Predictive markers of lung cancer – recent updates

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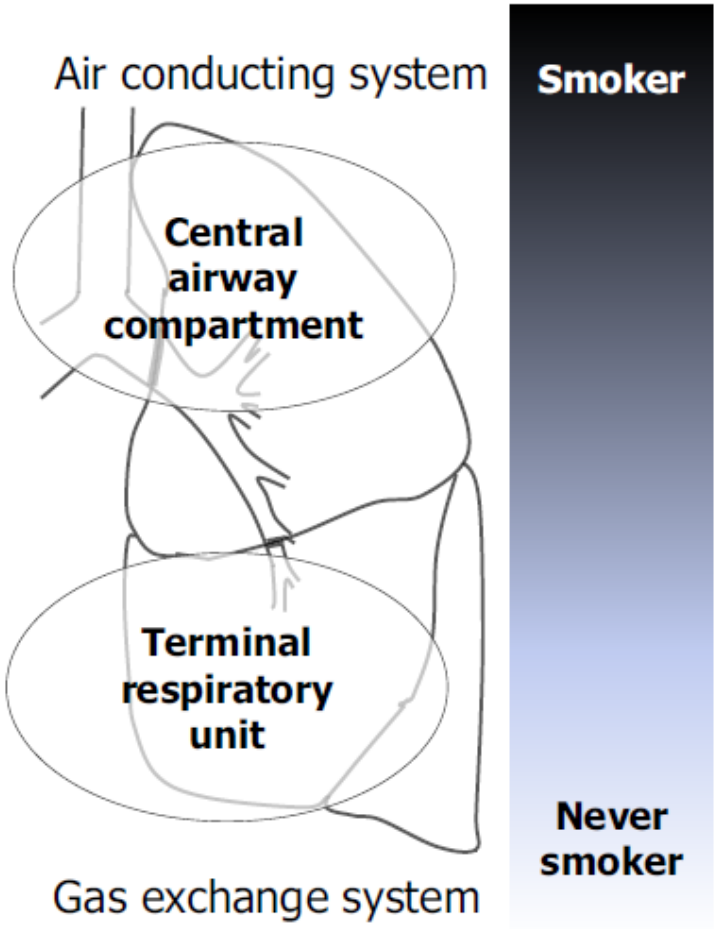
agenda

1. NSCLC genetic abnormalities and driver mutations
2. Molecular biomarker testing
   Methods, early stages, emerging biomarkers, resistance mechanisms
3. PD-L1
4. Liquid biopsy
5. Multiple lung tumours
6. Testing implementation and obstacles

Lung adenocarcinoma is not one disease



Repeated exposure of Tabacco carcinogens

Accumulation of genetic alterations

Escaping from immune surveillance against neoantigens

SCLC, SQC

KRAS activation

ADC

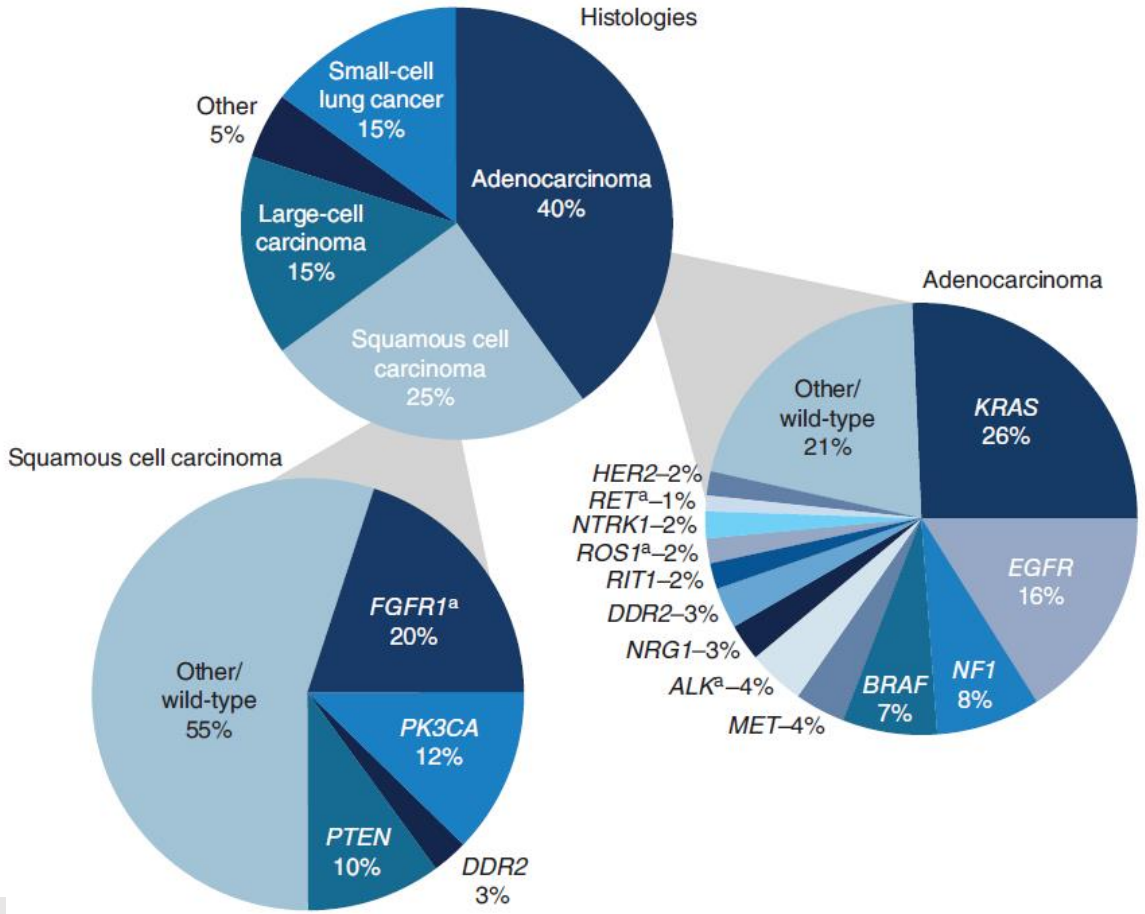
Driver alterations with powerful transformation activities

