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REVIEW

Predictive immunohistochemistry in non-small cell lung cancer – Biomarkers, companion diagnostics, and molecular testing: A narrative review

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Immunohistochemistry (IHC) is central to precision oncology in advanced non-small cell lung cancer (NSCLC), although its predictive value and the need for molecular confirmation differ across biomarkers. This narrative review aimed to summarize the clinical utility, validated assays, scoring systems, diagnostic performance, and testing algorithms for predictive IHC biomarkers in NSCLC. Recent guidelines, regulatory documents, and selected analytical and clinical studies were reviewed for programmed death-ligand 1 (PD-L1), anaplastic lymphoma kinase (ALK), ROS proto-oncogene 1 receptor tyrosine kinase (ROS1), mesenchymal–epithelial transition factor receptor (c-Met), B-Raf proto-oncogene serine/threonine kinase V600E (BRAF V600E), pan-tropomyosin receptor kinase (pan-TRK), human epidermal growth factor receptor 2 (HER2), and epidermal growth factor receptor (EGFR). PD-L1 and ALK are established IHC-based predictive assays, although PD-L1 interpretation remains assay-, platform-, and cutoff-specific. VENTANA ALK D5F3 and VENTANA MET SP44 RxDx are clinically validated companion diagnostics, whereas HER2 IHC score 3+ may support eligibility for trastuzumab deruxtecan in the tumor-agnostic setting. ROS1, BRAF V600E, pan-TRK, and non-companion-diagnostic c-Met or HER2 assays are best used for screening or triage and generally require molecular confirmation. EGFR mutation-specific IHC is not recommended because of insufficient diagnostic performance. Overall, IHC enables rapid, tissue-sparing biomarker assessment but should be integrated with broad genomic and transcriptomic next-generation sequencing (NGS), particularly when alteration-specific findings are negative, equivocal, or discordant.

Keywords: Non-small cell lung cancer, biomarkers, precision oncology, immunohistochemistry.

Introduction

Lung cancer is the most prevalent cancer globally, accounting for approximately 2.5 million new cases and 1.8 million deaths annually, making it the leading cause of cancer-related mortality worldwide [1–4]. Non-small cell lung cancer (NSCLC) constitutes the majority of diagnoses (80%–85%), with over 70% of cases presenting as metastatic at initial diagnosis [5]. The sole approved early-stage lung cancer screening method is annual low-dose computed tomography (LDCT) for individuals aged 50–80 with a smoking history of more than 20 pack-years, who are current smokers or have quit within the last 15 years. Large-scale trials, such as The National Lung Screening Trial (NLST) and NELSON, have demonstrated that LDCT reduces lung cancer mortality by approximately 20%–24%, primarily through the detection of Stage I cancers [6–9].

Significant advancements in the molecular characterization of NSCLC, particularly in adenocarcinoma subtypes, have led to the identification of critical genetic aberrations amenable to molecularly targeted therapies [10–12]. Genetic alterations in epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase *(ALK)*, ROS proto-oncogene 1, receptor tyrosine kinase *(ROS1)*, ret proto-oncogene *(RET)*, V-Raf murine sarcoma viral oncogene homolog B *(BRAF)*, KRAS proto-oncogene, GTPase *(KRAS)*, human epidermal growth factor receptor 2 *(HER2)*/erb-b2 receptor tyrosine kinase 2 *(ERBB2)*, and neurotrophic receptor tyrosine kinase (*NTRK*) are strong predictors of sensitivity to targeted treatments [11–18]. However, these alterations are detected in only about 30% of all NSCLC patients, though this increases to 50%–60% in patients with pulmonary adenocarcinomas [19]. Despite the remarkable success of molecularly targeted treatments, most NSCLC patients eventually develop acquired resistance and disease progression [20–23]. Nevertheless, these advancements have substantially improved NSCLC survival rates, rising from 12% in the 1970 s to approximately 30% between 2015 and 2021 [24], with further improvements anticipated. Small-cell lung cancer (SCLC), another subtype comprising 10%–15% of all lung cancers, remains a highly aggressive disease with limited targeted treatment options and an overall 5-year survival rate of less than 10% [24–27].

Molecular profiling is indispensable in the management of advanced NSCLC patients [28–30]. Various molecular tests

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are available, including next-generation sequencing (NGS), PCR-based assays, fluorescence in situ hybridization (FISH), and immunohistochemistry (IHC). These methods can be applied to tissue-based samples or, more recently, to liquid biopsies (circulating tumor DNA, ctDNA) to identify specific DNA mutations, gene rearrangements, gene fusions, and protein expression patterns [31–34].

IHC has long been a cornerstone in anatomic pathology [35, 36], primarily for diagnostic purposes. In lung pathology, for instance, it is used to detect thyroid transcription factor 1 (TTF-1) and Napsin-A positivity in adenocarcinomas, p63 and p40 positivity in squamous cell carcinomas, and neuroendocrine markers such as synaptophysin, chromogranin-A, and CD56 in small cell carcinomas. However, advances in precision oncology have significantly expanded its utility, enabling the assessment of predictive biomarkers across various cancers. Examples include steroid receptors, HER2, and Ki-67 in breast cancer; programmed death-ligand 1 (PD-L1) in multiple tumor types; HER2 in gastric and gastroesophageal carcinomas; and microsatellite instability/mismatch repair proteins in colorectal and endometrial carcinomas [37–39]. Similarly, in NSCLC, IHC has gained prominence as a valuable tool for evaluating predictive biomarkers (summarized in Table 1). Its advantages include widespread availability, pathologist familiarity, rapid turnaround time, cost-effectiveness, and general applicability to formalin-fixed, paraffin-embedded (FFPE) tissue specimens. It can also be effectively utilized with small tumor samples, including cytology specimens (cell blocks or smears). This review offers a concise and well-illustrated overview of key predictive biomarkers in NSCLC that can be successfully assessed using IHC alone or in combination with other ancillary techniques. This narrative review draws upon recent guidelines, regulatory documents, and selected studies evaluating clinically relevant IHC biomarkers in advanced NSCLC, with an emphasis on validated assays, predictive performance, and practical integration with molecular testing.

PD-L1 status and assessment

The programmed cell death protein 1 (PD-1)/PD-L1 pathway represents an adaptive immune-resistance mechanism through which cancers evade endogenous antitumor responses [40]. PD-1 is typically expressed on the surface of activated T-lymphocytes, while its ligands, PD-L1 and programmed death-ligand 2 (PD-L2), are expressed on the surface of dendritic cells, macrophages, and cancer cells [41–45].

PD-L1 is a well-established predictive biomarker for immune checkpoint inhibitors (ICIs) in NSCLC and various other cancers (e.g., breast, gastric/gastroesophageal, bladder). However, PD-L1 is not considered a standalone "master predictor," as a proportion of PD-L1-positive cases may not respond to ICIs, and vice versa [46–48]. Furthermore, several additional predictive biomarkers for ICIs have been validated, including tumor mutational burden (TMB) and microsatellite instability-high (MSI-H) status, both of which are also tumor-agnostic biomarkers in precision oncology. In some cancers (e.g., triple-negative breast cancer, MSI-H colorectal carcinoma, cutaneous malignant melanoma), tumor-infiltrating lymphocytes (TILs) may also predict the response to ICIs.

PD-L1 expression in NSCLC is assessed by IHC on tumor tissue samples using validated and clinically approved assays (Table 1). It has been reported in 50%–60% of NSCLC cases (higher in squamous cell carcinoma than in adenocarcinomas), with approximately 20%–30% of cases exhibiting high (>50%) PD-L1 expression [49]. The results of IHC testing are routinely interpreted alongside other clinicopathologic parameters (e.g., tumor histology, grade, stage) and molecular genomic biomarkers (e.g., *EGFR, ALK, ROS1, RET, KRAS*). A recent joint lung cancer guideline (2024) emphasizes that, in advanced NSCLC cases, pathologists should utilize a validated PD-L1 IHC assay and integrate testing with genomic biomarker workup where appropriate [50]. This integration is crucial because PD-L1 guides the use of ICIs, while oncogenic drivers such as *EGFR* or *ALK* may redirect first-line therapy toward targeted agents [50]. The recent European Society for Medical Oncology (ESMO) guideline (2025) also recommends PD-L1 testing prior to treatment decision-making in patients with Stage II-III NSCLC being considered for pre- or perioperative chemo-immunotherapy. Additionally, PD-L1 testing is recommended for all resected Stage II-IIIA NSCLC cases if preoperative chemo-immunotherapy is not administered, to inform adjuvant chemo-immunotherapy options [51].

PD-L1 testing in NSCLC is conceptually straightforward but practically complex. The major PD-L1 assays are not merely different antibody clones; they represent complete assay systems linked to specific platforms (Ventana and Dako/Agilent), detection methods, scoring systems, cutoffs, and therapeutic indications [52]. The most widely used assays include 22C3 and 28–8 antibodies on the Agilent/Dako platform, and SP142 and SP263 antibodies on the Ventana/Roche platform (Table 1). In practice, 22C3 is most closely associated with pembrolizumab-based treatment and is assessed using the tumor proportion score (TPS), defined by membranous PD-L1 expression in viable tumor cells (Figures 1 and 2). The 28–8 assay is also primarily interpreted using membranous expression in cancer cells and has been employed in nivolumab-related treatment settings. SP263 is likewise commonly applied using tumor-cell scoring cutoffs. In contrast, SP142 has a distinctive staining and scoring profile, incorporating both tumor-cell and immune-cell assessment in specific atezolizumab-related settings. The SP142 clone is also recognized to stain fewer tumor cells than the other three clones. Therefore, SP142 should not be considered analytically equivalent to the other major assays for tumor-cell TPS-based interpretation. Consequently, current regulatory status should always be verified against the relevant assay and drug label, as companion diagnostics (CDx) indications are therapy- and context-specific.

Analytical comparison studies have demonstrated relatively high concordance among 22C3, 28–8, and SP263 antibodies for tumor-cell PD-L1 expression, whereas SP142 and other clones (73–10 and E1L3N) generally show lower tumor-cell staining [reviewed in [53]]. This has led to the practical understanding that 22C3, 28–8, and SP263 are analytically

Table 1. Summary of common predictive biomarkers tested by IHC in non-small cell lung carcinoma

Biomarker	Clone/Assay	Cutoff/interpretation	Drug(s)	Regulatory status and notes
PD–L1	22C3 pharmDx	TPS ≥1%; TPS ≥ 50% in high-expression contexts	Pembrolizumab; cemiplimab contexts	FDA CDx in NSCLC; indication/cutoff depends on drug and regimen
	28–8 pharmDx	Tumor–cell staining ≥ 1%	Nivolumab + ipilimumab	FDA CDx in NSCLC for Opdivo + Yervoy context
	VENTANA PD–L1 (SP263)	TC ≥ 1% or TC ≥ 50%, context-specific	Atezolizumab; cemiplimab contexts	FDA CDx in selected NSCLC indications; also used in durvalumab/CE–IVD contexts
	VENTANA PD–L1 (SP142)	TC ≥50% or IC ≥10%	Atezolizumab	FDA CDx in NSCLC; lower tumor-cell staining than 22C3/28-8/SP263
ALK	VENTANA ALK D5F3	Strong granular cytoplasmic staining in tumor cells	ALK inhibitors	FDA CDx for crizotinib, ceritinib, alectinib, lorlatinib
c-Met protein expression	VENTANA MET SP44 RxDx	High: ≥ 50% tumor cells with strong 3+ membranous and/or cytoplasmic staining	Telisotuzumab vedotin-tllv/ Emrelis	FDA CDx for previously treated locally advanced/metastatic non-squamous NSCLC with high c-Met protein overexpression; not a surrogate for MET exon 14 skipping or *MET* amplification
HER2 protein expression	VENTANA anti-HER2/neu (4B5) or other validated assay (e.g., HercepTest)*	IHC score 3+	Fam-trastuzumab deruxtecan-nxki/Enhertu	FDA accelerated tumor-agnostic indication for HER2-positive IHC 3+ solid tumors after prior therapy/no satisfactory alternatives; no FDA-approved pan-tumor HER2 IHC 3+ test currently available; not a surrogate for *HER2* mutation
ROS1	ROS1 D4D6 or SP384	Cytoplasmic staining; threshold depends on local validation	Crizotinib, entrectinib, repotrectinib, taletrectinib	IHC screening only; not an FDA CDx. Positive/equivocal cases require confirmation by FISH or, preferably, RNA-based NGS. FDA-listed molecular CDx includes Oncomine Dx Target Test for crizotinib and FoundationOne CDx/Liquid CDx for entrectinib.
BRAF V600E	VE1	Unequivocal cytoplasmic tumor-cell staining	Dabrafenib + trametinib; encorafenib + binimetinib	IHC screening/triage only; not FDA CDx in NSCLC. Detects V600E only; non-V600E BRAF mutations are missed. Confirm by PCR or NGS before therapy.
NTRK fusion/pan-TRK	pan-TRK EPR17341 or A7H6R	Any convincing tumor-cell staining, nuclear, cytoplasmic, or membranous patterns may occur	Larotrectinib, entrectinib, repotrectinib	IHC screening only; not FDA CDx. Sensitivity is variable in common tumors and may be lower for some *NTRK3* fusions; confirm by RNA-based NGS or another molecular fusion assay.

