Short communication

Pathologic assessment of response to neoadjuvant therapy in melanoma – recommendations for the evaluation of post-resection specimens

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Introduction

Clinical significance of neoadjuvant therapy in stage III melanoma

Neoadjuvant therapy has rapidly emerged as a transformative component of care for patients with stage III melanoma presenting with locoregional metastases, particularly in the setting of borderline resectable or locally advanced disease [1, 2]. What was once considered an investigational strategy is now increasingly integrated into routine clinical practice, driven by compelling evidence demonstrating both biological activity and clinically meaningful benefit.

Prospective phase II and III trials have consistently shown that preoperative systemic therapy – either immune checkpoint blockade (anti-PD-1 monotherapy or combined anti-PD-1/anti-CTLA-4 inhibition) or molecularly targeted therapy with BRAF and MEK inhibitors in BRAF-mutant tumours – induces profound tumour regression in a substantial proportion of patients. Importantly, these responses are not merely radiographic phenomena but are reflected at the histopathologic level.

Across multiple independent studies, achievement of a complete or near-complete pathologic response has been strongly and reproducibly associated with superior clinical outcomes, including prolonged event-free survival and recurrence-free survival. Trials such as NADINA, SWOG S1801, OpACIN-neo, and PRADO have established pathologic response as a robust early surrogate of outcome and a biologically meaningful marker of treatment efficacy [3–6]. These findings provide a strong rationale for response-adapted strategies, in which postoperative management is tailored according to the depth of pathologic response observed in the resected specimen.

In Poland, a regimen consisting of two cycles of neoadjuvant immunotherapy for patients with clinical stage III melanoma is currently reimbursed under the national drug program B.59, reflecting the transition of this approach from experimental to standard-of-care practice in appropriately selected patients.

Challenges in pathologic assessment following neoadjuvant therapy

The introduction of neoadjuvant therapy imposes new demands on the pathologic evaluation of post-resection specimens. The morphologic landscape following immunotherapy or targeted therapy differs substantially from that of untreated metastatic deposits and is characterised by diverse patterns of tumour regression, including fibrosis, necrosis, and treatment-associated inflammatory infiltrates.

Accordingly, standardisation of specimen handling, histologic assessment, and reporting of pathologic response are essential to ensure reproducibility, clinical interpretability, and comparability across institutions [7].

The role of the index lymph node in response assessment

In contrast to sentinel lymph node biopsy performed in patients with clinically node-negative (cN0) disease, therapeutic decision-making and prognostication in patients treated with neoadjuvant therapy depend on evaluation of the index lymph node (ILN).

The index lymph node is defined as the largest lymph node, a clinically suspicious lymph node, or a lymph node previously confirmed to harbour metastatic disease. It represents the reference tumour site for assessment of treatment response.

It is important to emphasise that the concept of the ILN is not synonymous with that of the sentinel lymph node:

- the sentinel lymph node is the first lymph node within the lymphatic drainage basin of the primary tumour and serves primarily a staging function in patients with cN0 melanoma,
- the ILN is the reference node for response assessment in patients with clinically evident nodal metastases (stage III disease).

Although in some cases both terms may refer to the same lymph node, they should not be used interchangeably in the neoadjuvant setting – particularly in patients presenting with macroscopic nodal metastases (Figure 1).

Rationale for reduction protocols

Recent studies have demonstrated that the degree of pathologic response, expressed as the percentage of residual viable tumour (%RVT), represents one of the most powerful prognostic factors in patients treated with neoadjuvant therapy. At the same time, exhaustive sampling of large nodal conglomerates is labour-intensive and not invariably required for reliable assessment of treatment response.

On this basis, so-called reduction protocols have been proposed. These protocols focus detailed histopathologic evaluation on a representative tumour site – most commonly the ILN – while maintaining diagnostic reliability and clinical safety.