EGFR activation

Thoracic tumors. WHO 2021

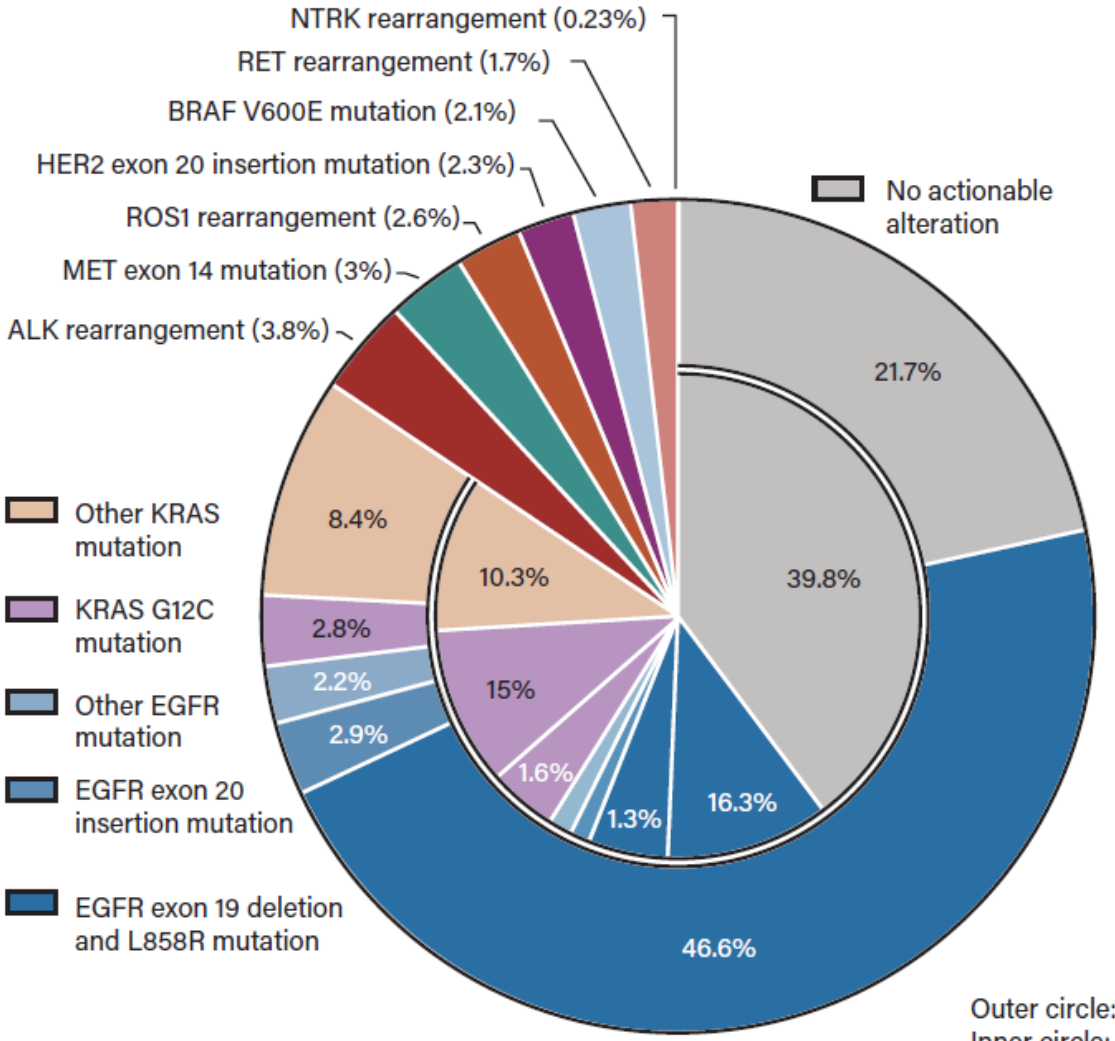
NSCLC and molecular drivers

Oncogenes		Tumor-suppressor genes
AKT1	*KIT*	*BRCA2*
AKT2	*KRAS*	*CDKN2A*
ALK	*MET*	*KEAP1*
BRAF	*NRAS*	*MLL2*
DDR2	*NRF2*	*NF1*
EGFR	*NTRK1/2/3*	*PTEN*
ERBB2	*PIK3CA*	*RB1*
FGFR1	*RET*	*STK11*
HRAS	*RICTOR*	*TP53*
JAK3	*ROS1*	*TSC1*
	SOX2	*VHL1*



Saller JJ. Cold Spring Harb Perspect Med 2022
Am Cancer Society

NSCLC oncogenic drivers



Outer circle: Asian populations
Inner circle: Western populations

EGFR	Exo 19 deletion L858R point mutation in exon 21 L861Q point mutation in exon 21 G719X point mutation in exon 18 T790M point mutation exon 20	NSCLC: ≅15% in Western populations NSCLC: ≅35–50% in Asian populations LUAD: 27% LSQCC: <9% LUAD never smoker: 42% LUAD former smoker: 13.5% LUAD current smoker: 5%
ALK	ALK rearrangement EML4-ALK (the most frequent) KIF5B-ALK KLC1-ALK	NSCLC: 3–5% LUAD: 3–7% LSQCC: 0.2–1% LUAD never smoker: 5–11% LUAD smoker: 0–0.8%
ROS1	ROS1 rearrangement CD74-ROS1 SDC4-ROS1 SLC34A2-ROS1 EZR-ROS1	NSCLC: 0.5–2% LUAD: 2.6% LSQCC: 0% LUAD never smoker: 5.8% LUAD smoker: 0.4%
RET	RET rearrangement	NSCLC: 1–2% LUAD: 1.4% LSQCC: 0% LUAD never-smoker: 1.8% LUAD smoker: 0.7%
BRAF	Point mutations $BRAF^{V600E}$ (30–40% of cases) $BRAF^{nonV600E}$ (60–70% of cases): $BRAF^{V469A}$, $BRAF^{D594G}$, $BRAF^{G466A}$ Class I, II and III BRAF mutants	NSCLC: 2.6% LUAD: 1.4% LSQCC: 0.2–1% LUAD never-smoker: 5–11% LUAD smoker: 0–0.8%
HER2	Point mutations HER2 amplifications	NSCLC: 1.8% LUYAD: 2.3% LSQCC: 0% LUAD never smoker: 4.5% LUAD smoker: 0.8%
MET	MET mutations (exon 14 skipping mutations) MET amplifications MET translocations	Met mutations, METex 14 NSCLC: 2.5–3% LUAD: 3% LSQCC: 0% LUAD never smoker: 4% LUAD smoker: 2.2%

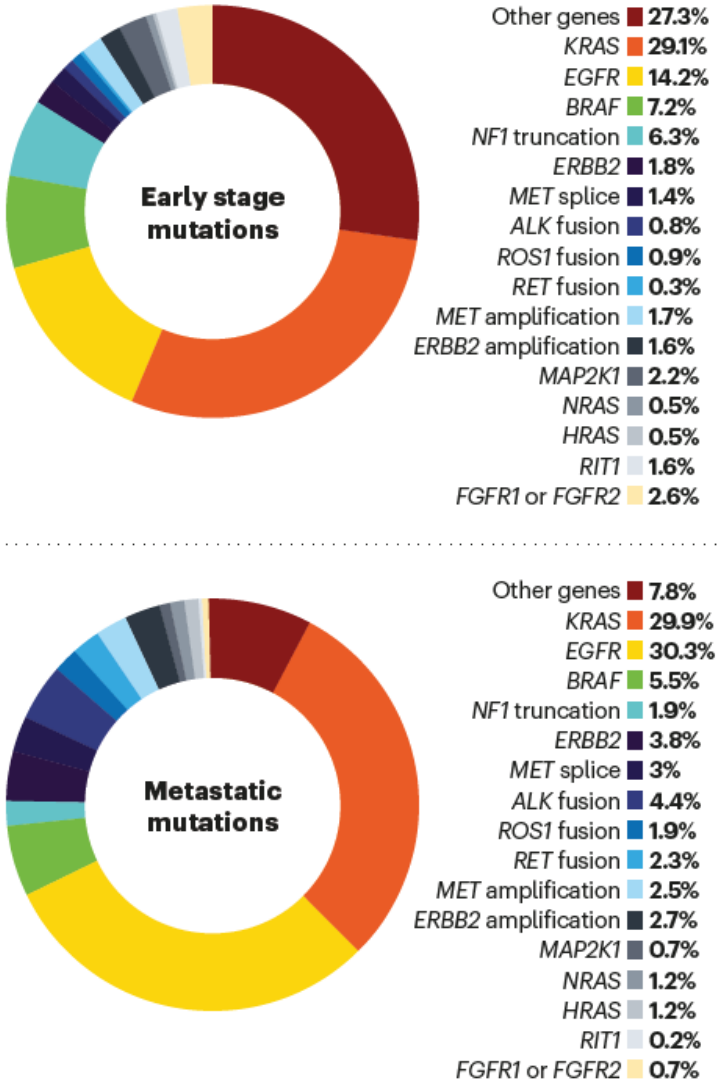
Tan AC, J Clin Oncol 2022

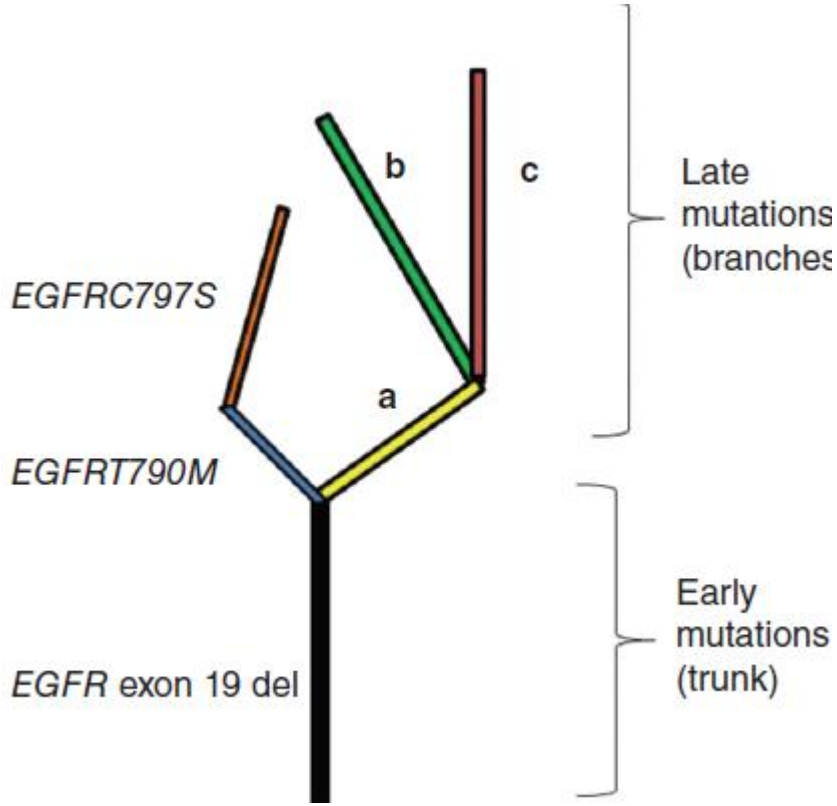
LC stage and driver alterations

Driver muta

Tumor type	W
Early-stage ADC (n=240)	
Entire cohort (n=1,125)	