*HercepTest (Agilent/Dako) served as the primary confirmatory assay in the DESTINY-PanTumor02 study, a pivotal trial supporting the pan-tumor indication. However, its use within the trial does not equate to Food and Drug Administration companion diagnostics (CDx) authorization. **Abbreviations:** ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene serine/threonine kinase; CDx, companion diagnostic; CE-IVD, Conformité Européenne *in vitro* diagnostic; c-Met, mesenchymal–epithelial transition factor receptor; FDA, Food and Drug Administration; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IC, immune cells; IHC, immunohistochemistry; MET, MET proto-oncogene receptor tyrosine kinase; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine receptor kinase; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; RNA, ribonucleic acid; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase; TC, tumor cells; TPS, tumor proportion score.

similar but not perfectly interchangeable, especially near clinically important thresholds such as 1% and 50%. These borderline categories are precisely where small differences in staining intensity, tumor sampling, fixation, section quality, or pathologist interpretation can substantially affect therapeutic eligibility. The problem is exacerbated when immune-cell scoring is required, as immune-cell assessment is generally more subjective than tumor-cell TPS and can be influenced by inflammation, necrosis, crush artifact, and the distinction between intratumoral, stromal, and peritumoral immune cells. For these reasons, laboratories should avoid informal clone substitution unless they have performed proper local validation

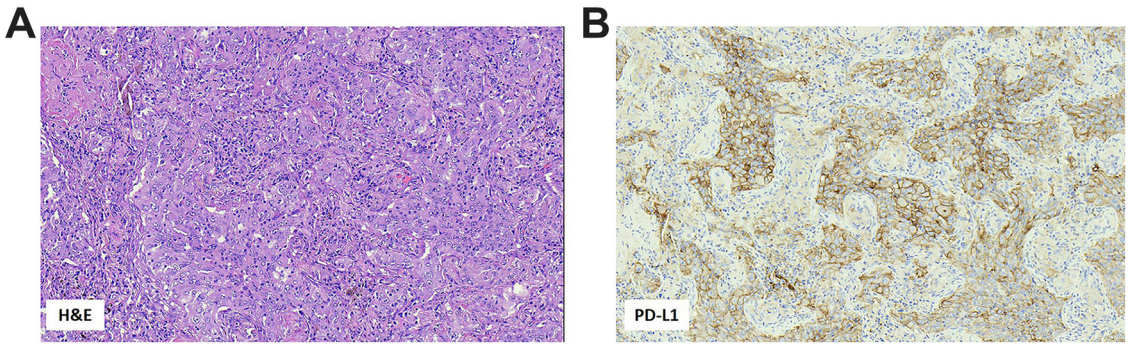


Figure 1. Histomorphologic features and programmed death-ligand 1 expression in high-grade pulmonary adenocarcinoma. (A) Hematoxylin and eosin (H&E) staining shows a high-grade pulmonary adenocarcinoma with a predominantly solid growth pattern and marked cytologic atypia. (B) Programmed death-ligand 1 (PD-L1) immunohistochemistry using the 22C3 pharmDx assay (Agilent/Dako) demonstrates membranous staining in more than 50% of viable tumor cells, consistent with high PD-L1 expression based on a tumor proportion score (TPS) of ≥50%. Original objective magnification, ×20. **Abbreviations**: H&E, hematoxylin and eosin; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

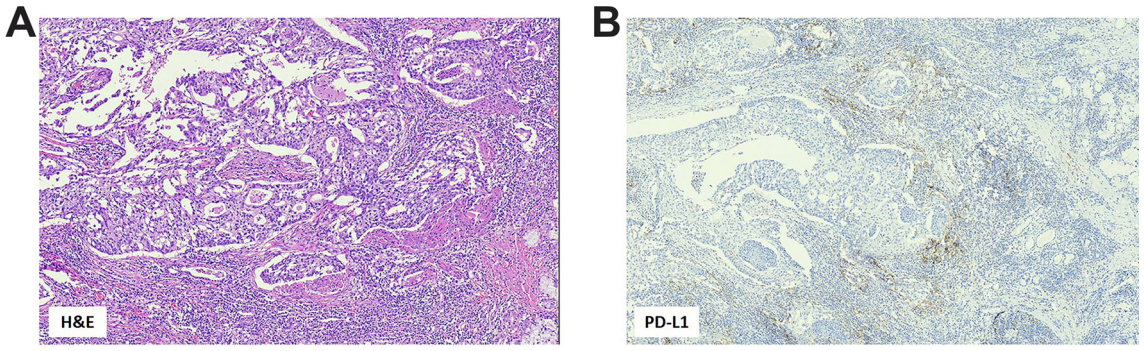


Figure 2. Histomorphologic features and PD-L1 immunoreactivity in moderately differentiated pulmonary adenocarcinoma. (A) H&E staining shows a moderately differentiated pulmonary adenocarcinoma with gland-forming architecture and a prominent inflammatory stromal infiltrate. (B) PD-L1 IHC using the 22C3 pharmDx assay (Agilent/Dako) shows immunoreactivity in TILs and macrophages but no convincing membranous staining in viable tumor cells. Because immune-cell staining is not included in TPS determination with this assay, the tumor is classified as PD-L1 negative (TPS <1%). Original magnification, ×20. **Abbreviations:** H&E, hematoxylin and eosin; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; TILs, tumor-infiltrating lymphocytes; TPS, tumor proportion score.

against a clinically validated assay and should report results using the scoring system appropriate to the assay and clinical indication.

Several preanalytical and interpretive issues are particularly important in routine lung cancer practice [54]. Many patients with advanced NSCLC are diagnosed via small biopsies or cytology cell blocks, leading to competition between PD-L1 testing and molecular profiling for limited tissue. While small biopsies are acceptable, intratumoral heterogeneity means that a small sample may not fully represent PD-L1 expression across the entire tumor. Cell blocks can also be used when properly validated, but laboratories must validate PD-L1 testing for each specimen type and fixative employed in their practice. Poor fixation, decalcification, low tumor cellularity, extensive necrosis, scant viable tumor cells, and crush artifact can significantly compromise PD-L1 scoring and interpretation. A recent joint guideline also emphasizes the use of validated PD-L1 IHC assays, appropriate validation for specimen types and fixatives, and the use of clinically validated assays as intended whenever feasible [50].

As with all IHC assays, the interpretation of PD-L1 is inherently subjective. Interobserver variability remains a recognized challenge, particularly in borderline cases and when differentiating weak, incomplete staining from true membranous positivity. Consequently, robust quality assurance measures, comprehensive pathologist training, participation in external quality assessment schemes, and meticulous review of cases near clinical cutoffs are imperative. A pragmatic reporting strategy should encompass the assay/clone utilized, platform (if applicable), specimen type, adequacy of tumor cellularity, scoring methodology, percentage score, and any limitations related to sample quality. Artificial intelligence (AI)-assisted tools may significantly enhance PD-L1 expression scoring in NSCLC, especially in complex and ambiguous cases, a novel approach supported by numerous studies [55–62].

Overall, PD-L1 IHC remains a critical predictive biomarker in NSCLC. However, its reliable application necessitates assay-specific interpretation, acknowledging that clones such as 22C3, 28–8, SP142, and SP263 are not universally interchangeable [63].

PD-L1 serves as a useful, albeit imperfect, predictive biomarker. Elevated PD-L1 expression in NSCLC frequently correlates with an improved response to PD-1/PD-L1 blockade, particularly within the TPS ≥50% subgroup. Nevertheless, responses can occur in tumors with lower expression, and some high-expressing tumors may not benefit. Similar to

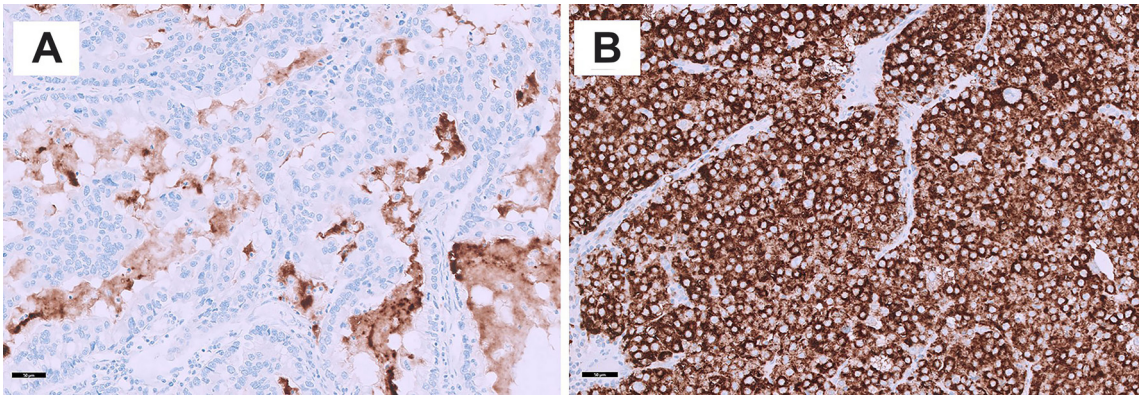


Figure 3. ALK IHC patterns and corresponding molecular findings in two pulmonary adenocarcinomas. (A) The first tumor shows no specific ALK expression in cancer cells using the VENTANA ALK (D5F3) assay, and subsequent NGS detected no *ALK* gene fusion. (B) The second tumor demonstrates diffuse, strong (3+), granular cytoplasmic ALK expression in cancer cells, consistent with a positive ALK IHC result; NGS subsequently confirmed an *EML4::ALK* fusion. **Abbreviations:** ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; IHC, immunohistochemistry; NGS, next-generation sequencing.

other predictive biomarkers, PD-L1 expression can fluctuate considerably between primary and metastatic sites and may be influenced by prior therapies and tumor heterogeneity [64–66]. Therefore, PD-L1 should not be interpreted in isolation. The optimal strategy involves integrated biomarker testing: PD-L1 IHC for immunotherapy selection, combined with broad molecular profiling (e.g., NGS) to identify actionable driver genomic alterations, thereby preventing exclusive reliance on immunotherapy in oncogene-addicted NSCLC cases (comprising 60%–70% of all cases) [67, 68].

In summary, three antibody clones (22C3, 28–8, and SP263) primarily function as TPS/tumor-cell assays, whereas the SP142 clone employs a distinct tumor-cell-plus-immune-cell (TC-plus-IC) method, rendering it the least interchangeable with the other three PD-L1 assays.

ALK status and assessment

The *ALK* gene encodes a receptor tyrosine kinase crucial for central nervous system development [69]. While largely inactive in adults, *ALK* rearrangements (fusions), mutations, or amplifications drive oncogenesis in several cancers, most notably NSCLC, anaplastic large-cell lymphomas (ALCL), neuroblastomas, and inflammatory myofibroblastic tumors [70–76].

ALK fusions are established actionable driver alterations in NSCLC, present in approximately 2%–7% of cases, predominantly in pulmonary adenocarcinomas [73]. The most common fusion partner is echinoderm microtubule-associated protein-like 4 (*EML4*), though over 20 different *ALK* gene partners have been described [77]. In these rearrangements, the ALK kinase domain is typically retained, generating an oncogenic fusion protein that promotes tumor growth and predicts sensitivity to ALK tyrosine kinase inhibitors. *ALK* gene fusions are clinically significant as they can be targeted with ALK inhibitors (e.g., crizotinib, alectinib) (Table 1), which block the activity of the abnormal ALK protein and substantially improve outcomes for NSCLC patients with *ALK* fusions [70, 73, 78, 79].

IHC for ALK protein expression plays a vital role in routine NSCLC workup because fusion-positive NSCLCs exhibit aberrant ALK protein expression detectable in FFPE samples rapidly and with minimal tissue requirements (small biopsies). Given the high sensitivity and specificity of the ALK IHC assay, the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology (CAP/IASLC/AMP) guidelines endorse ALK IHC as an equivalent alternative to molecular assays for ALK assessment (e.g., FISH or NGS) [80, 81] (Figure 3A–B). The VENTANA ALK (D5F3) IHC assay is a Food and Drug Administration (FDA)-approved CDx test for ALK testing in NSCLC (Table 1). With this assay, any percentage of cancer cells demonstrating strong (score 3+) granular cytoplasmic staining is interpreted as positive (Figure 3B). A broader, RNA-based NGS or another orthogonal assay should always be employed when results are equivocal, discordant, or when comprehensive molecular and fusion profiling is required.

ROS1 status and assessment

The *ROS1* gene, a proto-oncogene located on chromosome 6, encodes a receptor tyrosine kinase that functions as a transmembrane signaling protein [82, 83]. While its physiological role remains to be fully elucidated, ROS1 protein expression is highest in normal kidneys, cerebellum, peripheral neural tissues, stomach, small intestine, and colon, and absent in normal lungs [82].

ROS1 genomic alterations, such as chromosomal rearrangements where the 3' kinase domain of the *ROS1* gene on chromosome 6 fuses with a partner gene (e.g., CD74, ezrin [EZR], solute carrier family 34 member 2 [SLC34A2]), are oncogenic. These alterations have been reported in 1%–2% of lung adenocarcinomas and tend to be mutually exclusive with other major genomic alterations, such as *EGFR* mutations and *ALK* rearrangements [83–85]. *ROS1* alterations also appear more prevalent in women and never-smokers and are associated with advanced disease presentation [86].