It should be acknowledged, however, that treatment response may be spatially heterogeneous within a single tumour bed (TB). Small foci of residual viable melanoma may coexist with areas of complete regression, fibrosis, necrosis, or melanosis. Therefore, reduction protocols are intended to provide a reproducible and clinically practical estimate of response, but they cannot fully eliminate the inherent limitations related to sampling. This limitation is particularly relevant in large TB and should be considered when interpreting borderline response categories.

The validity of a reduction approach depends on the following prerequisites:

- accurate identification of the index lymph node,
- appropriate gross handling and sampling,
- standardised microscopic assessment,
- clear reporting of %RVT and response category.

Purpose of the document

The purpose of these recommendations is to standardise the following:

- gross examination of specimens following neoadjuvant therapy for melanoma,
- principles of tissue sampling from lymph nodes and extranodal sites,
- microscopic assessment of pathologic response,
- reporting of results in routine diagnostic practice.

These recommendations are based on current clinical trial data and international consensus statements, in particular those issued by the Society for Immunotherapy of Cancer, with consideration of national practice patterns [1, 3, 6, 8–10].

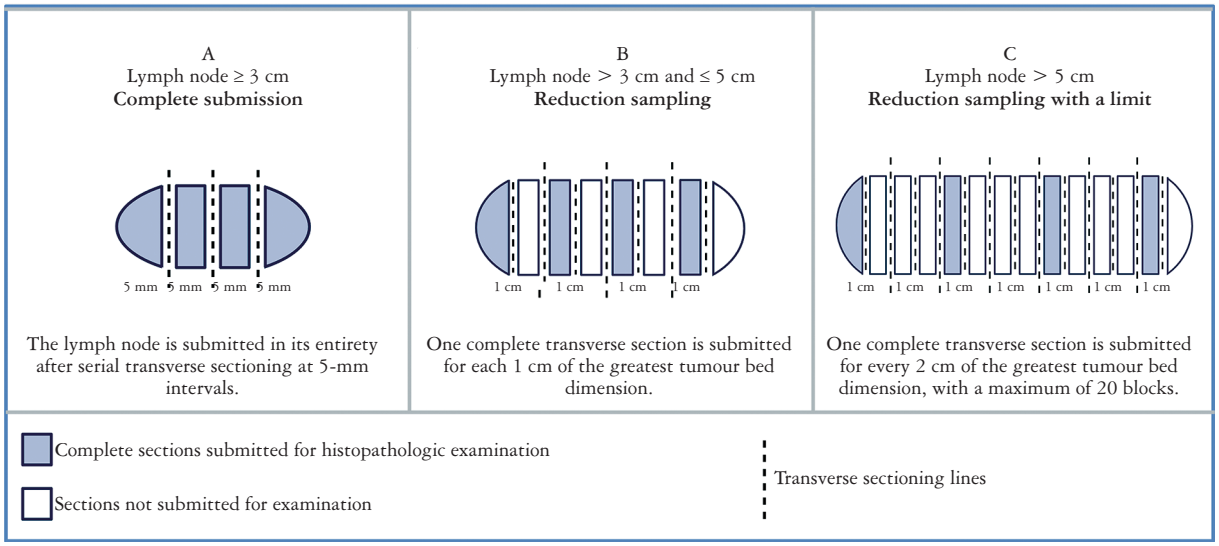

Figure 1. Sampling strategies for index lymph nodes and lymph nodes with macroscopic involvement on gross examination

Scope of application

These recommendations apply to the pathologic evaluation of post-resection specimens from patients with stage III melanoma and clinically evident locoregional metastases treated with neoadjuvant therapy, particularly nodal metastases.

They are primarily intended for surgical specimens that include the following:

- lymph nodes containing a TB, including the index lymph node,
- the primary cutaneous lesion following neoadjuvant therapy,
- in-transit metastases,
- selected visceral metastases resected after preoperative systemic treatment.

Limitations of application

The proposed reduction protocols do not apply to the following:

- conventional sentinel lymph node biopsy in patients with cN0 disease,
- specimens from patients not exposed to neoadjuvant therapy,
- cases in which reliable identification of the ILN is not feasible.

