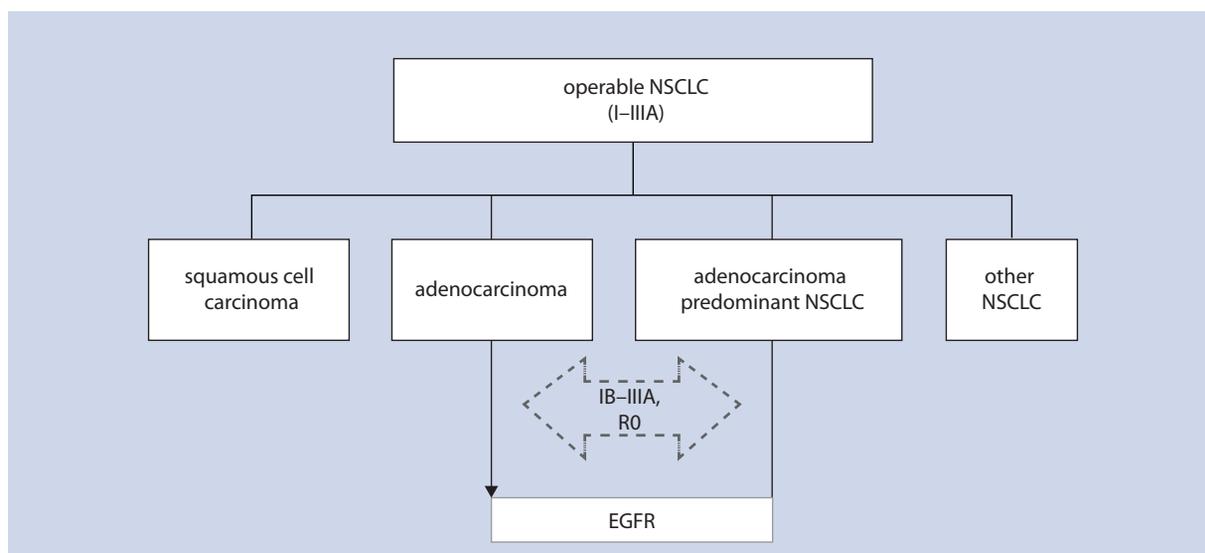


Figure 1. Qualification of patients treated surgically for adjuvant therapy with osimertinib; EGFR – epidermal growth factor receptor; NSCLC – non-small cell lung cancer

determining the value of longer use of osimertinib, and detecting resistance mechanisms and methods overcoming lower sensitivity to the drug (Fig. 1).

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References

1. Wojciechowska U, Barańska K, Michałek I, et al. Nowotwory złośliwe w Polsce w 2020 roku. Krajowy Rejestr Nowotworów. Warszawa 2022. https://onkologia.org.pl/sites/default/files/publications/2023-01/nowotwory_2020.pdf.
2. Goldstraw P, Chansky K, Crowley J, et al. International Association for the Study of Lung Cancer Staging and Prognostic Factors Committee, Advisory Boards, and Participating Institutions, International Association for the Study of Lung Cancer Staging and Prognostic Factors Committee Advisory Boards and Participating Institutions. The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer. *J Thorac Oncol*. 2016; 11(1): 39–51, doi: 10.1016/j.jtho.2015.09.009, indexed in Pubmed: 26762738.
3. Karacz CM, Yan J, Zhu H, et al. Timing, Sites, and Correlates of Lung Cancer Recurrence. *Clin Lung Cancer*. 2020; 21(2): 127–135.e3, doi: 10.1016/j.clcc.2019.12.001, indexed in Pubmed: 31932216.
4. Pignon JP, Tribodet H, Scagliotti GV, et al. LACE Collaborative Group. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol*. 2008; 26(21): 3552–3559, doi: 10.1200/JCO.2007.13.9030, indexed in Pubmed: 18506026.
5. Krzakowski M, Jassem J, Antczak A, et al. Thoracic neoplasms. *Oncol Clin Pract*. 2022; 18(1): 1–39, doi: 10.5603/OCP.2021.0022.
6. Sullivan I, Planchard D. Next-Generation Tyrosine Kinase Inhibitors for Treating -Mutant Lung Cancer beyond First Line. *Front Med (Lausanne)*. 2016; 3: 76, doi: 10.3389/fmed.2016.00076, indexed in Pubmed: 28149837.
7. Wu YL, Tsuboi M, He J, et al. Osimertinib in Resected EGFR-Mutated Non-Small-Cell Lung Cancer. *N Engl J Med*. 2020; 383(18): 1711–1723, doi: 10.1056/nejmoa2027071.
8. Wu YL, John T, Grohe C, et al. Postoperative Chemotherapy Use and Outcomes From ADAURA: Osimertinib as Adjuvant Therapy for Resected EGFR-Mutated NSCLC. *J Thorac Oncol*. 2022; 17(3): 423–433, doi: 10.1016/j.jtho.2021.10.014, indexed in Pubmed: 34740861.
9. Lardinois D, De Leyn P, Van Schil P, et al. ESTS guidelines for intraoperative lymph node staging in non-small cell lung cancer. *Eur J Cardiothorac Surg*. 2006; 30(5): 787–792, doi: 10.1016/j.ejcts.2006.08.008, indexed in Pubmed: 16971134.
10. Darling GE, Allen MS, Decker PA, et al. Number of lymph nodes harvested from a mediastinal lymphadenectomy: results of the randomized, prospective American College of Surgeons Oncology Group Z0030 trial. *Chest*. 2011; 139(5): 1124–1129, doi: 10.1378/chest.10-0859, indexed in Pubmed: 20829340.
11. Patomorfologia: standardy i przykłady dobrej praktyki oraz elementy diagnostyki różnicowej. Wytyczne dla zakładów/pracowni patomorfologii 2020.
12. Standardy organizacyjne oraz standardy postępowania w patomorfologii. Wytyczne dla zakładów/pracowni patomorfologii 2020.
13. Program Akredytacji Jednostki Diagnostyki Patomorfologicznej. Zestaw standardów. CMJ, Kraków 2021.