CD74-ROS1 is the most common fusion in NSCLC [87]. *ROS1* fusions have also been identified in other common cancers, including cholangiocarcinomas, colorectal carcinomas, gastric carcinomas, ovarian carcinomas, epithelioid hemangioendotheliomas [83, 88–90], angiosarcomas, glioblastomas, and inflammatory myofibroblastic tumors [91–93]. The fusion activates the ROS1 protein, which subsequently

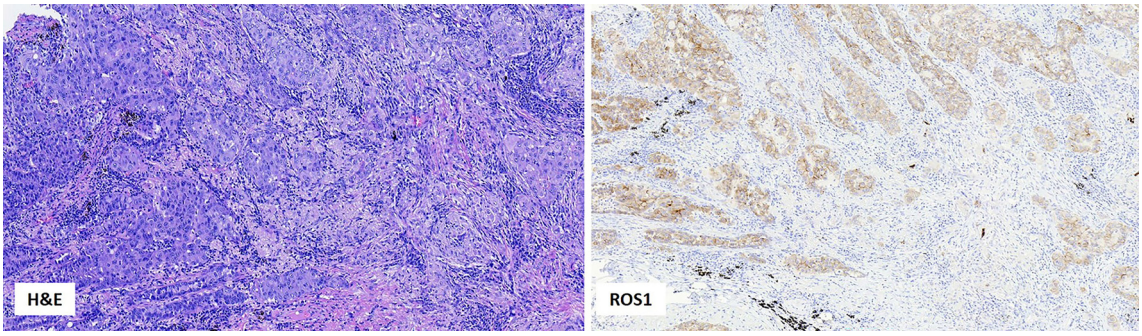


Figure 4. Histomorphologic and ROS1 IHC findings in high-grade pulmonary adenocarcinoma harboring an *EZR::ROS1* fusion. H&E staining shows a high-grade pulmonary adenocarcinoma with predominantly solid architecture and marked cytologic atypia (left). ROS1 IHC using rabbit mAb #3287 (Cell Signaling Technology) demonstrates diffuse, moderate (2+) cytoplasmic staining in >90% of tumor cells (right). Subsequent NGS confirmed an *EZR::ROS1* fusion. Original magnification, ×20. **Abbreviations:** *EZR*, ezrin; H&E, hematoxylin and eosin; IHC, immunohistochemistry; mAb, monoclonal antibody; NGS, next-generation sequencing; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase.

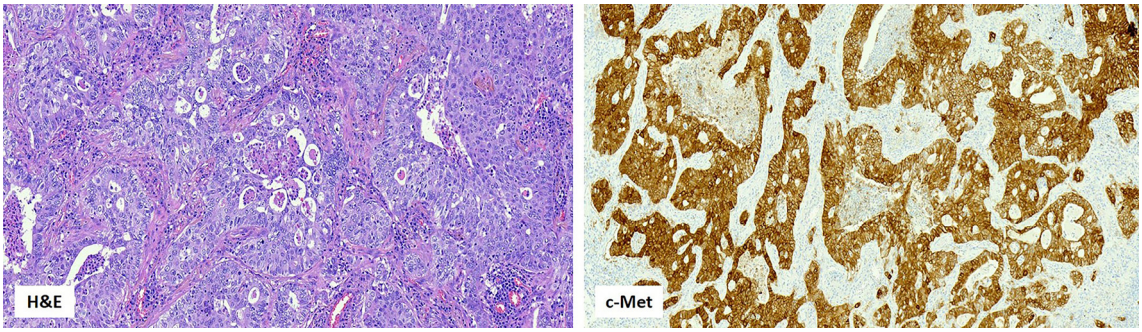


Figure 5. High c-Met protein expression without corresponding *MET* genomic alterations in pulmonary adenocarcinoma. H&E staining shows a moderately differentiated pulmonary adenocarcinoma with gland-forming architecture (left). c-Met IHC using the VENTANA MET (SP44) assay demonstrates diffuse, strong (3+) membranous and cytoplasmic staining in >90% of tumor cells (right). Subsequent NGS detected neither a *MET* exon 14 skipping alteration nor *MET* amplification, illustrating that c-Met protein overexpression is not a reliable surrogate for *MET* genomic alterations. Original magnification, ×20. **Abbreviations:** c-Met, mesenchymal–epithelial transition factor receptor; H&E, hematoxylin and eosin; IHC, immunohistochemistry; *MET*, MET proto-oncogene receptor tyrosine kinase; NGS, next-generation sequencing.

activates multiple downstream signaling pathways, including phosphoinositide 3-kinase/protein kinase B pathway (PI3K/AKT), mitogen-activated protein kinase/extracellular signal-regulated kinase pathway (MAPK/ERK), and Janus kinase/signal transducer and activator of transcription 3 pathway (JAK/STAT3), all implicated in sustained cancer cell survival and proliferation.

ROS1 rearrangements are clinically relevant due to their association with higher therapeutic responses to targeted therapies such as crizotinib and entrectinib [83, 94]. However, resistance may arise from secondary mutations in the *CD74-ROS1* fusion [95–97].

Similar to other genomic alterations, *ROS1* rearrangements can be detected using FISH, PCR, or more comprehensively, NGS [98]. Davies et al. compared the diagnostic utility of break-apart FISH, DNA-based hybrid capture library preparation followed by NGS, and RNA-based anchored multiplex PCR library preparation followed by NGS [98]. None of the three assays achieved 100% sensitivity in detecting *ROS1* alterations, including the NGS assay. A ROS1 IHC assay has been developed, demonstrating excellent sensitivity (90%–100%) and specificity (85%–94%) for *ROS1* rearrangements in NSCLC, making it a highly effective screening tool for identifying *ROS1* rearrangements in lung adenocarcinomas [99–102] (Table 1). The 2018 CAP/IASLC/AMP guideline strongly recommends routine ROS1 testing "on all lung adenocarcinoma patients, irrespective of clinical characteristics" [80]. Our experience with tissue microarray assay also supports the high predictive value of ROS1 IHC (Rabbit mAb #3287 by Cell Signaling) for identifying ROS1-fused lung adenocarcinomas [103] (Figure 4). Although ROS1 IHC is highly useful for initial detection of *ROS1* fusions in NSCLC, IHC-positive cases should be molecularly confirmed (NGS) before initiating targeted therapy [80].

Hepatocyte growth factor receptor (c-Met) status and assessment

MET is a proto-oncogene located on chromosome 7q31 [104]. The *MET* gene encodes a receptor tyrosine kinase (RTK, c-Met) that binds hepatocyte growth factor (HGF), thereby regulating various cellular processes, including cell growth, proliferation, motility, and survival [105]. Aberrant *MET* signaling has been described in multiple cancers, such as gastric, colorectal, hereditary papillary renal cell carcinomas (OMIM #605074), gliomas, metastatic uveal melanoma, and sarcomas; however, its most prominent role is in NSCLC [106–108].

Aberrant activation of *MET* in NSCLC involves several oncogenic mechanisms, including *MET* exon 14 (*MET*ex14) skipping

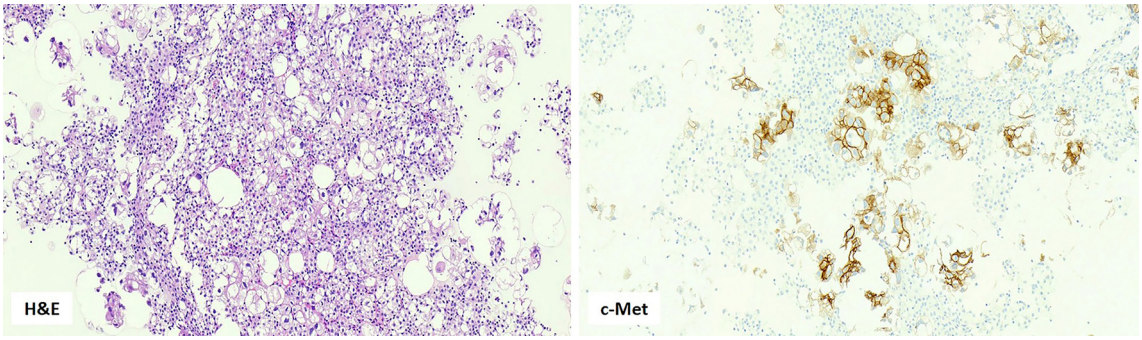


Figure 6. c-Met protein overexpression in pulmonary adenocarcinoma evaluated in a cell-block preparation. H&E staining shows cohesive clusters of malignant epithelial cells with glandular differentiation and abundant vacuolated cytoplasm (left). c-Met IHC using the VENTANA MET (SP44) assay demonstrates strong (3+) membranous and cytoplasmic staining in tumor-cell clusters, consistent with c-Met protein overexpression (right). Original magnification, ×10. **Abbreviations:** c-Met, mesenchymal–epithelial transition factor receptor; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

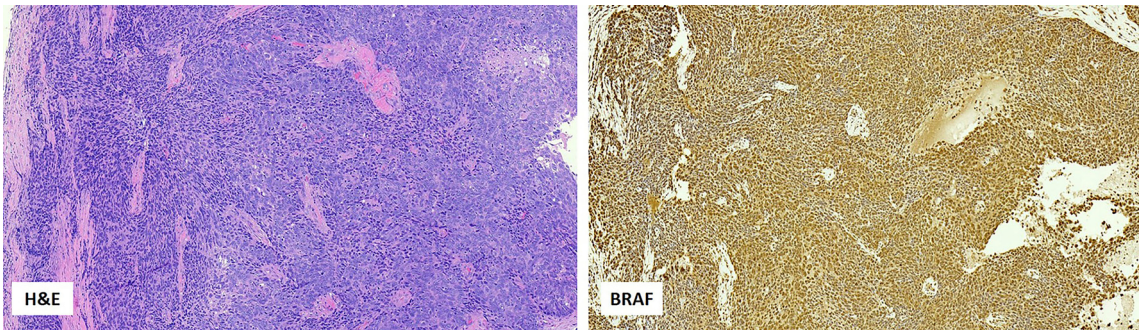


Figure 7. BRAF V600E protein expression in high-grade pulmonary squamous cell carcinoma. H&E staining shows a high-grade pulmonary squamous cell carcinoma with predominantly solid architecture and marked cytologic atypia (left). BRAF V600E IHC using the VE1 clone (VENTANA) demonstrates diffuse, strong cytoplasmic staining in tumor cells (right). Subsequent NGS confirmed a *BRAF* p.V600E mutation. Original magnification, ×10. **Abbreviations:** *BRAF*, B-Raf proto-oncogene serine/threonine kinase; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NGS, next-generation sequencing.

mutations (2%–4% of all NSCLC alterations), *MET* gene amplification (2%–5%), *MET* fusions (<1%), and recently recognized c-Met protein overexpression (13%–25% of NSCLC, depending on the cutoff value for overexpression) [109–111]. *MET*ex14 skipping mutations are clinically significant, given the substantial potential of MET inhibitors such as capmatinib (Tabrecta) and tepotinib (Tepmetko), both approved as first-line treatments for patients with MET-mutated NSCLC [112, 113]. Additionally, some multikinase inhibitors, such as crizotinib, demonstrate significant activity in MET-mutated NSCLC [114].

Conversely, c-Met protein overexpression frequently arises from transcriptional regulation rather than from genomic alterations within the *MET* gene (Figures 5 and 6). This distinction gained clinical significance following the FDA's accelerated approval of telisotuzumab vedotin-tllv (EMRELIS™). This antibody-drug conjugate (ADC) is indicated for adult patients with previously treated, locally advanced/metastatic NSCLC exhibiting high c-Met protein overexpression. The approval was based on compelling data from the LUMINOSITY trial, which defined high c-Met expression as positivity in ≥50% of cancer cells with 3+ intensity, demonstrating significant and durable clinical responses in pretreated patients [115, 116]. The LUMINOSITY trial initially established a cutoff for c-MET-overexpressing NSCLC as positivity in ≥25% of tumor cells with strong (3+) IHC staining, with high overexpression specifically defined as positivity in ≥50% of cancer cells with strong (3+) IHC staining [115]. Concurrently with the drug, the VENTANA MET (SP44) RxDx Assay received approval as a CDx IHC test [117] (Figures 5 and 6).

Several other clones are available for IHC assessment of c-Met protein expression, including the D1C2 rabbit monoclonal antibody (Cell Signaling Technology), anti-Met (c-Met) antibody EPR19067 (Abcam), and various anti-c-Met antibodies from ThermoFisher Scientific. The accurate evaluation of c-Met protein expression in NSCLC is complicated by the diversity of anti-Met clones and the absence of standardized scoring systems for defining c-Met protein overexpression [reviewed in [109]]. Beyond the approved VENTANA MET SP44 RxDx/telisotuzumab vedotin context, a universally accepted cutoff for c-Met protein overexpression in NSCLC does not exist. The use of different antibodies and scoring methodologies across studies likely contributes to the variability in reported prevalence and clinical correlations.

It is crucial to recognize that, despite its rapidity and cost-effectiveness, IHC assessment of c-Met protein expression is not a reliable surrogate for *MET* genomic alterations such as *MET* amplification or exon 14 mutations. Multiple studies have confirmed a lack of correlation between underlying *MET* genomic alterations and c-Met protein expression [118]. While the field is evolving rapidly, a unified approach to c-Met protein

assessment and scoring is anticipated. Nevertheless, IHC-based evaluation of c-MET expression may remain a valuable complementary predictive tool in cases lacking detectable *MET* genomic alterations.