In these situations, standard specimen processing protocols should be followed.

Definitions

Unless otherwise specified, the following definitions apply throughout this document.

Tumour bed

The total area of the lesion following neoadjuvant therapy, encompassing residual foci of viable melanoma cells (i.e. tumour with preserved morphology on haematoxylin staining) as well as areas of regression, including fibrotic and/or fibroinflammatory stroma, aggregates of melanin-laden macrophages (melanophages), and necrosis.

Viable tumour

Melanoma with preserved cellular morphology on haematoxylin staining.

Regression

Treatment-related changes within the TB, including stromal fibrosis and/or a fibroinflammatory component, as well as aggregates of melanin-laden macrophages (melanophages).

Deposit

An area corresponding to a prior focus of melanoma that has undergone complete regression or necrosis and contains no VT cells. In this document, the term *deposit* refers exclusively to lesions lacking VT and is not synonymous with the term *tumour deposit* as defined in the tumour-node-metastasis classification system.

Percentage of residual viable tumour

The percentage of viable melanoma (VT) relative to the total TB area.

$$\%\text{RVT} = \frac{\text{area of VT}}{\text{total area of TB}} \times 100\%$$

In routine practice, %RVT may be estimated by visual assessment on haematoxylin and eosin-stained sections, provided that all components of the TB are considered according to the TB balance principle. When available, morphometric assessment or digital image analysis may be used as adjunctive tools, particularly in borderline cases or for central review; however, these methods are not mandatory for routine reporting. The same measurement approach should be applied consistently within a given case. Because %RVT estimation may be subject to inter-observer variability, especially in cases with extensive fibrosis, necrosis, melanosis, or scattered residual tumour cells, correlation with the overall morphology and, where appropriate, review by a second pathologist is recommended.

Tumour bed balance principle

The sum of all components within the TB equals 100% and includes viable melanoma, regression, and necrosis.

Extranodal extension

The presence of viable melanoma or a deposit in perinodal soft tissue beyond the lymph node capsule.

Immunohistochemistry

Immunohistochemical staining may be used as an adjunct but is not mandatory, in distinguishing melanoma cells from melanophages and from lymphoid cells within germinal centres of lymphoid follicles. The preferred marker is SOX10 (nuclear staining pattern; high sensitivity and specificity). An alternative marker is Melan-A (cytoplasmic staining pattern; associated with a higher risk of false-positive staining in melanophages). Importantly, immunohistochemistry does not permit reliable distinction between necrotic tumour and viable melanoma.

Categories of pathologic response

Based on the %RVT, the following categories of pathologic response (pR) are defined:

- pathologic complete response (pCR): 0% RVT (no viable melanoma identified),
- near pathologic complete response (near-pCR): > 0% to ≤ 10% RVT,
- important: the categories pCR and near-pCR jointly define major pathologic response,
- partial pathologic response: > 10% to ≤ 50% RVT,
- no pathologic response: > 50% RVT.

Gross examination of specimens following neoadjuvant therapy for melanoma

Lymph nodes

Reduction protocols apply exclusively to lymph nodes constituting the TB, including ILN and macroscopically involved lymph nodes.

Identification of the index lymph node

Identification of the ILN is highly recommended. The node is defined as the largest clinically suspicious lymph node or a lymph node previously confirmed to harbour metastatic disease.

The method for identifying the ILN should be discussed and agreed upon with the operating surgeon.

Methods for designating the index lymph node

The following physical methods of marking the ILN before neoadjuvant treatment by the surgeon are acceptable:

- markers (magnetic, nitinol, hydrogel, radioactive I-125 seed),
- surgical clips,
- surgical ink,
- surgical sutures,
- others validated and implemented in routine practice.

The index lymph node and the remaining lymph nodes from the lymphadenectomy specimen should be submitted in separate containers, optimally with no more than one to two marked nodes per container.