ADC, adenocarcinoma; Early invasive ADC and entire cohort

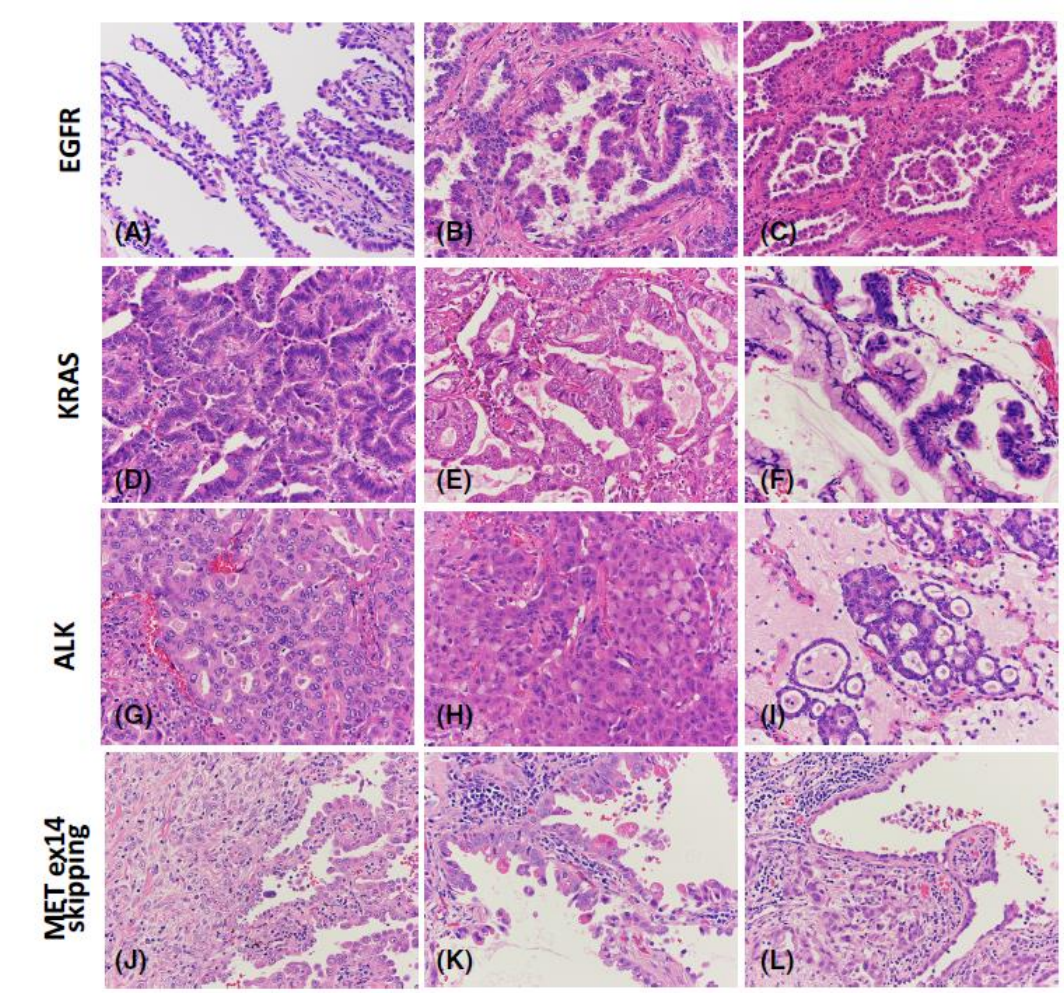


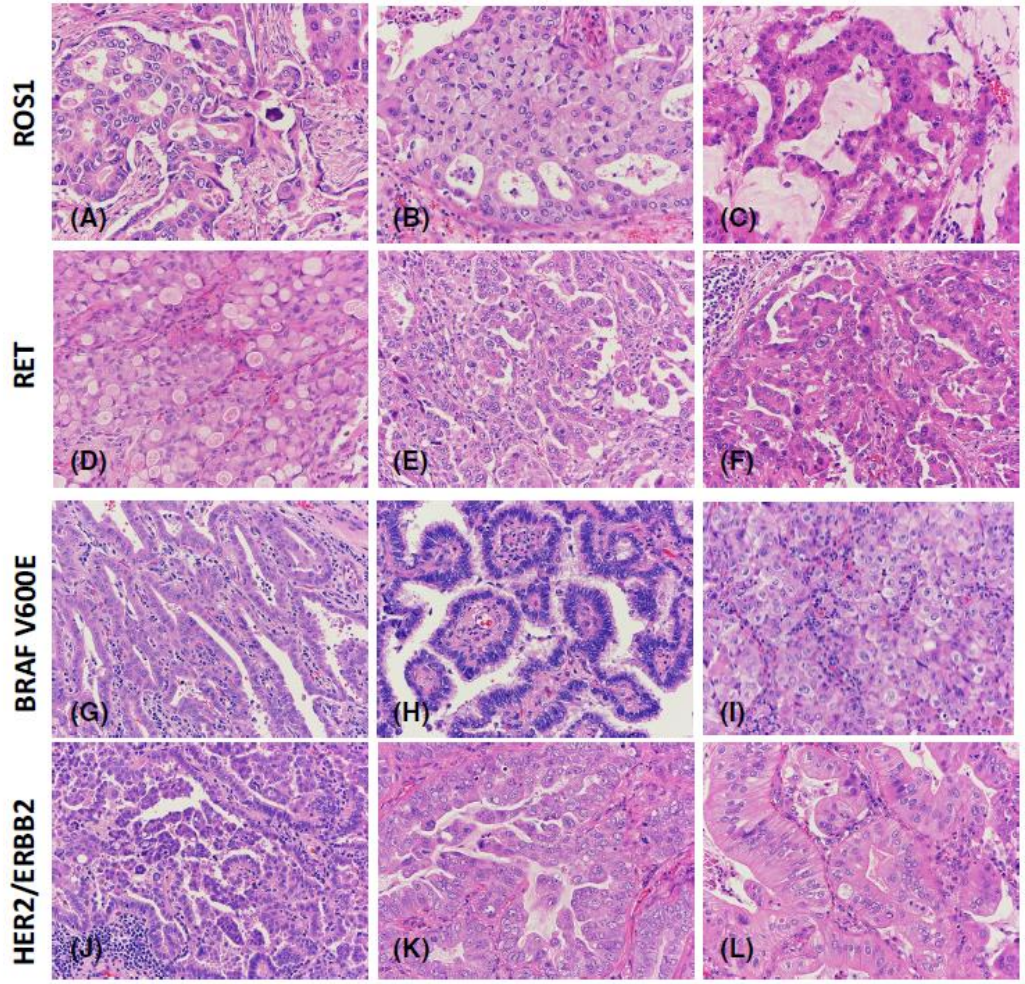


Skoulidis F. Nat Rev Cancer 2019
Yatabe Y. Histopathology 2024

Adenocarcinoma

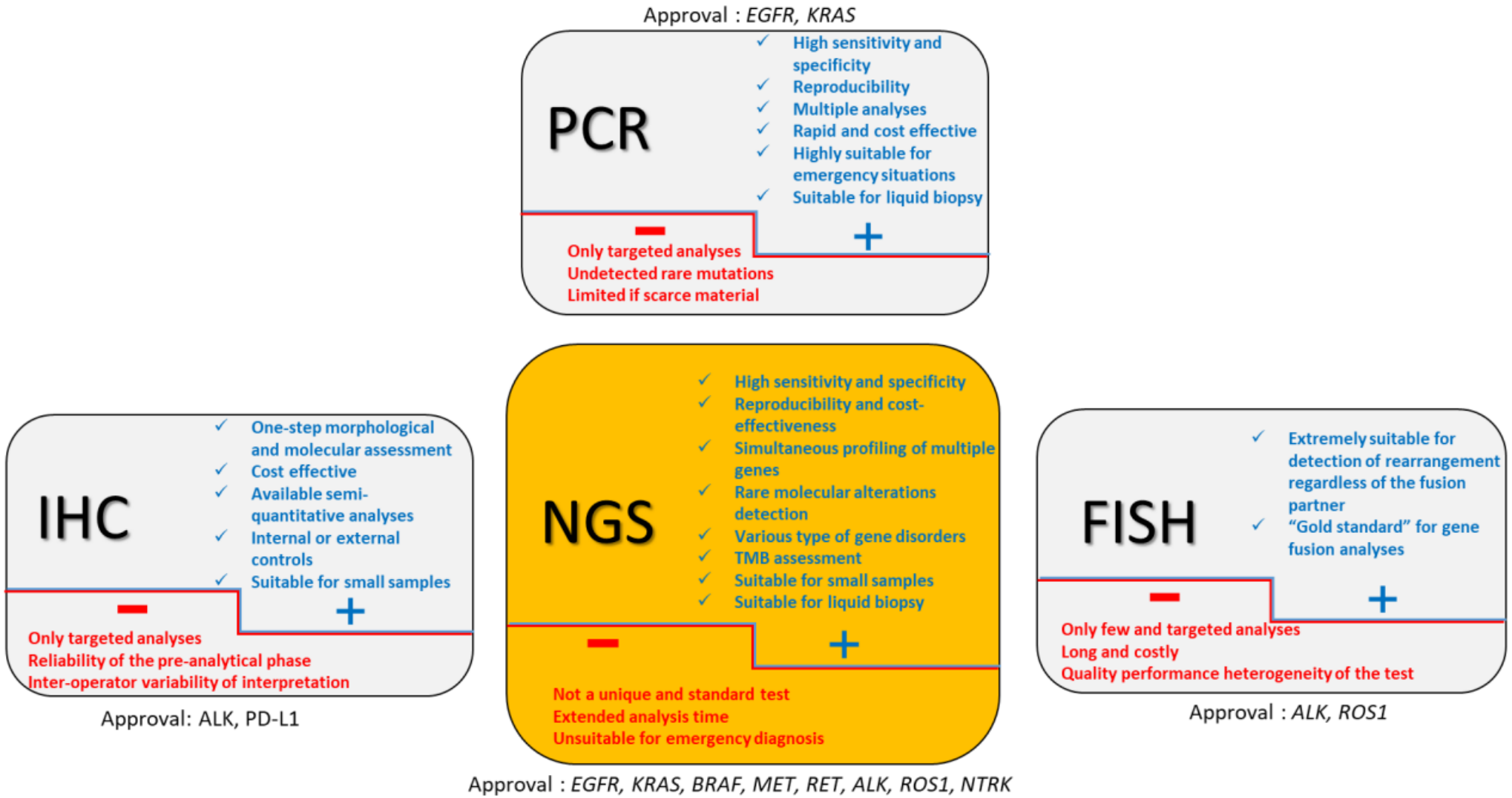
All targetable mutations identified in LC are associated with ADC