14. Darling GE. Current status of mediastinal lymph node dissection versus sampling in non-small cell lung cancer. *Thorac Surg Clin*. 2013; 23(3): 349–356, doi: 10.1016/j.thorsurg.2013.05.002, indexed in Pubmed: 23931018.
15. Langfort R, Chyczewski L, Szolkowska M, et al. Pluco. Standardy oceny mikroskopowej materiału biopsyjnego i operacyjnego u chorych na nowotwory złośliwe. *Pol J Pathol*. 2015; 66(suppl. 1): 22–24.
16. Boyle DP, Allen DC. *Histopathology reporting. Guidelines for surgical cancer*. 4th ed. Springer. 2020.
17. Edge SB, Byrd DR, Compton CC. *AJCC Cancer Staging Handbook*. 7th ed. AJCC, Springer 2010.
18. Pfeifer JD, Humphrey PA, Ritter JH, Dehner LP. *The Washington Manual of Surgical Pathology*. 3rd ed. Wolters Kluwer, Philadelphia 2020.
19. WHO Classification of Tumours Editorial Board. Thoracic tumours. In: WHO classification of tumours series, 5th ed.; vol. 5. International Agency for Research on Cancer, Lyon 2021.
20. Moreira AL, Ocampo PSS, Xia Y, et al. A Grading System for Invasive Pulmonary Adenocarcinoma: A Proposal From the International Association for the Study of Lung Cancer Pathology Committee. *J Thorac Oncol*. 2020; 15(10): 1599–1610, doi: 10.1016/j.jtho.2020.06.001, indexed in Pubmed: 32562873.
21. Travis WD, Brambilla E, Rami-Porta R, et al. International Staging Committee. Visceral pleural invasion: pathologic criteria and use of elastic stains: proposal for the 7th edition of the TNM classification for lung cancer. *J Thorac Oncol*. 2008; 3(12): 1384–1390, doi: 10.1097/JTO.0b013e31818e0d9f, indexed in Pubmed: 19057261.
22. Rami-Porta R. *Staging Manual in Thoracic Oncology*. 2nd ed. IASLC, North Fort Myers, FL 2016.
23. Travis WD, Asamura H, Bankier AA, et al. International Association for the Study of Lung Cancer Staging and Prognostic Factors Committee and Advisory Board Members. The IASLC Lung Cancer Staging Project: Proposals for Coding T Categories for Subsolid Nodules and Assessment of Tumor Size in Part-Solid Tumors in the Forthcoming Eighth Edition of the TNM Classification of Lung Cancer. *J Thorac Oncol*. 2016; 11(8): 1204–1223, doi: 10.1016/j.jtho.2016.03.025, indexed in Pubmed: 27107787.
24. Rami-Porta R. The Evolving Concept of Complete Resection in Lung Cancer Surgery. *Cancers (Basel)*. 2021; 13(11), doi: 10.3390/cancers13112583, indexed in Pubmed: 34070418.
25. Langfort R. Nowotwory klatki piersiowej. Rak płuca. In: Nasierowska-Guttmejer A, Górnicka B. ed. *Zalecenia do diagnostyki histopatologicznej nowotworów*. Centrum Onkologii, PTP 2013: 47–62.
26. Pisters K, Kris MG, Gaspar LE, et al. Adjuvant Systemic Therapy and Adjuvant Radiation Therapy for Stage I to IIIA NSCLC Guideline Expert Panel. Adjuvant Systemic Therapy and Adjuvant Radiation Therapy for Stage I-III A Completely Resected Non-Small-Cell Lung Cancer: ASCO Guideline Rapid Recommendation Update. *J Clin Oncol*. 2022; 40(10): 1127–1129, doi: 10.1200/JCO.22.00051, indexed in Pubmed: 35167335.
27. Solomon B, Ahn J, Barlesi F, et al. ALINA: A phase III study of alectinib versus chemotherapy as adjuvant therapy in patients with stage IB–IIIA anaplastic lymphoma kinase-positive (ALK+) non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2019; 37(15_suppl): TPS8569–TPS8569, doi: 10.1200/jco.2019.37.15_suppl.tps8569.