The pathology requisition form must clearly specify the method of marking used (e.g. "node marked with surgical clip – ILN").

If the ILN is not identified, the pathologist cannot reliably select lymph nodes during gross examination, creating a risk of a false-negative assessment. In such cases, all lymph nodes should be processed as a potential TB.

Required elements of the gross description

The gross description must include the following:

- the total number of lymph nodes identified,
- the three dimensions of the largest lymph node,
- the three dimensions of the largest TB.

Tissue sampling method for the index lymph node and macroscopically involved lymph nodes

For the index lymph node, and for any additional lymph nodes that are macroscopically involved on gross pathological examination, the following sectioning protocol is recommended:

- sectioning should be performed perpendicular to the long axis of the lymph node,
- each complete cross-section should be clearly labelled, allowing correlation of paraffin blocks with the specific slice. This is particularly important for potential consultations and central case review.

Sampling scheme

A. Lymph nodes ≤ 3 cm in the greatest dimension: Submit entirely after serial sectioning at 5-mm intervals (all slices embedded).

B. Lymph nodes > 3 cm and ≤ 5 cm in the greatest dimension: Submit one complete transverse section for each 1 cm of the greatest dimension of the TB (i.e. every second slice).

C. Lymph nodes > 5 cm in the greatest dimension: Submit one complete transverse section for every 2 cm of the greatest dimension of the TB (i.e. every fourth slice), with a minimum of one complete cross-section. Limit: no more than 20 blocks from the TB per patient.

Note: The sampling scheme is ultimately intended to apply to the TB, which forms the basis for pathologic response assessment. At the gross examination stage, however, precise delineation of the TB may not be feasible; therefore, approximate reference to the greatest dimension of the lymph node is acceptable.

Lymph nodes other than the index lymph node and without macroscopic involvement

Lymph nodes that are neither designated as ILN nor identified as macroscopically involved should be processed grossly according to standard procedures:

- each lymph node should be sectioned perpendicular to its long axis at approximately 5-mm intervals, and each resulting slice should be submitted in a separate, labelled cassette,
- the block legend must clearly indicate that the sections originate from the same lymph node,
- these principles apply to all lymph nodes that are neither designated as ILN nor macroscopically involved, regardless of size.

Specimens from extranodal sites (extranodal tumour bed)

Primary cutaneous lesion and skin/subcutaneous tissue with in-transit metastases

All lesions potentially corresponding to a TB should be identified and described, and three dimensions