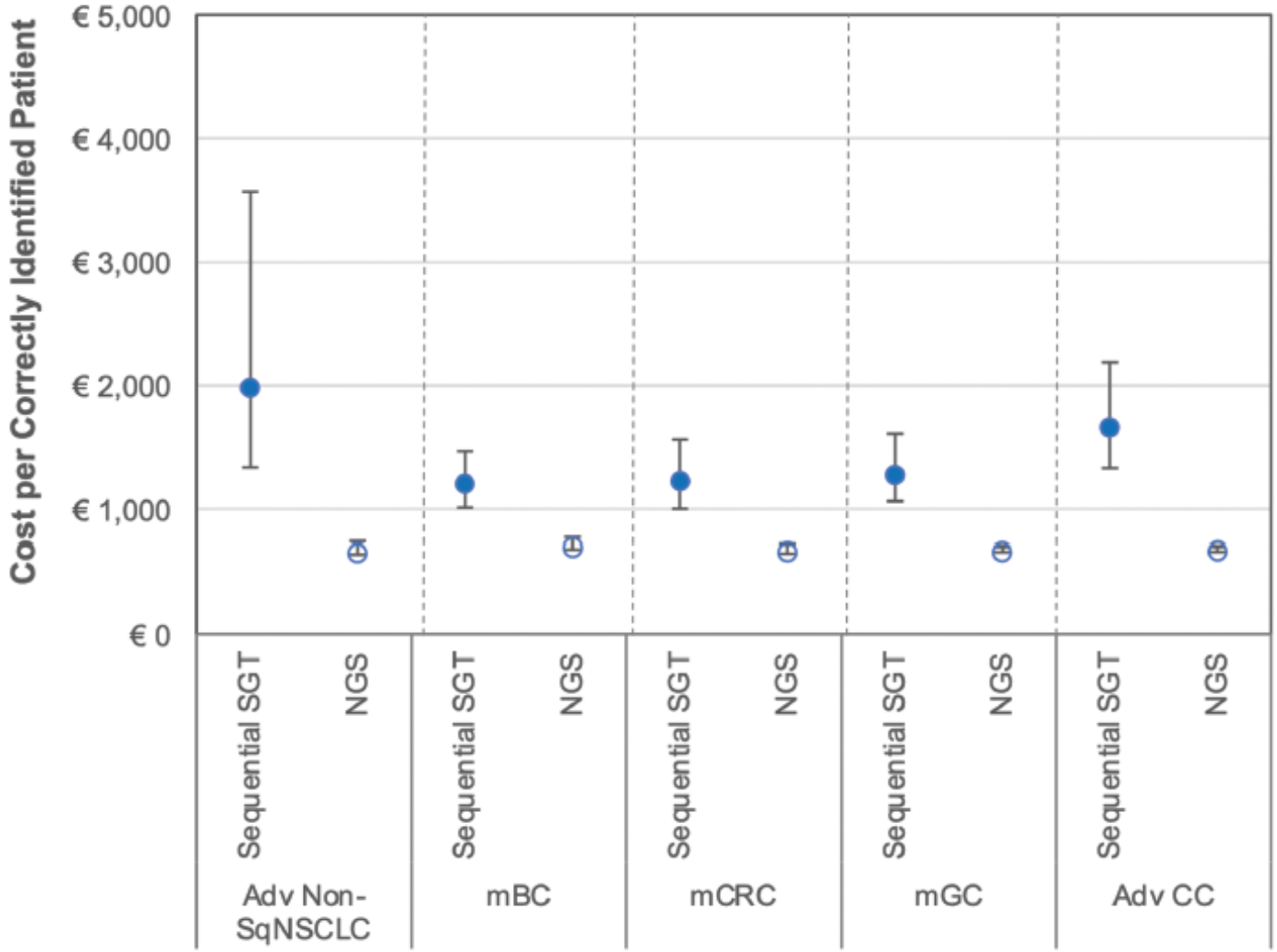
Yatabe Y. Histopathology 2024

Predictive biomarker testing methods



Pujol N. J Pers Med 2022

Predictive biomarker testing cost



Stenzinger A. Oncologist 2023

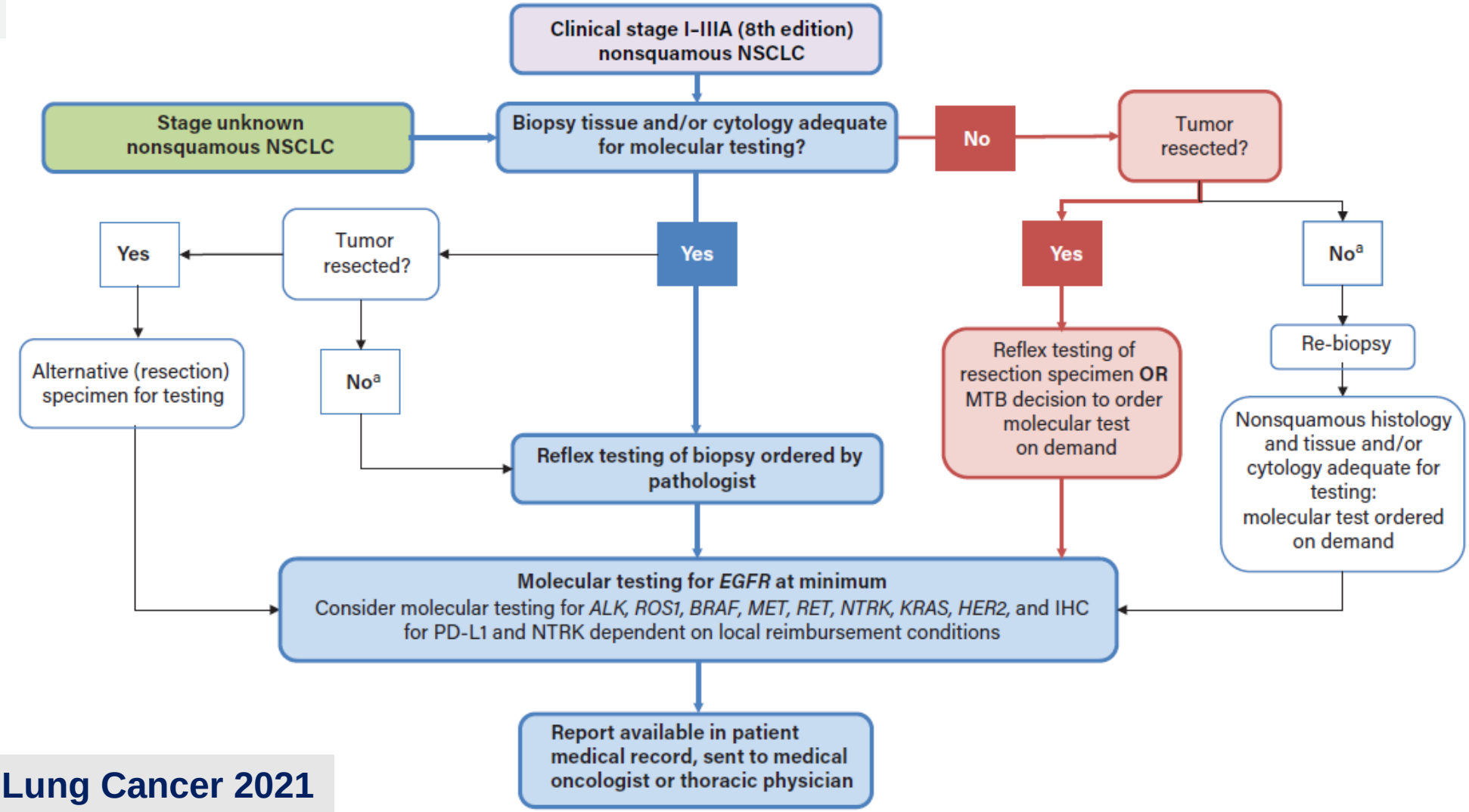
Predictive biomarker testing - status

Biomarker	Austria	Belgium	Czech Republic	England[&]	France	Germany	Netherlands	Portugal	Slovenia	Spain	Sweden
EGFR	2013	2010	2012	n/a	2008	2012	2011	2013	2010	2012	2009
ALK	2013	2013	2013	n/a	2012	2012	2015	2013	2013	2012	2013
ROS1	2013	2017	2016	n/a	2012	2015	2015	* 2022	2015	2020	2014
BRAF V600	2020	2017	*	n/a	2012	2016	2015	* 2022	2018	2020	2018
RET	2020	2022	*	n/a	2018	2016	2015	* 2022	2022	2023	2023
MET exon 14	2020	2020	*	n/a	2012	2018	2020	* 2022	2022	2023	2023
MET amp	2020	2023		n/a	[¶] 2023	[#] 2018		* 2022		2023	[§] 2023
KRAS G12C	2020	2021	*	n/a	2008	2021	2015	* 2022	2022	2023	2022
NTRK	2020	2021	*	n/a	2018	2016	2020	* 2022	2020	2023	2022
HER2/*ERBB2*	2020	2016	*		2022	2016	2015	* 2022	2022	2023	* 2018
NRG1	2020				2022	[#] 2019	2020				* 2019
PD-L1	2020	2018	2016	n/a	2015	2016	2020		2017	2020	2015

●, required by current national guidelines; ●, recommended by current national guidelines; ●*, not specified in current national guidelines but recommended by national expert consensus; ●, testing not recommended or biomarker not present in current national guidelines.