BRAF V600E status and assessment

The *BRAF* kinase, encoded by *the BRAF* gene, plays a critical role in cellular processes such as proliferation, growth, and survival [119]. Consequently, mutations in the *BRAF* gene are implicated in cancer development and progression [119, 120]. *BRAF* mutations are prevalent in various malignancies, including hairy cell leukemias (100%), metanephric adenomas (90%), cutaneous malignant melanomas (40%–50%), papillary thyroid carcinomas (50%), systemic histiocytosis (40%–70%), and colorectal adenocarcinomas (10%–15%).

In NSCLC, *BRAF* mutations occur in 1%–3% of cases, without significant differences based on sex or smoking status [121, 122]. The *BRAF V600E* mutation is the most common subtype, accounting for 30%–50% of *BRAF* mutations in NSCLC, with numerous non-V600E mutations also described in NSCLC [121] and other cancers, notably cutaneous malignant melanoma [123, 124]. The development of BRAF inhibitors, often combined with mitogen-activated protein kinase kinase (MEK) inhibitors, addresses anti-BRAF resistance mediated by MEK/MAPK activation [125]. A combined BRAF/MEK inhibitor regimen (dabrafenib/trametinib) was initially approved for melanoma patients [126] and recently received tumor-agnostic approval (2022) for all metastatic solid cancers harboring *BRAF V600E* mutations [120, 127].

BRAF mutations can be detected through NGS, allele-specific PCR assays (e.g., Cobas® 4800), and IHC using the VE1 antibody. VE1 IHC offers a rapid and cost-effective screening method for detecting the BRAF V600E mutation in various cancers, including NSCLC [128–132] (Figure 7). This method is particularly useful for screening small samples, such as those obtained via endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). However, the VE1 clone specifically targets the BRAF V600E mutation, which represents only 30%–50% of all *BRAF* mutations in NSCLC (Figure 7). It does not detect other pathogenic *BRAF* mutations, such as non-V600E variants (e.g., G469A, D594G). Moreover, several studies emphasize the importance of rigorous optimization of the VE1 antibody to prevent false-positive results and enhance concordance with molecular assays [131, 133]. Further efforts are needed to refine interpretation criteria and provide comprehensive training for pathologists in assessing BRAF IHC [133]. Importantly, current CAP/IASLC/AMP guidelines do not recommend routine *BRAF* molecular testing outside of clinical trials. Instead, BRAF testing is recommended as part of larger panels, either initially or when routine *EGFR*, *ALK*, and *ROS1* results are negative [80].

Pan-tropomyosin receptor kinase (pan-TRK) status and assessment

Fusions involving Neurotrophic Tyrosine Receptor Kinase 1–3 (*NTRK1, NTRK2*, and *NTRK3*) genes represent uncommon but clinically significant oncogenic alterations [134]. These genes encode the tropomyosin receptor kinases TRKA, TRKB, and TRKC, respectively [135]. TRK signaling is essential for normal neural development and cell survival [135, 136]. In cancer, chromosomal rearrangements can fuse the kinase-containing 3' portion of an NTRK gene with the 5' portion of a partner gene. The resulting chimeric fusion protein typically exhibits constitutive activity, leading to ligand-independent activation of downstream signaling pathways and promoting tumor growth and survival. These fusions are highly prevalent (80%–100%) in a small subset of rare tumors, including infantile fibrosarcoma, congenital mesoblastic nephroma, and secretory carcinomas of the breast and salivary glands, in which the fusion between ETS variant transcription factor 6 (*ETV6*) and *NTRK3*, designated *ETV6::NTRK3*, is characteristic [134]. Conversely, they are exceedingly rare in common cancers, such as NSCLC. Despite their low prevalence, their detection is clinically relevant because NTRK fusion-positive cancers often respond well to TRK inhibitors, including larotrectinib, entrectinib, and repotrectinib.

NTRK1-3 fusions constitute a very small molecular subset of NSCLC, accounting for less than 1% of cases [137–139]. Real-world screening data from 1068 unselected consecutive NSCLC patients identified *NTRK1-3* fusions in only 0.2% of cases [138]. Similar findings were reported in a recent large cohort comprising approximately 26,000 samples [139]. Radonić et al. observed *NTRK1-3* fusions to be exceptionally rare (0.03%) in NSCLC, noting reported false positivity of TRK IHC, particularly in pulmonary squamous cell carcinomas (approximately 4%) [139].

NTRK1-3 fusions predominantly affect lung adenocarcinomas, though rare instances have been described in other histologic subtypes. These fusions are mutually exclusive with other oncogenic drivers in NSCLC, such as *EGFR, ALK, ROS1, BRAF, RET*, and *KRAS*. Consequently, they are most frequently sought as part of comprehensive molecular profiling, especially in advanced non-squamous NSCLC without other actionable alterations.

Detection of *NTRK1-3* fusions can be achieved through various methods, including DNA/RNA-based NGS, FISH, reverse-transcription PCR, and pan-TRK IHC. However, RNA-based NGS is considered the preferred molecular method due to its ability to detect expressed fusion transcripts and identify diverse fusion partners [134, 139, 140]. ESMO recommendations endorse tumor NGS in advanced non-squamous NSCLC and for the detection of tumor-agnostic alterations when matched therapies are available [134].

Pan-TRK IHC serves an important practical role as a screening test. It is relatively rapid, cost-effective, tissue-sparing, and widely accessible in diagnostic pathology laboratories. It detects the conserved C-terminal region of TRKA/B/C protein, with staining patterns (cytoplasmic, membranous, nuclear, or perinuclear) varying depending on the specific fusion and tumor type [137, 140]. In tumors with a low prevalence of *NTRK1-3* fusions, IHC can be utilized to select cases for confirmatory molecular testing. ESMO guidance supports either front-line RNA-based sequencing or IHC screening followed by sequencing of IHC-positive cases when *NTRK1-3* fusions are uncommon in an unselected population [134].

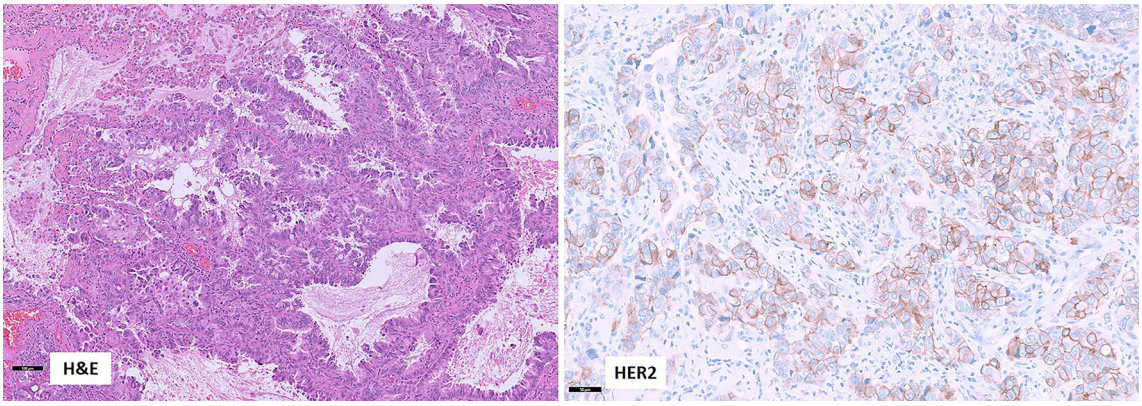


Figure 8. HER2 protein expression in pulmonary adenocarcinoma without corresponding *ERBB2* genomic alterations. H&E staining shows a moderately differentiated pulmonary adenocarcinoma with glandular architecture and focal mucin production (left). HER2 IHC using the VENTANA anti-HER2/neu (4B5) assay demonstrates moderate membranous staining in tumor cells, corresponding to an IHC score of 2+ (right). Subsequent NGS detected neither *ERBB2* amplification nor mutation but identified an *EGFR* exon 19 deletion, illustrating the limited concordance between HER2 protein expression and *ERBB2* genomic status in NSCLC. Original magnification, ×20. **Abbreviations:** *EGFR*, epidermal growth factor receptor; *ERBB2*, erb-b2 receptor tyrosine kinase 2; H&E, hematoxylin and eosin; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer.

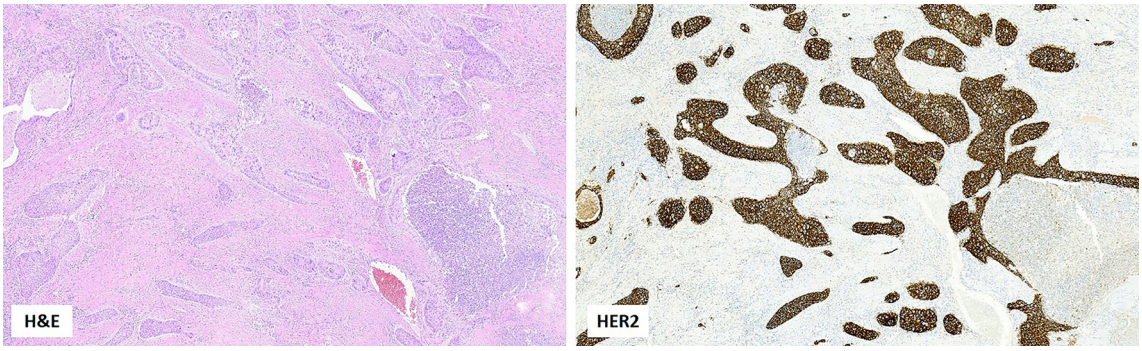


Figure 9. HER2 overexpression associated with *ERBB2* amplification in pulmonary adenocarcinoma. H&E staining shows a pulmonary adenocarcinoma with predominantly gland-forming architecture (left). HER2 IHC using the VENTANA anti-HER2/neu (4B5) assay demonstrates intense, complete membranous staining in 100% of tumor cells, corresponding to an IHC score of 3+ (right). Subsequent CNV analysis showed a copy-number ratio of 27.1, consistent with high-level *ERBB2* amplification. Original magnification, ×10. **Abbreviations:** CNV, copy-number variation; *ERBB2*, erb-b2 receptor tyrosine kinase 2; H&E, hematoxylin and eosin; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

However, IHC has significant limitations. It cannot determine which *NTRK* gene is rearranged, identify the fusion partner, or differentiate between wild-type TRK expression and oncogenic fusion protein expression. False-positive staining may occur in tumors with physiological TRK expression (e.g., tumors of neural origin or neuroendocrine differentiation) [140–142], while false-negative results can also arise, particularly with some *NTRK3* fusions. In a nationwide routine diagnostic cohort, pan-TRK IHC demonstrated a sensitivity of 77% and a specificity of 84% compared with molecular testing [143]. Radonić et al. similarly reported that pan-TRK IHC may yield false-positive results, particularly in squamous cell carcinomas, concluding that confirmatory NGS is necessary to validate pan-TRK IHC results and assess NTRK fusion functionality [139]. Therefore, a positive or equivocal pan-TRK IHC result should be confirmed by a molecular method, preferably RNA-based NGS. In advanced NSCLC, where multiplex RNA-based fusion testing is often integrated into the diagnostic workflow, upfront molecular profiling may be more efficient than IHC alone.

HER2 status and assessment

HER2 (ERBB2), a member of the epidermal growth factor receptor (ERBB/HER) family of transmembrane receptor tyrosine kinases, lacks a known direct ligand [144]. Its activation primarily occurs through heterodimerization with other ERBB family members, leading to robust activation of downstream signaling pathways [144]. This activation promotes cell proliferation, survival, and growth via critical pathways such as PI3K/AKT and rat sarcoma/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase pathway (RAS/RAF/MAPK) [145, 146]. *HER2* gene amplification, followed by HER2 protein overexpression, is a significant oncogenic driver in various cancers, notably breast and gastric/gastroesophageal adenocarcinomas [147–149]. It strongly predicts responsiveness to anti-HER2 targeted therapies like trastuzumab and pertuzumab. Notably, fam-trastuzumab deruxtecan-nxki has received FDA accelerated tumor-agnostic approval for unresectable or metastatic HER2-positive (IHC score 3+) solid tumors in patients who have received prior systemic therapy and lack satisfactory alternative treatment options

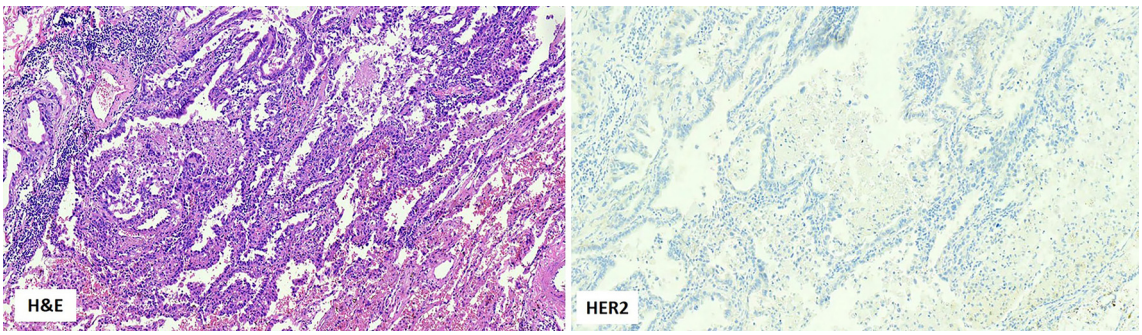


Figure 10. Absence of HER2 protein expression in pulmonary adenocarcinoma harboring an *ERBB2* mutation. H&E staining shows a moderately differentiated pulmonary adenocarcinoma with predominantly gland-forming architecture (left). HER2 IHC using the VENTANA anti-HER2/neu (4B5) assay demonstrates no membranous staining in tumor cells, corresponding to an IHC score of 0 (right). Subsequent NGS identified an *ERBB2* c.2313_2324dup mutation, illustrating that negative HER2 IHC does not exclude an underlying *ERBB2* genomic alteration. Original magnification, ×10. **Abbreviations:** *ERBB2*, erb-b2 receptor tyrosine kinase 2; H&E, hematoxylin and eosin; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NGS, next-generation sequencing.