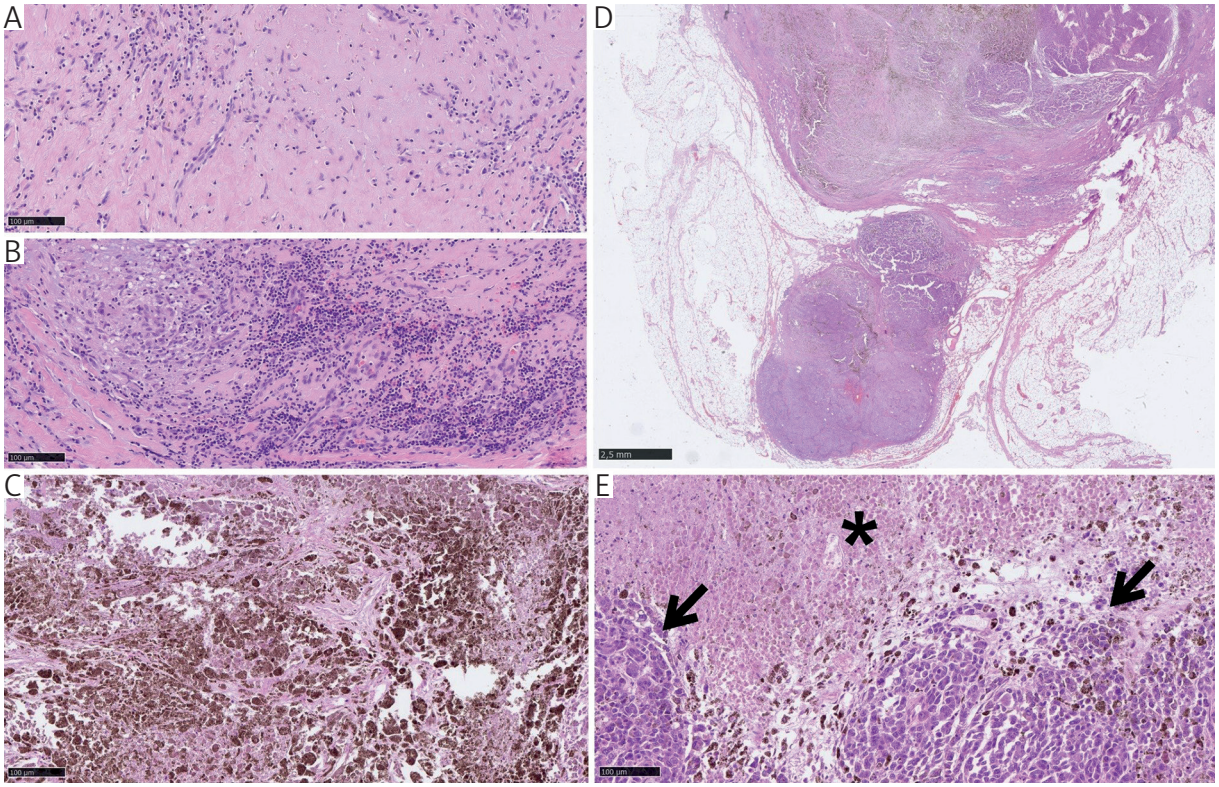

Figure 2. Representative histopathologic findings in a lymph node following neoadjuvant therapy for melanoma. **A**) Advanced collagen fibrosis without unequivocal residual viable tumour. **B**) Dense fibroinflammatory infiltrate within the tumour bed. **C**) Aggregates of melanophages admixed with necrotic melanoma cells. **D**) Low-power view of the lymph node. **E**) Transition zone between necrosis (*) and residual viable melanoma (arrows)

Panels A–C and E show higher-magnification images of selected regions from the lymph node shown in panel D.

of each TB should be recorded. In cutaneous specimens, the presence of residual melanoma – both invasive and *in situ* – should be assessed and appropriately documented.

Surgical margins must be evaluated in relation to the following: all TB; foci of residual invasive melanoma; foci of melanoma *in situ*; and in-transit metastases.

Visceral metastases (lung, liver, other sites)

All lesions potentially corresponding to a TB should be identified, and three dimensions of each TB should be recorded. Surgical margins must be assessed in relation to all TB.

Sampling methods for extranodal sites

Lesions identified as TB should be sampled according to the principles described above for the index lymph node, taking into account the specific anatomic context.

Documentation of sampling sites

It is recommended that documentation be provided to allow unequivocal localisation of submitted tissue sections, including, in particular, specimen photographs, gross diagrams, or – alternatively – a detailed block legend. The documentation should enable correlation of individual paraffin blocks with complete cross-sections and with any additional targeted samples.

Microscopic assessment of specimens following neoadjuvant therapy for melanoma

Microscopic assessment includes evaluation of the TB, with estimation of residual viable melanoma and treatment-related regressive changes, including fibrosis/fibroinflammatory stroma, necrosis, and tumoural melanosis (melanophage-rich areas); representative examples are shown in Figure 2.

Note: If multiple anatomical sites are present within the histologic specimen, a separate microscopic assessment of response to neoadjuvant therapy should be provided for each site, according to the guidelines outlined below.