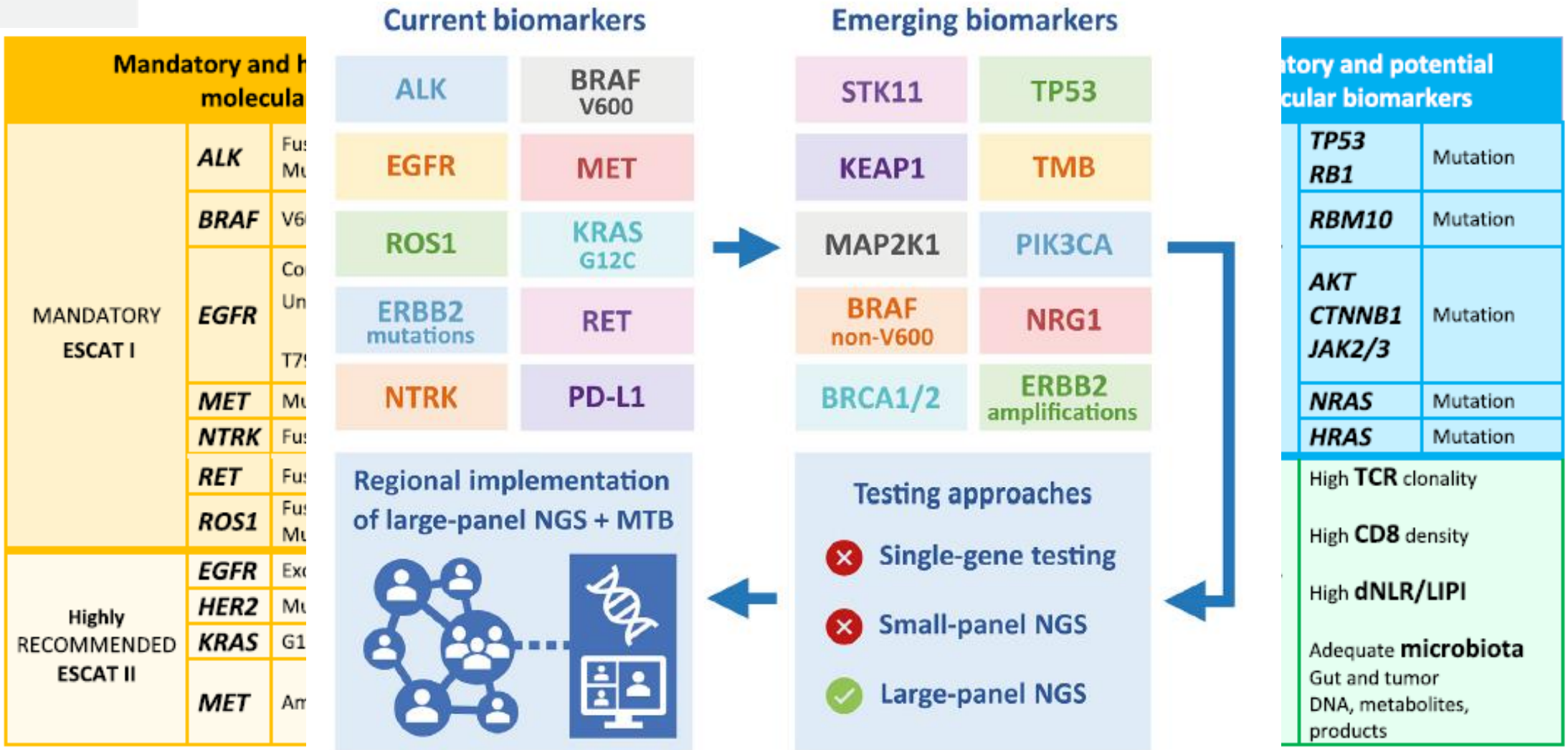
De Jager V. Lancet Reg 2024

Reflex testing in early stages



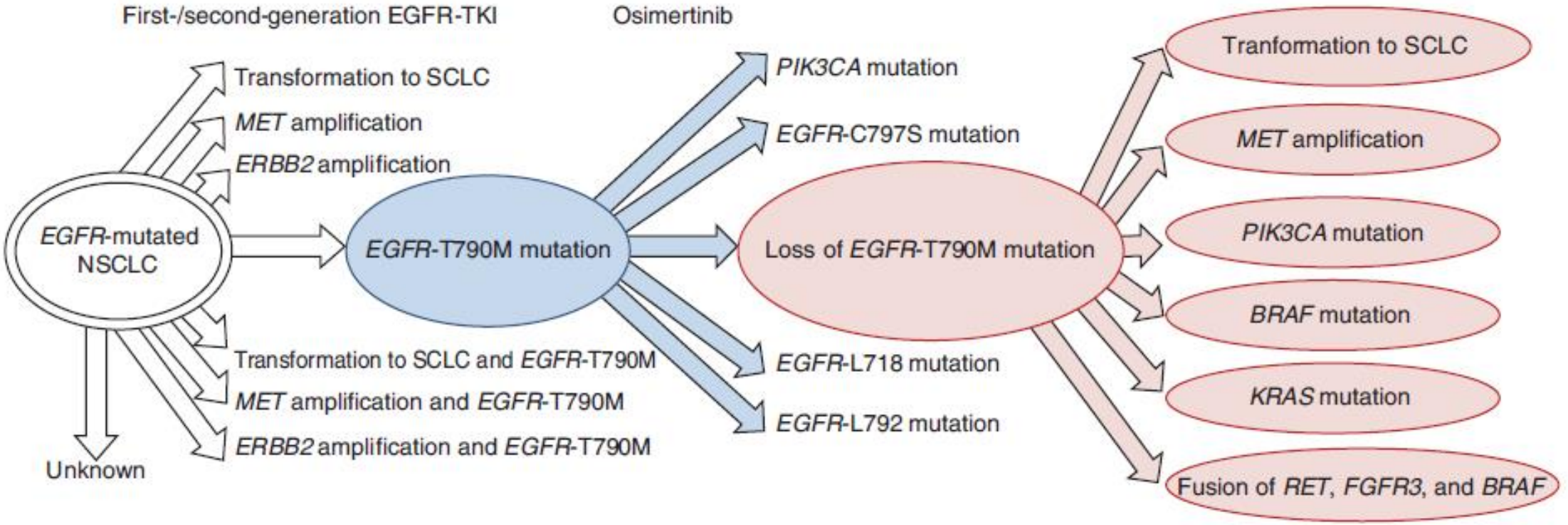
Aggarwal C. Lung Cancer 2021
Gosney JR. ESMO open 2023