(DESTINY-PanTumor02, DESTINY-Lung01, and DESTINY-CRC02 phase 2 trials) [150–152].

In NSCLC, particularly lung adenocarcinomas and in patients without a smoking history, the *HER2/ERBB2* gene is a crucial oncogenic driver and therapeutic target. HER2 activation, including protein expression in NSCLC, correlates with a more aggressive clinical course and poorer overall prognosis [153, 154]. HER2 alterations in NSCLC encompass gene mutations (1%–4%), amplifications (2%–5%), and protein overexpression (20%–30%) [153, 155–158]. While HER2 protein expression (scores 2+ and 3+) is most prevalent (Figures 8 and 9), *HER2* mutations, especially exon 20 insertions (e.g., A775_G776insYVMA), are more clinically significant oncogenic drivers due to their responsiveness to targeted therapies such as ADCs like fam-trastuzumab deruxtecan (Enhertu). However, HER2-positive NSCLC (score 3+, defined as strong, complete membranous staining in ≥10% cancer cells, Figure 9) without underlying *HER2* genomic alterations may also be eligible for treatment with fam-trastuzumab deruxtecan (Enhertu). This represents the first FDA-approved tumor-agnostic therapy among HER2-directed ADCs, offering a new option for patients with HER2-positive (IHC score 3+) solid cancers who have exhausted other treatment avenues [159, 160].

It is important to acknowledge that *HER2* mutations and HER2 protein overexpression in NSCLC represent distinct molecular events with a weak correlation [156, 161, 162] (Figures 8 and 10). Furthermore, a poor correlation between HER2 protein expression and *HER2* gene amplification is a well-characterized feature in NSCLC [154, 155, 163], even when different scoring systems are employed [154]. HER2 IHC does not reliably predict *HER2* mutation status in NSCLC [163]; thus, HER2-mutant NSCLC necessitates molecular testing. Conversely, HER2 IHC 3+ alone is relevant in the tumor-agnostic trastuzumab deruxtecan setting (Figure 9). A negative IHC result does not exclude the presence of HER2 genomic alterations (mutations or amplification) (Figures 8 and 10).

EGFR status and assessment

EGFR (HER1), like HER2 (ERBB2), belongs to the erbB family of receptor tyrosine kinases. Mutations represent the most common oncogenic mechanism of EGFR activation in NSCLC, occurring in 12%–17% of NSCLC cases, with a substantially higher incidence in the Asian-Pacific population (averaging 47%) [164]. EGFR-mutated NSCLC predominantly presents as adenocarcinoma by morphology. Exon 19 deletions and exon 21 L858R alterations are the most frequent, predicting therapeutic benefits from EGFR tyrosine-kinase inhibitors [165]. Less common *EGFR* alterations, including G719X, S768I, and L861Q, as well as exon 20 insertions, are associated with variable drug sensitivity. Acquired T790M and C797S mutations are typically linked to resistance to EGFR inhibitors [165].

EGFR gene mutations are detected using PCR-based assays or comprehensive molecular profiling assays such as NGS. Extensive research has focused on developing EGFR mutation-specific IHC assays to identify the most common *EGFR* mutations [166–176]. These assays aim to detect or predict specific *EGFR* gene alterations [167]. For instance, clones such as SP111 and D6B6/6B6 are designed to detect exon 19 p.E746_A750del alterations, while clones SP125 and 43B2 target exon 21 p.L858R [177, 178]. Despite some promising results [167, 170–174, 176], current CAP/IASLC/AMP guidelines do not endorse EGFR mutation-specific IHC testing. This is due to suboptimal antibody performance, which can miss clinically relevant *EGFR* alterations and produce false-positive results [80]. Therefore, while EGFR mutation-specific IHC testing may occasionally serve as an adjunct or provisional test, molecular testing (NGS) remains the necessary gold standard.

Practical considerations and recommendations

In clinical practice, IHC offers a rapid initial screen for predictive biomarkers, but it necessitates thorough validation and control. The use of CDx kits (Table 1) is strongly recommended due to their extensive clinical validation. Adherence to strict pre-analytic protocols, including cold ischemia time, use of approved fixatives, recommended fixation times, and proper control stains, is also advised.

Pathologists should score IHC according to companion guidelines (e.g., PD-L1 TPS cutoffs at 1% and 50%) and recognize the variability among available assays (e.g., SP142 typically stains fewer tumor cells) and their applicability for therapy selection.

ALK and PD-L1 IHC remain established and validated predictive assays in NSCLC. In contrast, ROS1, BRAF V600E, pan-TRK, non-CDx HER2, and non-CDx c-Met IHC are best regarded as screening or triage tools that require integration with molecular-genomic testing. Notably, VENTANA MET SP44 RxDx is an FDA-approved CDx for high c-Met protein overexpression in the telisotuzumab vedotin setting, and HER2 score 3+ is approved for fam-trastuzumab deruxtecan-nxki/Enhertu. EGFR mutation-specific IHC testing is not recommended.

A negative IHC result (except for PD-L1) should prompt comprehensive panel testing (e.g., NGS) to identify potential false negatives and other genomic alterations. Throughout this process, rigorous quality control and interdisciplinary communication (e.g., tumor board/molecular tumor board discussions) are essential to ensure accurate communication and optimize patient care.

Conclusion

IHC remains an indispensable tool in NSCLC precision oncology, particularly for PD-L1, ALK, c-Met, and HER2. ROS1, BRAF V600E, pan-TRK, and non-CDx c-Met/HER2 IHC assays should be interpreted as screening or triage tools, requiring integration with broad DNA/RNA-based molecular profiling. VENTANA MET SP44 RxDx serves as a CDx for high c-Met protein overexpression in the telisotuzumab vedotin setting, while HER2 IHC score 3+ may be relevant in the tumor-agnostic trastuzumab deruxtecan (Enhertu) context but should not be used as a surrogate for *HER2* mutations in NSCLC.

In every instance, using the correct clones and cutoffs is paramount. Moving forward, harmonization of testing protocols (e.g., CAP/IASLC/AMP initiatives), the adoption of multiplex technologies, and the integration of digital image analysis/AI will help overcome current limitations. Our updated sections, tables, and practical recommendations aim to facilitate the implementation of best practices. As new biomarkers and therapies emerge, ongoing research and guideline updates will be necessary. Ultimately, combining IHC with comprehensive genomic profiling will ensure the identification of all actionable alterations, enabling patients to receive the most effective precision therapy.

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References

[1] Ji Y, Zhang Y, Liu S, Li J, Jin Q, Wu J, et al. The epidemiological landscape of lung cancer: current status, temporal trend and future projections based on the latest estimates from GLOBOCAN 2022. J Natl Cancer Cent 2025;5(3):278–86. https://doi.org/10.1016/j.jncc.2025.01.003

[2] Hamran S, Alnoubani Z, Elsherif R, Gatalica Z, Vranic S, Babu GR. Epidemiology and Prevention of Lung Cancer in Europe: a Review of Burden, Disparities, and the Path to Equitable Early Detection. Acta Med Acad. 2026. https://doi.org/10.5644/ama2006-124.511

[3] Zhou J, Xu Y, Liu J, Feng L, Yu J, Chen D. Global burden of lung cancer in 2022 and projections to 2050: Incidence and mortality estimates from GLOBOCAN. Cancer Epidemiol 2024;93:102693. https://doi.org/10.1016/j.canep.2024.102693

[4] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022:GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74(3):229–63. https://doi.org/10.3322/caac.21834

[5] Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 2016;5(3):288–300. https://doi.org/10.21037/tlcr.2016.06.07

[6] Aberle DR, Abtin F, Brown K. Computed tomography screening for lung cancer: has it finally arrived? Implications of the national lung screening trial. J Clin Oncol 2013;31(8):1002–8. https://doi.org/10.1200/JCO.2012.43.3110

[7] National Lung Screening Trial Research T, Aberle DR, Adams AM, Berg CD, Clapp JD, Clingan KL, et al. Baseline characteristics of participants in the randomized national lung screening trial. J Natl Cancer Inst 2010;102(23):1771–9. https://doi.org/10.1093/jnci/djq434

[8] de Koning HJ, van der Aalst CM, de Jong PA, Scholten ET, Nackaerts K, Heuvelmans MA, et al. Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial. N Engl J Med 2020;382(6):503–13. https://doi.org/10.1056/NEJMoa1911793

[9] National Lung Screening Trial Research T, Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365(5):395–409. https://doi.org/10.1056/NEJMoa1102873

[10] Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12(2):175–80. https://doi.org/10.1016/S1470-2045(10)70087-5

[11] Araghi M, Mannani R, Heidarnejad Maleki A, Hamidi A, Rostami S, Safa SH, et al. Recent advances in non-small cell lung cancer targeted therapy; an update review. Cancer Cell Int 2023;23(1):162. https://doi.org/10.1186/s12935-023-02990-y

[12] Huang Q, Li Y, Huang Y, Wu J, Bao W, Xue C, et al. Advances in molecular pathology and therapy of non-small cell lung cancer. Signal Transduct Target Ther 2025;10(1):186. https://doi.org/10.1038/s41392-025-02243-6

[13] Mok TS, Wu YL, Yu CJ, Zhou C, Chen YM, Zhang L, et al. Randomized, placebo-controlled, phase II study of sequential erlotinib and chemotherapy as first-line treatment for advanced non-small-cell lung cancer. J Clin Oncol 2009;27(30):5080–7. https://doi.org/10.1200/JCO.2008.21.5541

[14] Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 2014;371(23):2167–77. https://doi.org/10.1056/NEJMoa1408440

[15] Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 2014;371(21):1963–71. https://doi.org/10.1056/NEJMoa1406766

[16] Planchard D, Besse B, Groen HJM, Souquet PJ, Quoix E, Baik CS, et al. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. Lancet Oncol 2016;17(7):984–93. https://doi.org/10.1016/S1470-2045(16)30146-2

[17] Tan AC, Tan DSW. Targeted Therapies for Lung Cancer Patients With Oncogenic Driver Molecular Alterations. J Clin Oncol 2022;40(6):611–25. https://doi.org/10.1200/JCO.21.01626

[18] Riely GJ, Wood DE, Aisner DL, Axtell AL, Bauman JR, Bharat A, et al. Non-Small Cell Lung Cancer, Version 4.2026, NCCN Clinical Practice Guidelines In Oncology. J Natl Compr Canc Netw 2026;24(4).

[19] Friedlaender A, Perol M, Banna GL, Parikh K, Addeo A. Oncogenic alterations in advanced NSCLC: a molecular super-highway. Biomark Res 2024;12(1):24. https://doi.org/10.1186/s40364-024-00566-0

[20] Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. Nat Rev Clin Oncol 2014;11(8):473–81. https://doi.org/10.1038/nrclinonc.2014.104

[21] Hirsch FR, Suda K, Wiens J, Bunn PA, Jr. New and emerging targeted treatments in advanced non-small-cell lung cancer. Lancet 2016;388(10048):1012–24. https://doi.org/10.1016/S0140-6736(16)31473-8

[22] Rotow J, Bivona TG. Understanding and targeting resistance mechanisms in NSCLC. Nat Rev Cancer 2017;17(11):637–58. https://doi.org/10.1038/nrc.2017.84

[23] Aldea M, Andre F, Marabelle A, Dogan S, Barlesi F, Soria JC. Overcoming Resistance to Tumor-Targeted and Immune-Targeted Therapies. Cancer Discov 2021;11(4):874–99. https://doi.org/10.1158/2159-8290.CD-20-1638

[24] American Cancer Society. Lung cancer survival rates [Internet]. 2026. Available from: https://www.cancer.org/cancer/types/lung-cancer/detection-diagnosis-staging/survival-rates.html

[25] Krpina K, Vranic S, Tomic K, Samarzija M, Baticic L. Small Cell Lung Carcinoma: Current Diagnosis, Biomarkers, and Treatment Options with Future Perspectives. Biomedicines 2023;11(7). https://doi.org/10.3390/biomedicines11071982

[26] Patel SR, Das M. Small Cell Lung Cancer: Emerging Targets and Strategies for Precision Therapy. Cancers (Basel) 2023;15(16). https://doi.org/10.3390/cancers15164016

[27] Tomic K, Begagic E, Voloder E, Naletilic MP, Jozic GB, Cale S, et al. Outcomes of extensive-stage small cell lung cancer treatment in a real-world clinical setting: a single-center experience. Contemp Oncol (Pozn) 2025;29(3):271–80. https://doi.org/10.5114/wo.2025.153755