28. Goldman J, Besse B, Wu Y, et al. P01.01 LIBRETTO-432: A Placebo-Controlled Phase 3 Study of Adjuvant Selpercatinib in Stage IB-III A RET Fusion-Positive NSCLC. *J Thorac Oncol*. 2021; 16(10): S975–S976, doi: 10.1016/j.jtho.2021.08.262.
29. <https://www.clinicaltrials.gov/ct2/show/NCT04302025>.
30. <https://www.clinicaltrials.gov/ct2/show/NCT04926831>.
31. Yuan M, Huang LL, Chen JH, et al. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. *Signal Transduct Target Ther*. 2019; 4: 61, doi: 10.1038/s41392-019-0099-9, indexed in Pubmed: 31871778.
32. Isla D, Lozano MD, Paz-Ares L, et al. New update to the guidelines on testing predictive biomarkers in non-small-cell lung cancer: a National Consensus of the Spanish Society of Pathology and the Spanish Society of Medical Oncology. *Clin Transl Oncol*. 2022 [Epub ahead of print], doi: 10.1007/s12094-022-03046-9, indexed in Pubmed: 36571695.
33. Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020; 31(11): 1491–1505, doi: 10.1016/j.annonc.2020.07.014, indexed in Pubmed: 32853681.
34. Schrock AB, Frampton GM, Herndon D, et al. Comprehensive Genomic Profiling Identifies Frequent Drug-Sensitive EGFR Exon 19 Deletions in NSCLC not Identified by Prior Molecular Testing. *Clin Cancer Res*. 2016; 22(13): 3281–3285, doi: 10.1158/1078-0432.CCR-15-1668, indexed in Pubmed: 26933124.
35. Suh JH, Schrock AB, Johnson A, et al. Hybrid Capture-Based Comprehensive Genomic Profiling Identifies Lung Cancer Patients with Well-Characterized Sensitizing Epidermal Growth Factor Receptor Point Mutations That Were Not Detected by Standard of Care Testing. *Oncologist*. 2018; 23(7): 776–781, doi: 10.1634/theoncologist.2017-0493, indexed in Pubmed: 29540602.
36. <https://www.clinicaltrials.gov/ct2/show/NCT04302025> (13.04.2023).
37. <https://www.clinicaltrials.gov/ct2/show/NCT04926831> (13.04.2023).
38. Wolff HB, Steeghs EMP, Mfumbilwa ZA, et al. Cost-Effectiveness of Parallel Versus Sequential Testing of Genetic Aberrations for Stage IV Non-Small-Cell Lung Cancer in the Netherlands. *JCO Precis Oncol*. 2022; 6: e2200201, doi: 10.1200/PO.22.00201, indexed in Pubmed: 35834758.
39. Steeghs EMP, Groen HJM, Schuurin Ed, et al. PATH consortium. Mutation-tailored treatment selection in non-small cell lung cancer patients in daily clinical practice. *Lung Cancer*. 2022; 167: 87–97, doi: 10.1016/j.lungcan.2022.04.001, indexed in Pubmed: 35461050.
40. Dall’Olio FG, Conci N, Rossi G, et al. Comparison of Sequential Testing and Next Generation Sequencing in advanced Lung Adenocarcinoma patients - A single centre experience. *Lung Cancer*. 2020; 149: 5–9, doi: 10.1016/j.lungcan.2020.08.008, indexed in Pubmed: 32932213.
41. Kuang S, Fung AS, Perdrizet KA, et al. Upfront Next Generation Sequencing in Non-Small Cell Lung Cancer. *Curr Oncol*. 2022; 29(7): 4428–4437, doi: 10.3390/curroncol29070352, indexed in Pubmed: 35877212.
42. Tsuboi M, Wu YL, Grohe C, et al. Osimertinib as adjuvant therapy in patients with resected EGFR-mutated non-small-cell lung cancer: updated results from ADAURA. *Ann Oncol*. 2022; 33(suppl_7).