NSCLC – predictive biomarker testing



Hofman P. Virchows Archiv 2024
De Jager V. Lancet Reg 2024

Mechanisms of resistance

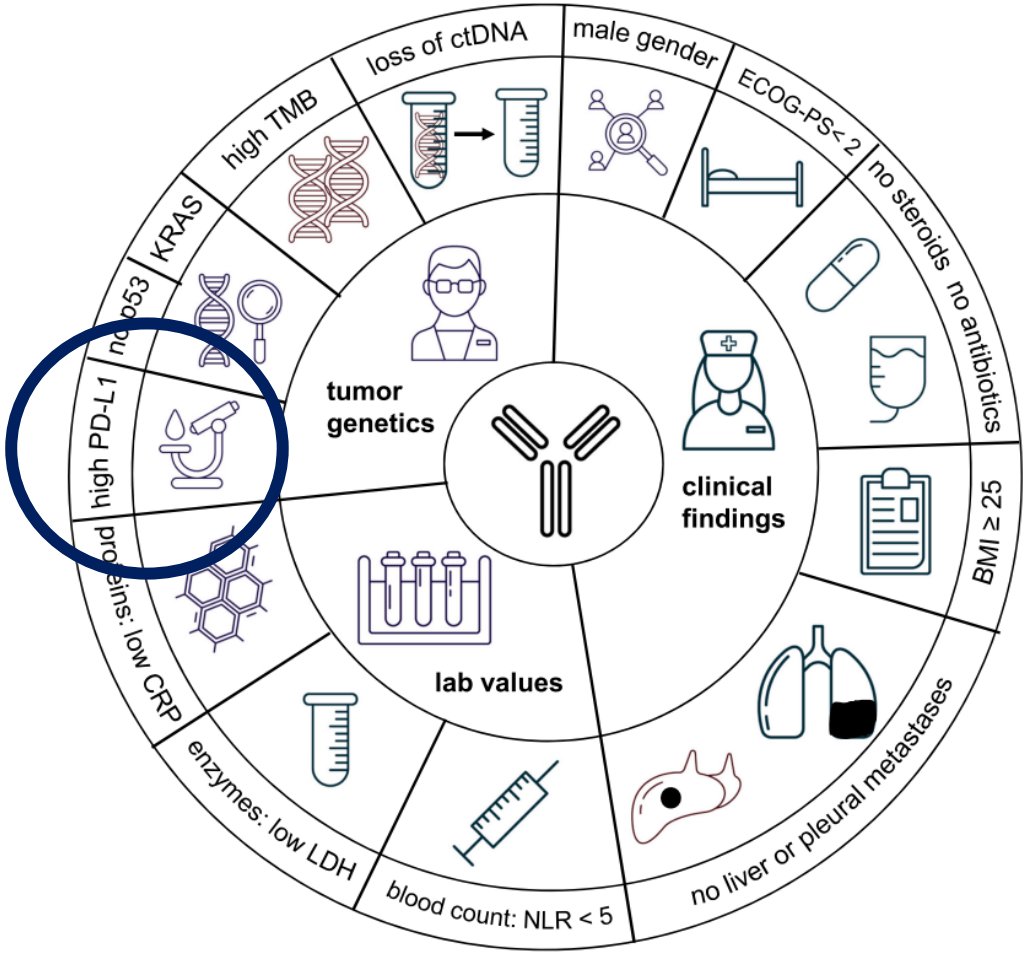


Saller JJ. Cold Spring Harb Perspect Med 2022

PD-L1 testing

- Established predictive biomarker for immunotherapy
  (CPI targeting PD-1 & PD-L1)
- Higher the PD-L1 expression, higher the clinical ORR
  15-25% response, ↑PFS and OS
- Testing technology is IHC
- Positivity defined with >1% PD-L1 expression on tumor cells

Positive predictive and prognostic factors for ICI therapy



Biomarker of Interest	Assay Details	Outcomes/Literature Support
Tissue-based		
PD-L1 Expression	Immunohistochemisty (IHC) to determine proportion of PD-L1 positivity/expression.	Greater PD-L1 positivity/expression associated with improved outcomes in first-line and second-line advanced NSCLC trials using IO.[13–15]
Tumor Mutational Burden (TMB)	Whole exome sequencing or FoundationOne CDx assay to quantify the number of somatic mutations per coding area of a tumor genome.	Higher TMB associated with improved PFS, though not OS, with first-line ipilimumab/nivolumab in advanced NSCLC, irrespective of PD-L1 expression. [17]
Tumor Infiltrating Lymphocytes (TILs)	Assessment of lymphocyte infiltration seen within tumor tissue.	Higher TIL density associated with improved survival in NSCLC.[47,48] Extent of PD-L1 expression on TILs associated with response to atezolizumab. [50]
Tumor Specific Genotypes	Fluorescence in situ hybridization (FISH) or next generation sequencing to identify genomic alterations in EGFR, ALK, KRAS etc.	EGFR and ALK mutated tumors associated with poorer outcomes in second-line IO trials. [54] STK11/LKB1 co-mutation associated with IO resistance.[61]
Gene Expression Signatures	Multi-gene profiling to identify immunogenic gene signatures, e.g. activated T-cell, IFN-y	High expression of T-effector and INF-y related gene signature associated with improved OS with second-line atezolizumab in advanced NSCLC.[27]
Serum-based		
Complete Blood Count (CBC) Markers (NLR, PLR, etc.)	Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), absolute eosinophil count, and others as calculated from CBC differential.	Higher NLR associated with poorer prognosis in advanced NSCLC.[71,72] NLR correlated to treatment response in second-line nivolumab studies.[74,75]
Blood Tumor Mutational Burden (bTMB)	FoundationOne CDx with quantification of single nucleotide variants, GuardantOMNI CDx assay.	Higher bTMB associated with longer PFS with second-line atezolizumab in advanced NSCLC.[78] High bTMB subgroup with improved OS with first-line tremelimumab/durvulumab.[19]

Brueckl, BMC Cancer 2020
Brodor, Cancer 2020

Neoadjuvant / perioperative treatment

- NSCLC resectable
- Early stages (IIa – IIIb)
  - Tumor size > 4 cm
  - Tumor size < 4 cm + lymph node metastasis
- Chemotherapy + immunotherapy
- **TPS PD-L1 >1%*** → IHC
  * EMA approval (nivolumab)
- **EGFR & ALK neg** → RT-PCR + IHC/FISH or RT-PCR or NGS

For patients being considered for neoadjuvant or adjuvant systemic therapy,
at a minimum, determination of EGFR and ALK alteration status is required.
Tumor proportion score measurement for determination of PD-L1 status should also be considered.

- Other biomarkers testing is encouraged → NGS

IASLC consensus. JTO 2024.
NCCN v3. 2025

- Upfront /reflex in primary specimen
  - Should be considered
  - Accelerate time to treatment
  - Optimal lab processing logistics

or/and

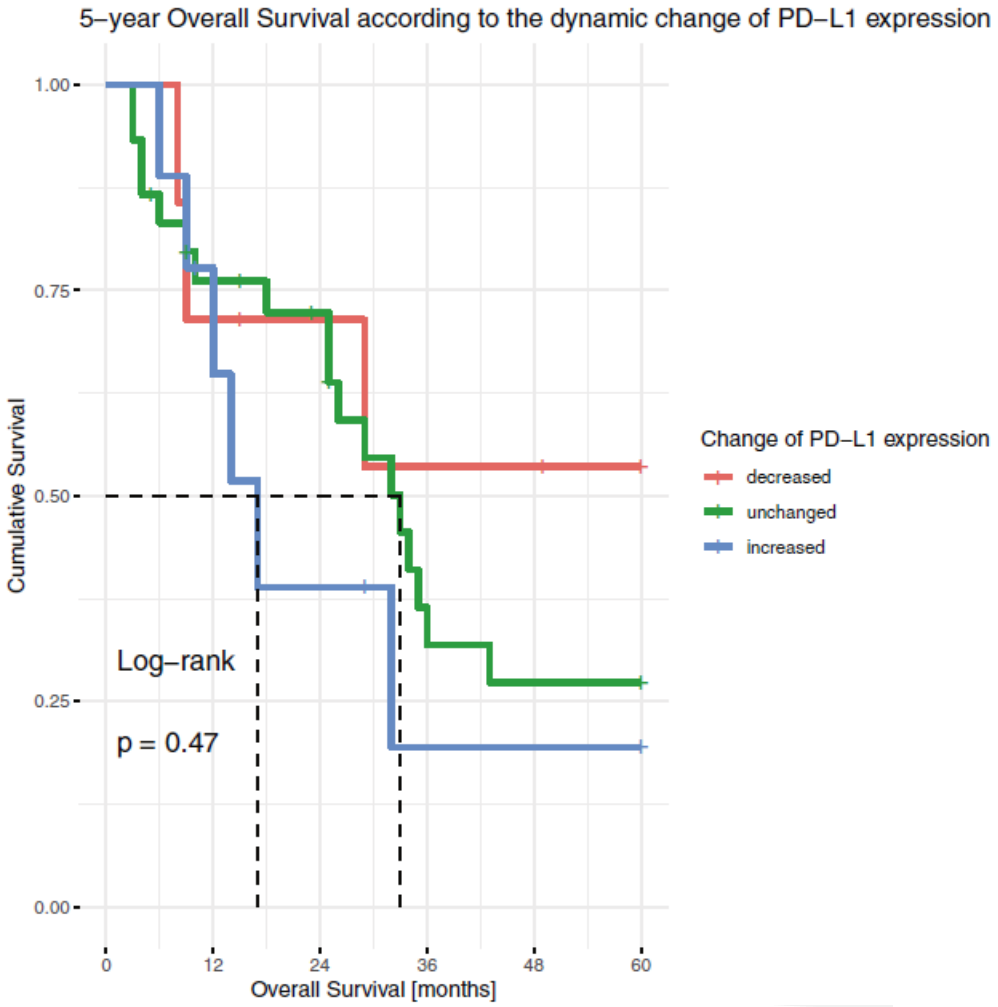
- Retesting in surgical specimen
  - For adjuvant treatment decision