[28] Penault-Llorca F, Socinski MA. Emerging molecular testing paradigms in non-small cell lung cancer management-current perspectives and recommendations. Oncologist 2025;30(3). https://doi.org/10.1093/oncolo/oyae357

[29] Mosele MF, Westphalen CB, Stenzinger A, Barlesi F, Bayle A, Bieche I, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024:a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024;35(7):588–606. https://doi.org/10.1016/j.annonc.2024.04.005

[30] Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, 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):1491–505. https://doi.org/10.1016/j.annonc.2020.07.014

[31] Aredo JV, Jamali A, Zhu J, Heater N, Wakelee HA, Vaklavas C, et al. Liquid Biopsy Approaches for Cancer Characterization, Residual Disease Detection, and Therapy Monitoring. Am Soc Clin Oncol Educ Book 2025;45(3):e481114. https://doi.org/10.1200/EDBK-25-481114

[32] Pascual J, Attard G, Bidard FC, Curigliano G, De Mattos-Arruda L, Diehn M, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2022;33(8):750–68. https://doi.org/10.1016/j.annonc.2022.05.520

[33] Heitzer E, van den Broek D, Denis MG, Hofman P, Hubank M, Mouliere F, et al. Recommendations for a practical implementation of circulating tumor DNA mutation testing in metastatic non-small-cell lung cancer. ESMO Open 2022;7(2):100399. https://doi.org/10.1016/j.esmoop.2022.100399

[34] Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. Arch Pathol Lab Med 2018;142(10):1242–53. https://doi.org/10.5858/arpa.2018-0901-SA

[35] Taylor CR. Immunohistochemistry in surgical pathology: principles and practice. Methods Mol Biol 2014;1180:81–109. https://doi.org/10.1007/978-1-4939-1050-2/_5

[36] Kim SW, Roh J, Park CS. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J Pathol Transl Med 2016;50(6):411–8. https://doi.org/10.4132/jptm.2016.08.08

[37] Gatalica Z, Feldman R, Vranic S, Spetzler D. Immunohistochemistry-Enabled Precision Medicine. Cancer Treat Res 2019;178:111–35. https://doi.org/10.1007/978-3-030-16391-4/_4

[38] Lassmann S, Werner M. Predictive pathology in routine diagnostics of solid tumors. Histol Histopathol 2012;27(3):289–96.

[39] Sheffield BS. Immunohistochemistry as a Practical Tool in Molecular Pathology. Arch Pathol Lab Med 2016;140(8):766–9. https://doi.org/10.5858/arpa.2015-0453-RA

[40] Fares CM, Van Allen EM, Drake CG, Allison JP, Hu-Lieskovan S. Mechanisms of Resistance to Immune Checkpoint Blockade: Why Does Checkpoint Inhibitor Immunotherapy Not Work for All Patients? Am Soc Clin Oncol Educ Book 2019;39:147–64. https://doi.org/10.1200/EDBK/_240837

[41] Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev 2014;23(12):2965–70. https://doi.org/10.1158/1055-9965.EPI-14-0654

[42] Wang X, Teng F, Kong L, Yu J. PD-L1 expression in human cancers and its association with clinical outcomes. Onco Targets Ther 2016;9:5023–39. https://doi.org/10.2147/OTT.S105862

[43] Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. J Immunother Cancer 2019;7(1):278. https://doi.org/10.1186/s40425-019-0768-9

[44] Wang Y, Du J, Gao Z, Sun H, Mei M, Wang Y, et al. Evolving landscape of PD-L2:bring new light to checkpoint immunotherapy. Br J Cancer 2023;128(7):1196–207. https://doi.org/10.1038/s41416-022-02084-y

[45] Cha JH, Chan LC, Li CW, Hsu JL, Hung MC. Mechanisms Controlling PD-L1 Expression in Cancer. Mol Cell 2019;76(3):359–70. https://doi.org/10.1016/j.molcel.2019.09.030

[46] Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. BMJ 2018;362:k3529. https://doi.org/10.1136/bmj.k3529

[47] Zdrenka M, Kowalewski A, Ahmadi N, Sadiqi RU, Chmura L, Borowczak J, et al. Refining PD-1/PD-L1 assessment for biomarker-guided immunotherapy: a review. Biomol Biomed 2024;24(1):14–29. https://doi.org/10.17305/bb.2023.9265

[48] Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther 2015;14(4):847–56. https://doi.org/10.1158/1535-7163.MCT-14-0983

[49] Ngo P, Cooper WA, Wade S, Fong KM, Canfell K, Karikios D, et al. Why PD-L1 expression varies between studies of lung cancer: results from a Bayesian meta-analysis. Sci Rep 2025;15(1):4166. https://doi.org/10.1038/s41598-024-80301-9

[50] Sholl LM, Awad M, Basu Roy U, Beasley MB, Cartun RW, Hwang DM, et al. Programmed Death Ligand-1 and Tumor Mutation Burden Testing of Patients With Lung Cancer for Selection of Immune Checkpoint Inhibitor Therapies: Guideline From the College of American Pathologists, Association for Molecular Pathology, International Association for the Study of Lung Cancer, Pulmonary Pathology Society, and LUNGevity Foundation. Arch Pathol Lab Med 2024;148(7):757–74. https://doi.org/10.5858/arpa.2023-0536-CP

[51] Zer A, Ahn MJ, Barlesi F, Bubendorf L, De Ruysscher D, Garrido P, et al. Early and locally advanced non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2025;36(11):1245–62. https://doi.org/10.1016/j.annonc.2025.08.003

[52] Vranic S, Gatalica Z. PD-L1 testing by immunohistochemistry in immuno-oncology. Biomol Biomed 2023;23(1):15–25. https://doi.org/10.17305/bjbms.2022.7953

[53] Chebib I, Mino-Kenudson M. PD-L1 immunohistochemistry: Clones, cutoffs, and controversies. APMIS 2022;130(6):295–313. https://doi.org/10.1111/apm.13223

[54] Lantuejoul S, Sound-Tsao M, Cooper WA, Girard N, Hirsch FR, Roden AC, et al. PD-L1 Testing for Lung Cancer in 2019:Perspective From the IASLC Pathology Committee. J Thorac Oncol 2020;15(4):499–519. https://doi.org/10.1016/j.jtho.2019.12.107

[55] Ito H, Yoshizawa A, Terada K, Nakakura A, Rokutan-Kurata M, Sugimoto T, et al. A Deep Learning-Based Assay for Programmed Death Ligand 1 Immunohistochemistry Scoring in Non-Small Cell Lung Carcinoma: Does it Help Pathologists Score? Mod Pathol 2024;37(6):100485. https://doi.org/10.1016/j.modpat.2024.100485

[56] Huang Z, Chen L, Lv L, Fu CC, Jin Y, Zheng Q, et al. A new AI-assisted scoring system for PD-L1 expression in NSCLC. Comput Methods Programs Biomed 2022;221:106829. https://doi.org/10.1016/j.cmpb.2022.106829

[57] Kabir S, Chowdhury MEH, Sarmun R, Vranic S, Al Saady RM, Rose I, et al. A novel deep learning framework for automatic scoring of PD-L1 expression in non-small cell lung cancer. Biomol Biomed 2025;26(1):79–90. https://doi.org/10.17305/bb.2025.12056

[58] Wu J, Liu C, Liu X, Sun W, Li L, Gao N, et al. Artificial intelligence-assisted system for precision diagnosis of PD-L1 expression in non-small cell lung cancer. Mod Pathol 2022;35(3):403–11. https://doi.org/10.1038/s41379-021-00904-9

[59] Das A, Tomita N, Syme KJ, Ma W, O'Connor P, Corbett KN, et al. Cross-Modality Learning for Predicting Immunohistochemistry Biomarkers from Hematoxylin and Eosin-Stained Whole Slide Images. Am J Pathol 2025;195(12):2400–10. https://doi.org/10.1016/j.ajpath.2025.08.014

[60] Ge C, Shi Y, Wang W, Zhang A, Huang M, Zhao F, et al. Artificial Intelligence-driven image analysis for standardised programmed death-ligand 1 expression evaluation in non-small cell lung cancer. Diagn Pathol 2025;20(1):106. https://doi.org/10.1186/s13000-025-01707-1

[61] Zhang P, Zhang B, Liu N, Huang D, Zhong H, Wu J, et al. Prediction of PD-L1 expression in nonsmall cell lung cancer using artificial intelligence models based on radiomics: a systematic review and meta-analysis. Eur Respir Rev 2026;35(181). https://doi.org/10.1183/16000617.0299-2025

[62] Maniewski M, Borowczak J, Durslewicz J, Zdrenka M, Kowalewski A, Szylberg L. Clinical relevance of AI-based PD-L1 scoring in non-small cell lung cancer. Front Oncol 2026;16:1790571. https://doi.org/10.3389/fonc.2026.1790571

[63] Hendry S, Byrne DJ, Wright GM, Young RJ, Sturrock S, Cooper WA, et al. Comparison of Four PD-L1 Immunohistochemical Assays in Lung Cancer. J Thorac Oncol 2018;13(3):367–76. https://doi.org/10.1016/j.jtho.2017.11.112

[64] Moutafi MK, Tao W, Huang R, Haberberger J, Alexander B, Ramkissoon S, et al. Comparison of programmed death-ligand 1 protein expression between primary and metastatic lesions in patients with lung cancer. J Immunother Cancer 2021;9(4). https://doi.org/10.1136/jitc-2020-002230

[65] Frank MS, Bodtger U, Hoegholm A, Stamp IM, Gehl J. Re-biopsy after first line treatment in advanced NSCLC can reveal changes in PD-L1 expression. Lung Cancer 2020;149:23–32. https://doi.org/10.1016/j.lungcan.2020.08.020

[66] Sakai H, Takeda M, Sakai K, Nakamura Y, Ito A, Hayashi H, et al. Impact of cytotoxic chemotherapy on PD-L1 expression in patients with non-small cell lung cancer negative for EGFR mutation and ALK fusion. Lung Cancer 2019;127:59–65. https://doi.org/10.1016/j.lungcan.2018.11.025

[67] Smolle E, Leithner K, Olschewski H. Oncogene addiction and tumor mutational burden in non-small-cell lung cancer: Clinical significance and limitations. Thorac Cancer 2020;11(2):205–15. https://doi.org/10.1111/1759-7714.13246

[68] Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2023;34(4):339–57. https://doi.org/10.1016/j.annonc.2022.12.009

[69] Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer 2008;8(1):11–23. https://doi.org/10.1038/nrc2291

[70] Hallberg B, Palmer RH. The role of the ALK receptor in cancer biology. Ann Oncol 2016;27 Suppl 3:iii4–iii15. https://doi.org/10.1093/annonc/mdw301

[71] Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, et al. Oncogenic mutations of ALK kinase in neuroblastoma. Nature 2008;455(7215):971–4. https://doi.org/10.1038/nature07399

[72] Stein H, Foss HD, Durkop H, Marafioti T, Delsol G, Pulford K, et al. CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood 2000;96(12):3681–95. https://doi.org/10.1182/blood.V96.12.3681

[73] Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363(18):1693–703. https://doi.org/10.1056/NEJMoa1006448

[74] Lawrence B, Perez-Atayde A, Hibbard MK, Rubin BP, Dal Cin P, Pinkus JL, et al. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. Am J Pathol 2000;157(2):377–84. https://doi.org/10.1016/S0002-9440(10)64550-6

[75] Hiwatari M, Seki M, Matsuno R, Yoshida K, Nagasawa T, Sato-Otsubo A, et al. Novel TENM3-ALK fusion is an alternate mechanism for ALK activation in neuroblastoma. Oncogene 2022;41(20):2789–97. https://doi.org/10.1038/s41388-022-02301-1

[76] Morris SW, Naeve C, Mathew P, James PL, Kirstein MN, Cui X, et al. ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). Oncogene 1997;14(18):2175–88. https://doi.org/10.1038/sj.onc.1201062

[77] Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements: a new therapeutic target in a molecularly defined subset of non-small cell lung cancer. J Thorac Oncol 2009;4(12):1450–4. https://doi.org/10.1097/JTO.0b013e3181c4dedb

[78] Voena C, Ambrogio C, Iannelli F, Chiarle R. ALK in cancer: from function to therapeutic targeting. Nat Rev Cancer 2025;25(5):359–78. https://doi.org/10.1038/s41568-025-00797-9

[79] Peng L, Zhu L, Sun Y, Stebbing J, Selvaggi G, Zhang Y, et al. Targeting ALK Rearrangements in NSCLC: Current State of the Art. Front Oncol 2022;12:863461. https://doi.org/10.3389/fonc.2022.863461

[80] Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med 2018;142(3):321–46. https://doi.org/10.5858/arpa.2017-0388-CP

[81] Thorne-Nuzzo T, Williams C, Catallini A, Clements J, Singh S, Amberson J, et al. A Sensitive ALK Immunohistochemistry Companion Diagnostic Test Identifies Patients Eligible for Treatment with Crizotinib. J Thorac Oncol 2017;12(5):804–13. https://doi.org/10.1016/j.jtho.2017.01.020

[82] Davies KD, Doebele RC. Molecular pathways: ROS1 fusion proteins in cancer. Clin Cancer Res 2013;19(15):4040–5. https://doi.org/10.1158/1078-0432.CCR-12-2851

[83] Davies KD, Le AT, Theodoro MF, Skokan MC, Aisner DL, Berge EM, et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. Clin Cancer Res 2012;18(17):4570–9. https://doi.org/10.1158/1078-0432.CCR-12-0550