Discordant nodal responses. In some cases, the ILN may show complete or near-complete response, whereas residual viable melanoma is present in another lymph node, or conversely, the ILN may contain RVT while other lymph nodes show only deposits or complete regression. In such cases, the response in the ILN should be documented separately, but the overall nodal response category should be based on the cumula-

Lymph nodes (applies to index lymph node, macroscopically involved lymph nodes, and other lymph nodes obtained during lymphadenectomy)*

PRESENCE OF VIABLE MELANOMA (VT)	ABSENCE OF VIABLE MELANOMA (VT)
Number of lymph nodes with metastatic involvement: _____ Total number of lymph nodes examined: _____ Largest focus of VT: • dimensions (mm)**: _____ × _____ • location: subcapsular/intraparenchymal (specify) Extranodal extension: yes/no **Summary** %RVT _____ % → category: _____	Number of lymph nodes with deposits: _____ Total number of lymph nodes examined: _____ Largest deposit: • dimensions (mm)**: _____ × _____ • location of the largest deposit: subcapsular/intraparenchymal (specify) Extranodal extension: yes/no **Summary** %RVT = 0% → category: pCR

%RVT – the percentage of residual viable tumour, pCR – pathologic complete response, TB – tumour bed
** In cases where viable tumour and/or deposits are present in more than one lymph node, %RVT should be calculated as a cumulative value:*

Methodological notes

$$\%\text{RVT} = \frac{\sum \text{area of VT}}{\sum \text{area of TB across all ILNs}}$$

*** Use of a uniform measurement scale is recommended, preferably in millimetres (mm).*

Primary cutaneous lesion and skin/subcutaneous tissue with in-transit metastases

PRESENCE OF VIABLE MELANOMA (VT)	ABSENCE OF VIABLE MELANOMA (VT)
Largest focus of VT: • dimensions (mm): _____ × _____ Residual invasive melanoma: • largest dimension: ___ mm, • thickness: ___ mm, • ulceration: yes/no, • mitotic activity: ___/mm^2, • angioinvasion: yes/no, • perineural invasion: yes/no Melanoma *in situ* present: yes/no Surgical margins: assessed with respect to VT and invasive melanoma/melanoma *in situ* **Summary** %RVT _____ % → category: _____ #	Largest deposit: • dimensions (mm): _____ × _____ Melanoma *in situ* present: yes/no Surgical margins: assessed with respect to the deposit and any melanoma *in situ* **Summary** %RVT = 0% → category: pCR

%RVT – the percentage of residual viable tumour, pCR – pathologic complete response
 # In cases with multiple foci of viable tumour (VT) and/or deposits, the percentage of residual VT should be calculated as a cumulative value:

Methodological notes

$$\%\text{RVT} = \frac{\sum \text{area of VT}}{\sum \text{area of all corresponding TB}}$$

Visceral metastases (lung, liver, other sites)

PRESENCE OF VIABLE MELANOMA (VT)	ABSENCE OF VIABLE MELANOMA (VT)
Largest focus of VT: • dimensions (mm): _____ × _____ Angioinvasion: yes/no Perineural invasion: yes/no Surgical margins: assessed with respect to VT **Summary** %RVT _____ % → category: _____ #	Largest deposit: • dimensions (mm): _____ × _____ Surgical margins: assessed with respect to the deposit **Summary** %RVT = 0% → category: pCR

%RVT – the percentage of residual viable tumour, pCR – pathologic complete response
 # In cases with multiple foci of viable tumour (VT) and/or deposits, the percentage of residual VT should be calculated as a cumulative value:

Methodological notes

$$\%\text{RVT} = \frac{\sum \text{area of VT}}{\sum \text{area of all corresponding TB}}$$

tive %RVT calculated across all lymph node TB with VT and/or deposits. The presence of viable melanoma in any lymph node precludes classification of the overall nodal response as pCR, even if the ILN shows complete response.

Conclusions

Accurate pathological assessment after neoadjuvant therapy is essential for reliable response classification, and it should be based on standardised grossing and microscopic evaluation of the TB. This assessment provides clinically relevant information on residual viable melanoma and treatment-induced regression, which may support postoperative risk stratification and further therapeutic decision-making.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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