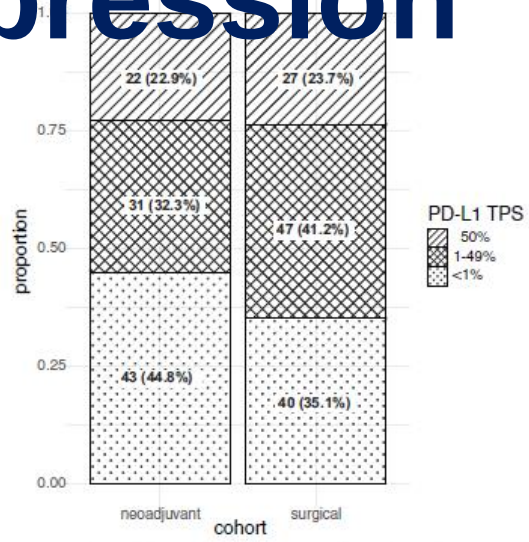
PD-L1 TPS
- **metric for regulatory approval**
- **lack of outcome prediction in early stage NSCLC**

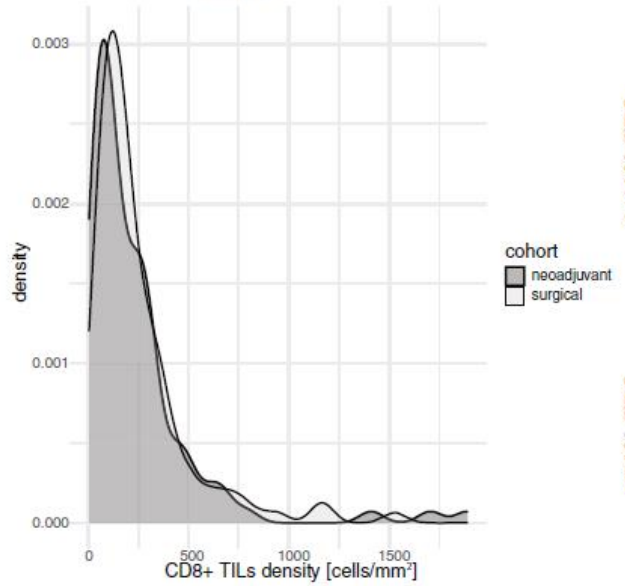
Patients with stage II or IIIA EGFR- and ALK-wild-type disease
who have undergone complete resection followed by chemotherapy
should be considered for adjuvant immunotherapy based on PD-L1 results as follows:
- PD-L1 < 1%: Discourage
- PD-L1 1%–49%: Consider
- PD-L1 > 50%: Recommend