[84] Yoshida A, Kohno T, Tsuta K, Wakai S, Arai Y, Shimada Y, et al. ROS1-rearranged lung cancer: a clinicopathologic and molecular study of 15 surgical cases. Am J Surg Pathol 2013;37(4):554–62. https://doi.org/10.1097/PAS.0b013e3182758fe6

[85] Gainor JF, Shaw AT. Novel targets in non-small cell lung cancer: ROS1 and RET fusions. Oncologist 2013;18(7):865–75. https://doi.org/10.1634/theoncologist.2013-0095
[86] Bi H, Ren D, Ding X, Yin X, Cui S, Guo C, et al. Clinical characteristics of patients with ROS1 gene rearrangement in non-small cell lung cancer: a meta-analysis. Transl Cancer Res 2020;9(7):4383–92. https://doi.org/10.21037/tcr-20-1813
[87] Muminovic M, Carracedo Uribe CR, Alvarez-Pinzon A, Shan K, Raez LE. Importance of ROS1 gene fusions in non-small cell lung cancer. Cancer Drug Resist 2023;6(2):332–44. https://doi.org/10.20517/cdr.2022.105
[88] Lee J, Lee SE, Kang SY, Do IG, Lee S, Ha SY, et al. Identification of ROS1 rearrangement in gastric adenocarcinoma. Cancer 2013;119(9):1627–35. https://doi.org/10.1002/cncr.27967
[89] Aisner DL, Nguyen TT, Paskulin DD, Le AT, Haney J, Schulte N, et al. ROS1 and ALK fusions in colorectal cancer, with evidence of intratumoral heterogeneity for molecular drivers. Mol Cancer Res 2014;12(1):111–8. https://doi.org/10.1158/1541-7786.MCR-13-0479-T
[90] Aydin HA, Pestereli E, Ozcan M, Bayramoglu Z, Erdogan G, Simsek T. A study detection of the ROS1 gene fusion by FISH and ROS1 protein expression by IHC methods in patients with ovarian malignant or borderline serous tumors. Pathol Res Pract 2018;214(11):1868–72. https://doi.org/10.1016/j.prp.2018.09.016
[91] Schoot RA, Orbach D, Minard Colin V, Alaggio R, Di Carlo D, Corradini N, et al. Inflammatory Myofibroblastic Tumor With ROS1 Gene Fusions in Children and Young Adolescents. JCO Precis Oncol 2023;7:e2300323. https://doi.org/10.1200/PO.23.00323
[92] Davare MA, Henderson JJ, Agarwal A, Wagner JP, Iyer SR, Shah N, et al. Rare but Recurrent ROS1 Fusions Resulting From Chromosome 6q22 Microdeletions are Targetable Oncogenes in Glioma. Clin Cancer Res 2018;24(24):6471–82. https://doi.org/10.1158/1078-0432.CCR-18-1052
[93] Jain P, Iyer S, Straka J, Surrey LF, Pogoriler J, Han H, et al. Discovery and functional characterization of the oncogenicity and targetability of a novel NOTCH1-ROS1 gene fusion in pediatric angiosarcoma. Cold Spring Harb Mol Case Stud 2022;8(6).
[94] Kim HR, Lim SM, Kim HJ, Hwang SK, Park JK, Shin E, et al. The frequency and impact of ROS1 rearrangement on clinical outcomes in never smokers with lung adenocarcinoma. Ann Oncol 2013;24(9):2364–70. https://doi.org/10.1093/annonc/mdt220
[95] Awad MM, Katayama R, McTigue M, Liu W, Deng YL, Brooun A, et al. Acquired resistance to crizotinib from a mutation in CD74-ROS1. N Engl J Med 2013;368(25):2395–401. https://doi.org/10.1056/NEJMoa1215530
[96] Gerlinger M, Norton L, Swanton C. Acquired resistance to crizotinib from a mutation in CD74-ROS1. N Engl J Med 2013;369(12):1172–3. https://doi.org/10.1056/NEJMc1309091
[97] Yu ZQ, Wang M, Zhou W, Mao MX, Chen YY, Li N, et al. ROS1-positive non-small cell lung cancer (NSCLC): biology, diagnostics, therapeutics and resistance. J Drug Target 2022;30(8):845–57. https://doi.org/10.1080/1061186X.2022.2085730
[98] Davies KD, Le AT, Sheren J, Nijmeh H, Gowan K, Jones KL, et al. Comparison of Molecular Testing Modalities for Detection of ROS1 Rearrangements in a Cohort of Positive Patient Samples. J Thorac Oncol 2018;13(10):1474–82. https://doi.org/10.1016/j.jtho.2018.05.041
[99] Sholl LM, Sun H, Butaney M, Zhang C, Lee C, Janne PA, et al. ROS1 immunohistochemistry for detection of ROS1-rearranged lung adenocarcinomas. Am J Surg Pathol 2013;37(9):1441–9. https://doi.org/10.1097/PAS.0b013e3182960fa7
[100] Shan L, Lian F, Guo L, Qiu T, Ling Y, Ying J, et al. Detection of ROS1 gene rearrangement in lung adenocarcinoma: comparison of IHC, FISH and real-time RT-PCR. PLoS One 2015;10(3):e0120422. https://doi.org/10.1371/journal.pone.0120422
[101] Yoshida A, Tsuta K, Wakai S, Arai Y, Asamura H, Shibata T, et al. Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. Mod Pathol 2014;27(5):711–20. https://doi.org/10.1038/modpathol.2013.192
[102] Su Y, Goncalves T, Dias-Santagata D, Hoang MP. Immunohistochemical Detection of ROS1 Fusion. Am J Clin Pathol 2017;147(1):77–82. https://doi.org/10.1093/ajcp/aqw201
[103] Gatalica Z, Rose I, Vranic S. Detection of fusion oncogenes in routinely collected biorepository samples. Cancer Research 2026;86:A005–A. https://doi.org/10.1158/1538-7445.FUSIONPOSITIVE26-A005
[104] Duh FM, Scherer SW, Tsui LC, Lerman MI, Zbar B, Schmidt L. Gene structure of the human MET proto-oncogene. Oncogene 1997;15(13):1583–6. https://doi.org/10.1038/sj.onc.1201338
[105] Di Renzo MF, Narsimhan RP, Olivero M, Bretti S, Giordano S, Medico E, et al. Expression of the Met/HGF receptor in normal and neoplastic human tissues. Oncogene 1991;6(11):1997–2003.
[106] Ichimura E, Maeshima A, Nakajima T, Nakamura T. Expression of c-met/HGF receptor in human non-small cell lung carcinomas in vitro and in vivo and its prognostic significance. Jpn J Cancer Res 1996;87(10):1063–9. https://doi.org/10.1111/j.1349-7006.1996.tb03111.x
[107] Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010;11(12):834–48. https://doi.org/10.1038/nrm3012
[108] Recondo G, Che J, Janne PA, Awad MM. Targeting MET Dysregulation in Cancer. Cancer Discov 2020;10(7):922–34. https://doi.org/10.1158/2159-8290.CD-19-1446
[109] Tsao MS, Sholl L, Shiller M, Illei P, Wistuba, II, Beasley MB, et al. MET (c-Met) protein overexpression is an emerging protein biomarker in non-small cell lung cancer. NPJ Precis Oncol 2025;9(1):369. https://doi.org/10.1038/s41698-025-01144-9
[110] Liang H, Wang M. MET Oncogene in Non-Small Cell Lung Cancer: Mechanism of MET Dysregulation and Agents Targeting the HGF/c-Met Axis. Onco Targets Ther 2020;13:2491–510. https://doi.org/10.2147/OTT.S231257
[111] Bubendorf L, Dafni U, Schobel M, Finn SP, Tischler V, Sejda A, et al. Prevalence and clinical association of MET gene overexpression and amplification in patients with NSCLC: Results from the European Thoracic Oncology Platform (ETOP) Lungscape project. Lung Cancer 2017;111:143–9. https://doi.org/10.1016/j.lungcan.2017.07.021
[112] Brazel D, Zhang S, Nagasaka M. Spotlight on Tepotinib and Capmatinib for Non-Small Cell Lung Cancer with MET Exon 14 Skipping Mutation. Lung Cancer (Auckl) 2022;13:33–45. https://doi.org/10.2147/LCTT.S360574
[113] Mathieu LN, Larkins E, Akinboro O, Roy P, Amatya AK, Fiero MH, et al. FDA Approval Summary: Capmatinib and Tepotinib for the Treatment of Metastatic NSCLC Harboring MET Exon 14 Skipping Mutations or Alterations. Clin Cancer Res 2022;28(2):249–54. https://doi.org/10.1158/1078-0432.CCR-21-1566
[114] Drilon A, Clark JW, Weiss J, Ou SI, Camidge DR, Solomon BJ, et al. Antitumor activity of crizotinib in lung cancers harboring a MET exon 14 alteration. Nat Med 2020;26(1):47–51. https://doi.org/10.1038/s41591-019-0716-8
[115] Camidge DR, Bar J, Horinouchi H, Goldman J, Moiseenko F, Filippova E, et al. Telisotuzumab Vedotin Monotherapy in Patients With Previously Treated c-Met Protein-Overexpressing Advanced Nonsquamous EGFR-Wildtype Non-Small Cell Lung Cancer in the Phase II LUMINOSITY Trial. J Clin Oncol 2024;42(25):3000–11. https://doi.org/10.1200/JCO.24.00720
[116] US Food and Drug Administration. FDA grants accelerated approval to telisotuzumab vedotin-tllv for NSCLC with high c-Met protein overexpression [Internet]. 2025. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-telisotuzumab-vedotin-tllv-nsclc-high-c-met-protein-overexpression
[117] US Food and Drug Administration. VENTANA MET (SP44) RxDx assay [Internet]. 2025. Available from: https://www.accessdata.fda.gov/cdrh/_docs/pdf24/P240037D.pdf
[118] Baldacci S, Figeac M, Antoine M, Descarpentries C, Kherrouche Z, Jamme P, et al. High MET Overexpression Does Not Predict the presence of MET exon 14 Splice Mutations in NSCLC: Results From the IFCT PREDICT.amm study. J Thorac Oncol 2020;15(1):120–4. https://doi.org/10.1016/j.jtho.2019.09.196
[119] Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417(6892):949–54. https://doi.org/10.1038/nature00766
[120] Wang W, Lian B, Xu C, Wang Q, Li Z, Zheng N, et al. Expert consensus on the diagnosis and treatment of solid tumors with BRAF mutations. Innovation (Camb) 2024;5(6):100661. https://doi.org/10.1016/j.xinn.2024.100661
[121] Chen D, Zhang LQ, Huang JF, Liu K, Chuai ZR, Yang Z, et al. BRAF mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis. PLoS One 2014;9(6):e101354. https://doi.org/10.1371/journal.pone.0101354
[122] Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrero R, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 2002;62(23):6997–7000.
[123] Dong J, Phelps RG, Qiao R, Yao S, Benard O, Ronai Z, et al. BRAF oncogenic mutations correlate with progression rather than initiation of human melanoma. Cancer Res 2003;63(14):3883–5.

[124] Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, et al. The role of BRAF V600 mutation in melanoma. J Transl Med 2012;10:85. https://doi.org/10.1186/1479-5876-10-85
[125] Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 2010;18(6):683–95. https://doi.org/10.1016/j.ccr.2010.11.023
[126] Broderick J. FDA approves adjuvant dabrafenib/trametinib for BRAF+ melanoma [Internet]. 2014. Available from: https://www.onclive.com/view/fda-approves-adjuvant-dabrafenibtrametinib-for-braf-melanoma
[127] Vranic S, Basu GD, Hall DW, Gatalica Z. Tumor-Type Agnostic, Targeted Therapies: BRAF Inhibitors Join the Group. Acta Med Acad 2022;51(3):217–31. https://doi.org/10.5644/ama2006-124.392
[128] Pearlstein MV, Zedek DC, Ollila DW, Treece A, Gulley ML, Groben PA, et al. Validation of the VE1 immunostain for the BRAF V600E mutation in melanoma. J Cutan Pathol 2014;41(9):724–32. https://doi.org/10.1111/cup.12364
[129] Suh MS, Choi YD, Lee JB, Lee SC, Won YH, Yun SJ. Sensitivity and Usefulness of VE1 Immunohistochemical Staining in Acral Melanomas with BRAF Mutation. Ann Dermatol 2018;30(5):556–61. https://doi.org/10.5021/ad.2018.30.5.556
[130] Capper D, Berghoff AS, Magerle M, Ilhan A, Wohrer A, Hackl M, et al. Immunohistochemical testing of BRAF V600E status in 1,120 tumor tissue samples of patients with brain metastases. Acta Neuropathol 2012;123(2):223–33. https://doi.org/10.1007/s00401-011-0887-y
[131] Kuan SF, Navina S, Cressman KL, Pai RK. Immunohistochemical detection of BRAF V600E mutant protein using the VE1 antibody in colorectal carcinoma is highly concordant with molecular testing but requires rigorous antibody optimization. Hum Pathol 2014;45(3):464–72. https://doi.org/10.1016/j.humpath.2013.10.026
[132] Campar B, Miladinovic M, Raonic J, Curovic I, Panjkovic M. VE1 immunohistochemistry as a screening tool for BRAFV600E mutation in lung adenocarcinoma: evidence from a Montenegro National Cohort. Folia Histochem Cytobiol 2025;63(4):177–84. https://doi.org/10.5603/fhc.108990
[133] Chen YA, Lai JY, Hsu CY, Lien HC, Hang JF. Proficiency test of BRAF immunohistochemistry as a surrogate marker of p.V600E mutation: Assessment of staining and interpretation quality in Taiwan. Virchows Arch 2026;488(5):1125–35. https://doi.org/10.1007/s00428-025-04192-5
[134] Marchio C, Scaltriti M, Ladanyi M, Iafrate AJ, Bibeau F, Dietel M, et al. ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research. Ann Oncol 2019;30(9):1417–27. https://doi.org/10.1093/annonc/mdz204
[135] Reichardt LF. Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 2006;361(1473):1545–64. https://doi.org/10.1098/rstb.2006.1894
[136] Nakagawara A. Trk receptor tyrosine kinases: a bridge between cancer and neural development. Cancer Lett 2001;169(2):107–14. https://doi.org/10.1016/S0304-3835(01)00530-4
[137] Gatalica Z, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. Mod Pathol 2019;32(1):147–53. https://doi.org/10.1038/s41379-018-0118-3
[138] Overbeck TR, Reiffert A, Schmitz K, Rittmeyer A, Korber W, Hugo S, et al. NTRK Gene Fusions in Non-Small-Cell Lung Cancer: Real-World Screening Data of 1068 Unselected Patients. Cancers (Basel) 2023;15(11). https://doi.org/10.3390/cancers15112966
[139] Radonic T, Rahmani-Khajouei S, Dafni U, Stenzinger A, Haberecker M, Madsen LB, et al. Prevalence and characteristics of NTRK fusions in 25,946 patients with non-small cell lung cancer. Lung Cancer 2026;218:109514. https://doi.org/10.1016/j.lungcan.2026.109514
[140] Solomon JP, Linkov I, Rosado A, Mullaney K, Rosen EY, Frosina D, et al. NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls. Mod Pathol 2020;33(1):38–46. https://doi.org/10.1038/s41379-019-0324-7
[141] Cimic A, Vranic S, Arguello D, Contreras E, Gatalica Z, Swensen J. Molecular Profiling Reveals Limited Targetable Biomarkers in Neuroendocrine Carcinoma of the Cervix. Appl Immunohistochem Mol Morphol 2021;29(4):299–304. https://doi.org/10.1097/PAI.0000000000000884
[142] Vranic S, Palazzo J, Sanati S, Florento E, Contreras E, Xiu J, et al. Potential Novel Therapy Targets in Neuroendocrine Carcinomas of the Breast. Clin Breast Cancer 2019;19(2):131–6. https://doi.org/10.1016/j.clbc.2018.09.001
[143] Koopman B, Kuijpers C, Groen HJM, Timens W, Schuuring E, Willems SM, et al. Detection of NTRK Fusions and TRK Expression and Performance of pan-TRK Immunohistochemistry in Routine Diagnostics: Results from a Nationwide Community-Based Cohort. Diagnostics (Basel) 2022;12(3). https://doi.org/10.3390/diagnostics12030668
[144] Yarden Y. Biology of HER2 and its importance in breast cancer. Oncology 2001;61(suppl 2):1–13. https://doi.org/10.1159/000055396
[145] Pan L, Li J, Xu Q, Gao Z, Yang M, Wu X, et al. HER2/PI3K/AKT pathway in HER2-positive breast cancer: a review. Medicine (Baltimore) 2024;103(24):e38508. https://doi.org/10.1097/MD.0000000000038508
[146] Ruiz-Saenz A, Dreyer C, Campbell MR, Steri V, Gulizia N, Moasser MM. HER2 Amplification in Tumors Activates PI3K/Akt Signaling Independent of HER3. Cancer Res 2018;78(13):3645–58. https://doi.org/10.1158/0008-5472.CAN-18-0430
[147] Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. Cancer Metastasis Rev 2015;34(1):157–64. https://doi.org/10.1007/s10555-015-9552-6
[148] Bartlett JM, Going JJ, Mallon EA, Watters AD, Reeves JR, Stanton P, et al. Evaluating HER2 amplification and overexpression in breast cancer. J Pathol 2001;195(4):422–8. https://doi.org/10.1002/path.971
[149] Tanner M, Hollmen M, Junttila TT, Kapanen AI, Tommola S, Soini Y, et al. Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIalpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. Ann Oncol 2005;16(2):273–8. https://doi.org/10.1093/annonc/mdi064
[150] Meric-Bernstam F, Makker V, Oaknin A, Oh DY, Banerjee S, Gonzalez-Martin A, et al. Efficacy and Safety of Trastuzumab Deruxtecan in Patients With HER2-Expressing Solid Tumors: Primary Results From the DESTINY-PanTumor02 Phase II Trial. J Clin Oncol 2024;42(1):47–58. https://doi.org/10.1200/JCO.23.02005
[151] Li BT, Smit EF, Goto Y, Nakagawa K, Udagawa H, Mazieres J, et al. Trastuzumab Deruxtecan in HER2-Mutant Non-Small-Cell Lung Cancer. N Engl J Med 2022;386(3):241–51. https://doi.org/10.1056/NEJMoa2112431
[152] Raghav K, Siena S, Takashima A, Kato T, Van den Eynde M, Pietrantonio F, et al. Trastuzumab deruxtecan in patients with HER2-positive advanced colorectal cancer (DESTINY-CRC02): primary results from a multicentre, randomised, phase 2 trial. Lancet Oncol 2024;25(9):1147–62. https://doi.org/10.1016/S1470-2045(24)00380-2
[153] Kim EK, Kim KA, Lee CY, Shim HS. The frequency and clinical impact of HER2 alterations in lung adenocarcinoma. PLoS One 2017;12(2):e0171280. https://doi.org/10.1371/journal.pone.0171280
[154] Tan AC, Saw SPL, Chen J, Lai GGY, Oo HN, Takano A, et al. Clinical and Genomic Features of HER2 Exon 20 Insertion Mutations and Characterization of HER2 Expression by Immunohistochemistry in East Asian Non-Small-Cell Lung Cancer. JCO Precis Oncol 2022;6:e2200278. https://doi.org/10.1200/PO.22.00278
[155] Lee Y, Lee B, Choi YL, Kang DW, Han J. Clinicopathologic and Molecular Characteristics of HER2 (ERBB2)-Altered Non-Small Cell Lung Cancer: Implications for Precision Medicine. Mod Pathol 2024;37(6):100490. https://doi.org/10.1016/j.modpat.2024.100490
[156] Suzuki M, Shiraishi K, Yoshida A, Shimada Y, Suzuki K, Asamura H, et al. HER2 gene mutations in non-small cell lung carcinomas: concurrence with Her2 gene amplification and Her2 protein expression and phosphorylation. Lung Cancer 2015;87(1):14–22. https://doi.org/10.1016/j.lungcan.2014.10.014
[157] Arcila ME, Chaft JE, Nafa K, Roy-Chowdhuri S, Lau C, Zaidinski M, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res 2012;18(18):4910–8. https://doi.org/10.1158/1078-0432.CCR-12-0912
[158] Mehta A, Nathany S, Tripathi R, Sharma SK, Saifi M, Batra U. Non-amplification genetic alterations of HER2 gene in non-small cell lung carcinoma. J Clin Pathol 2021;74(2):106–10. https://doi.org/10.1136/jclinpath-2020-206730
[159] Vranic S, Gatalica Z. Tumor-type agnostic, targeted therapies make a new step forward: the first tumor-agnostic approval of a HER2-targeted therapy. Biomol Biomed 2024;24(4):673–5. https://doi.org/10.17305/bb.2024.10641

[160] US Food and Drug Administration. FDA grants accelerated approval to fam-trastuzumab deruxtecan-nxki for unresectable or metastatic HER2-positive solid tumors [Internet]. 2024. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-fam-trastuzumab-deruxtecan-nxki-unresectable-or-metastatic-her2
[161] Cardillo AB, Kovar S, Roper N, Hicks DG, Velez MJ. Exploration of HER2 (ERBB2) immunohistochemistry in non-small cell lung cancer: correlation with ERBB2 mutational status: experimental research. Ann Med Surg (Lond) 2023;85(6):2640–6. https://doi.org/10.1097/MS9.0000000000000719
[162] Li BT, Ross DS, Aisner DL, Chaft JE, Hsu M, Kako SL, et al. HER2 Amplification and HER2 Mutation Are Distinct Molecular Targets in Lung Cancers. J Thorac Oncol 2016;11(3):414–9. https://doi.org/10.1016/j.jtho.2015.10.025
[163] Wen W, Chen WS, Xiao N, Bender R, Ghazalpour A, Tan Z, et al. Mutations in the Kinase Domain of the HER2/ERBB2 Gene Identified in a Wide Variety of Human Cancers. J Mol Diagn 2015;17(5):487–95. https://doi.org/10.1016/j.jmoldx.2015.04.003
[164] Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). Am J Cancer Res 2015;5(9):2892–911.
[165] Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene 2009;28 Suppl 1(Suppl 1):S24–31. https://doi.org/10.1038/onc.2009.198
[166] Kitamura A, Hosoda W, Sasaki E, Mitsudomi T, Yatabe Y. Immunohistochemical detection of EGFR mutation using mutation-specific antibodies in lung cancer. Clin Cancer Res 2010;16(13):3349–55. https://doi.org/10.1158/1078-0432.CCR-10-0129
[167] Chen Z, Liu HB, Yu CH, Wang Y, Wang L, Song Y. Diagnostic value of mutation-specific antibodies for immunohistochemical detection of epidermal growth factor receptor mutations in non-small cell lung cancer: a meta-analysis. PLoS One 2014;9(9):e105940. https://doi.org/10.1371/journal.pone.0105940
[168] Allo G, Bandarchi B, Yanagawa N, Wang A, Shih W, Xu J, et al. Epidermal growth factor receptor mutation-specific immunohistochemical antibodies in lung adenocarcinoma. Histopathology 2014;64(6):826–39. https://doi.org/10.1111/his.12331
[169] Kawahara A, Yamamoto C, Nakashima K, Azuma K, Hattori S, Kashihara M, et al. Molecular diagnosis of activating EGFR mutations in non-small cell lung cancer using mutation-specific antibodies for immunohistochemical analysis. Clin Cancer Res 2010;16(12):3163–70. https://doi.org/10.1158/1078-0432.CCR-09-3239.
[170] Hitij NT, Kern I, Sadikov A, Knez L, Stanic K, Zwitter M, et al. Immunohistochemistry for EGFR Mutation Detection in Non-Small-Cell Lung Cancer. Clin Lung Cancer 2017;18(3):e187–e96. https://doi.org/10.1016/j.cllc.2016.11.021.
[171] Houang M, Sioson L, Clarkson A, Watson N, Farzin M, Toon CW, et al. EGFR mutation specific immunohistochemistry is a useful adjunct which helps to identify false negative mutation testing in lung cancer. Pathology 2014;46(6):501–8. https://doi.org/10.1097/PAT.0000000000000141.
[172] Cooper WA, Yu B, Yip PY, Ng CC, Lum T, Farzin M, et al. EGFR mutant-specific immunohistochemistry has high specificity and sensitivity for detecting targeted activating EGFR mutations in lung adenocarcinoma. J Clin Pathol 2013;66(9):744–8. https://doi.org/10.1136/jclinpath-2013-201607.
[173] Kato Y, Peled N, Wynes MW, Yoshida K, Pardo M, Mascaux C, et al. Novel epidermal growth factor receptor mutation-specific antibodies for non-small cell lung cancer: immunohistochemistry as a possible screening method for epidermal growth factor receptor mutations. J Thorac Oncol 2010;5(10):1551–8. https://doi.org/10.1097/JTO.0b013e3181e9da60.
[174] Wen YH, Brogi E, Hasanovic A, Ladanyi M, Soslow RA, Chitale D, et al. Immunohistochemical staining with EGFR mutation-specific antibodies: high specificity as a diagnostic marker for lung adenocarcinoma. Mod Pathol 2013;26(9):1197–203. https://doi.org/10.1038/modpathol.2013.53.
[175] Hofman P, Ilie M, Hofman V, Roux S, Valent A, Bernheim A, et al. Immunohistochemistry to identify EGFR mutations or ALK rearrangements in patients with lung adenocarcinoma. Ann Oncol 2012;23(7):1738–43. https://doi.org/10.1093/annonc/mdr535.
[176] Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. J Mol Diagn 2010;12(2):169–76. https://doi.org/10.2353/jmoldx.2010.090140.
[177] Seo AN, Park TI, Jin Y, Sun PL, Kim H, Chang H, et al. Novel EGFR mutation-specific antibodies for lung adenocarcinoma: highly specific but not sensitive detection of an E746_A750 deletion in exon 19 and an L858R mutation in exon 21 by immunohistochemistry. Lung Cancer 2014;83(3):316–23. https://doi.org/10.1016/j.lungcan.2013.12.008.
[178] Gaber R, Watermann I, Kugler C, Vollmer E, Perner S, Reck M, et al. Preselection of EGFR mutations in non-small-cell lung cancer patients by immunohistochemistry: comparison with DNA-sequencing, EGFR wild-type expression, gene copy number gain and clinicopathological data. Rom J Morphol Embryol 2017;58(4):1175–84.

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