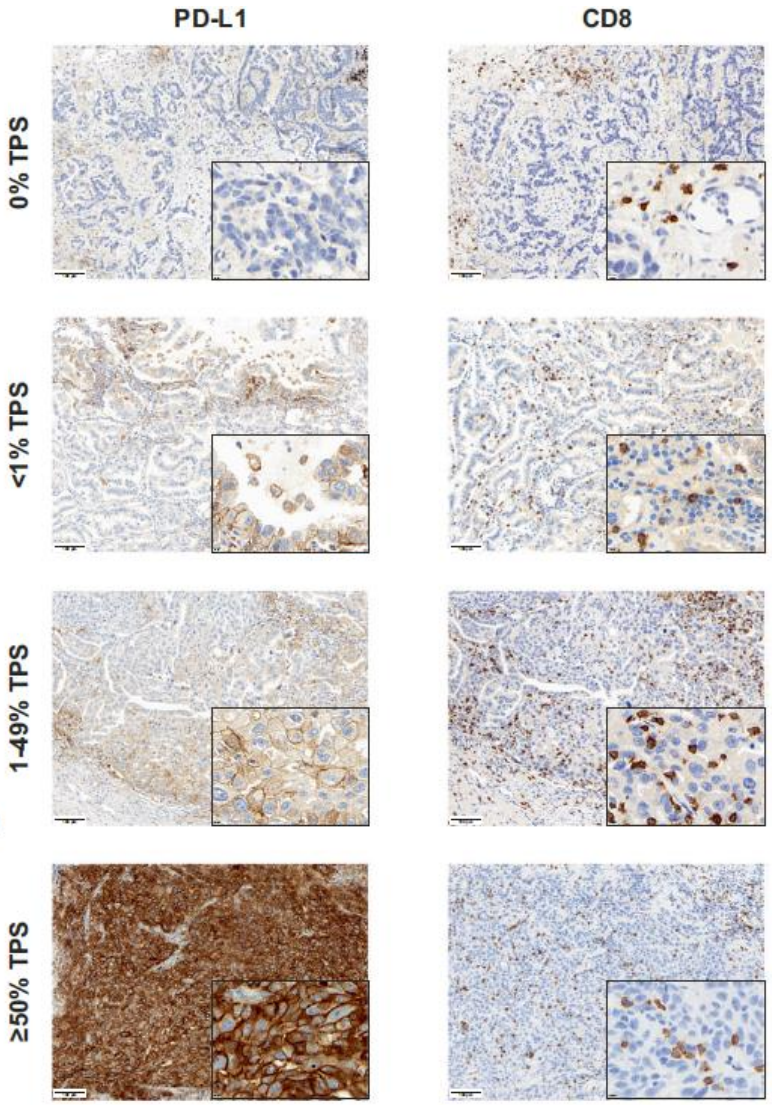
IASLC consensus. JTO 2024

Effect of neoadjuvant therapy on PD-L1 expression



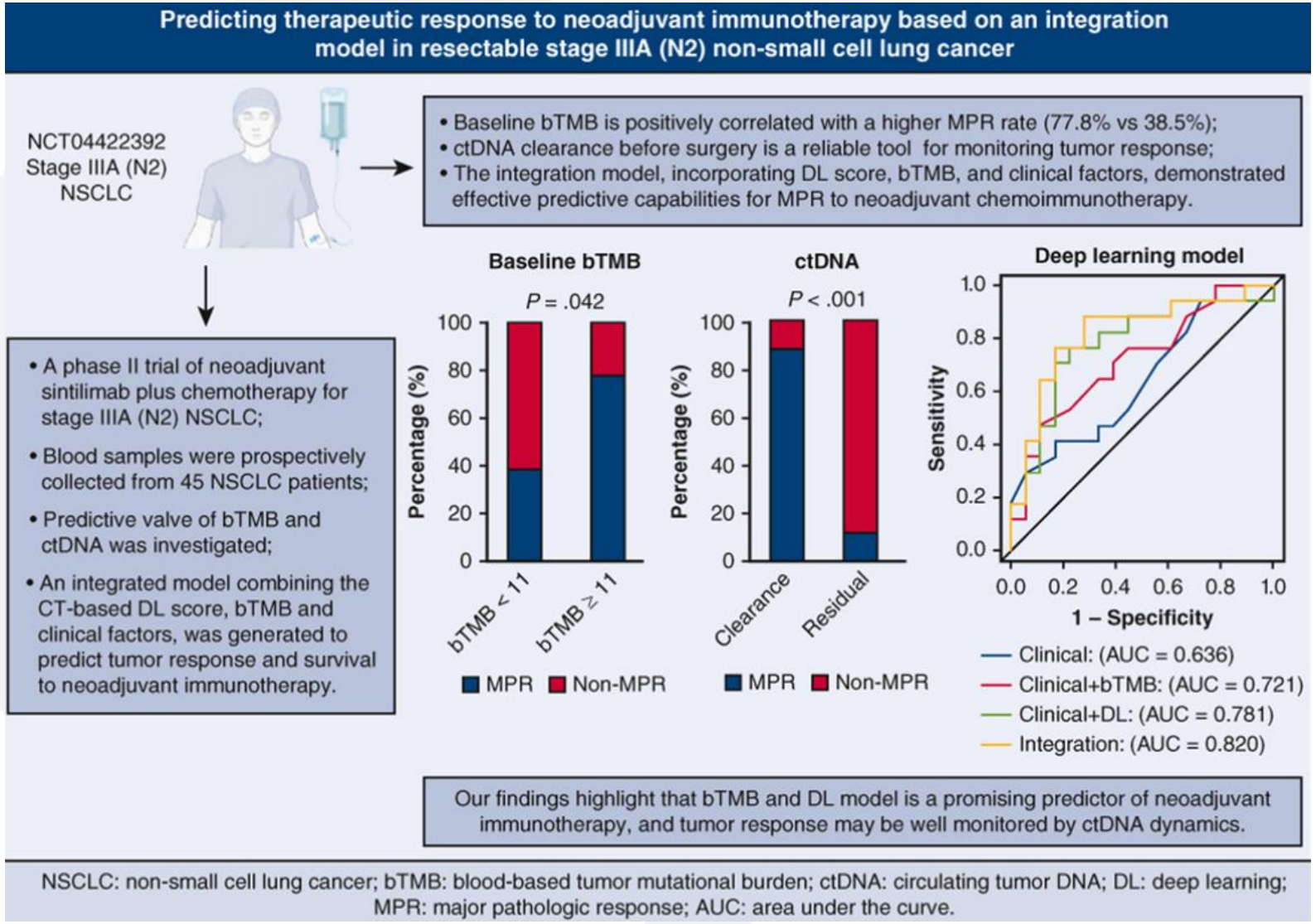






Zens, Modern Pathol 2023

TMB instead of PD-L1



Xu L. J Thorac Cardiovasc Surg 2025

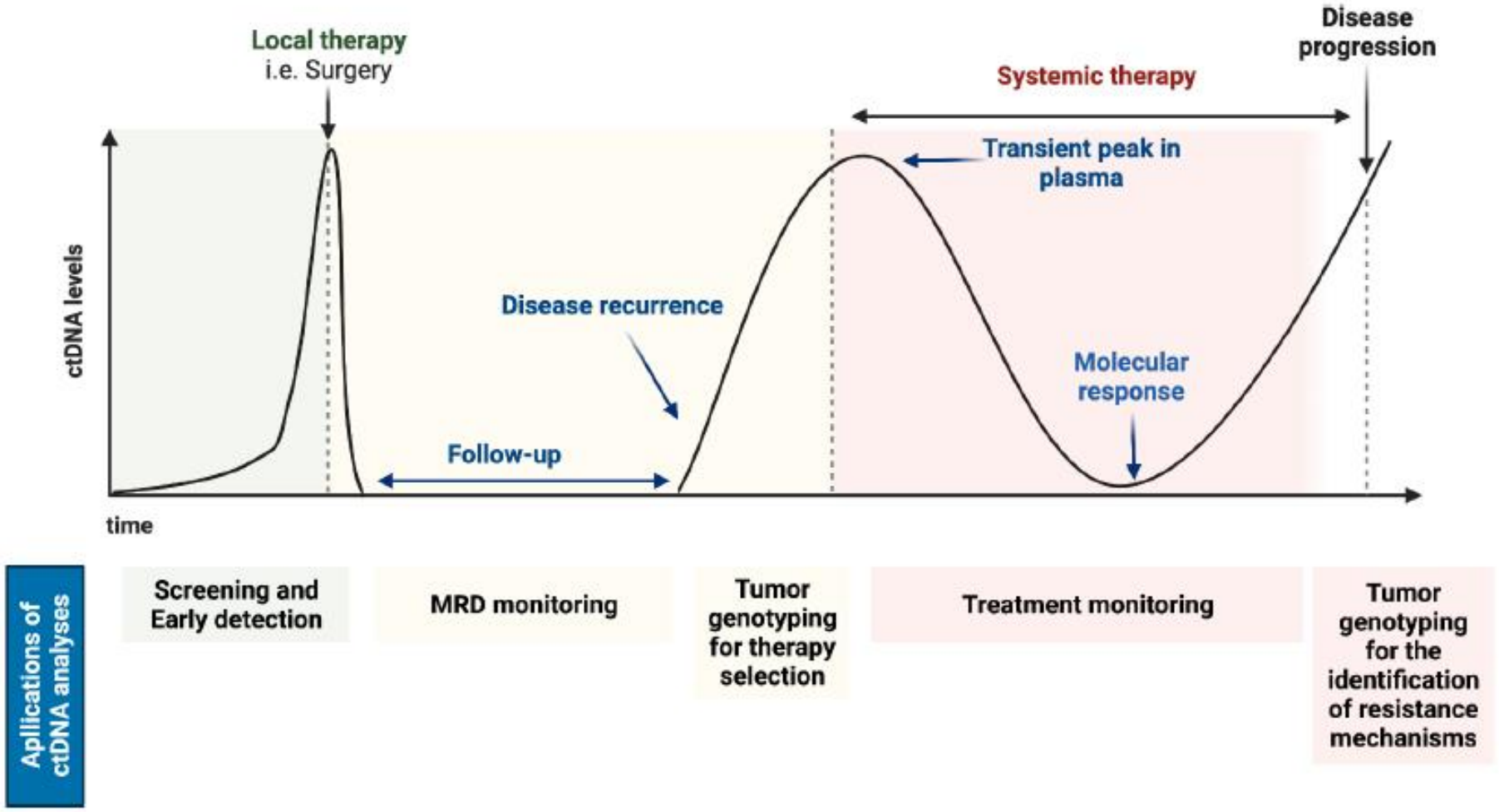
Programmed Death Ligand-1 and Tumor Mutation Burden Testing of Patients With Lung Cancer for Selection of Immune Checkpoint Inhibitor Therapies

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

Guideline Statement	Strength of Recommendation
1. In patients with advanced NSCLC, pathologists should use a validated PD-L1 IHC expression assay, in conjunction with other targetable genomic biomarker assays where appropriate, to optimize selection for treatment with ICIs.	Strong recommendation
2. Pathologists should ensure appropriate validation has been performed on all specimen types and fixatives. *Note:* Specific validation requirements are out of the scope of this guideline, and laboratories should refer to the Principles of Analytic Validation of Immunohistochemical Assays Guideline[57] for details on how to validate IHC specimens.	Conditional recommendation
3. When feasible, pathologists should use clinically validated PD-L1 IHC assays as intended.	Conditional recommendation
4. Pathologists who choose to use LDTs for PD-L1 expression should validate according to the requirements of their accrediting body.	Strong recommendation
5. Pathologists should report PD-L1 IHC results using a percentage expression score.	Conditional recommendation
6. Clinicians should not use tumor mutation burden alone to select patients with advanced NSCLC for ICIs, based on insufficient evidence in this population.	Conditional recommendation

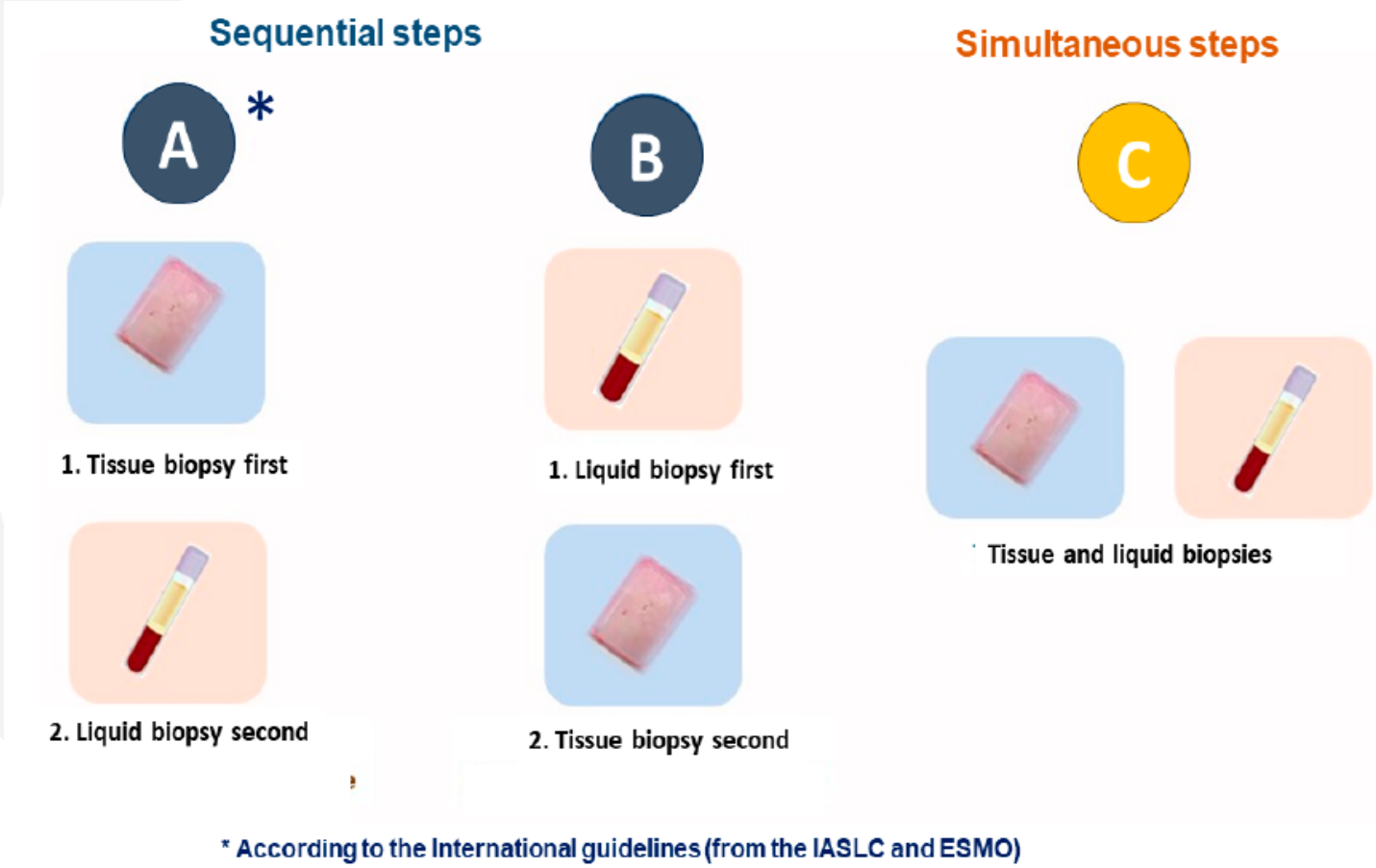
Scholl, Arch Pathol Lab Med 2024

Liquid biopsy



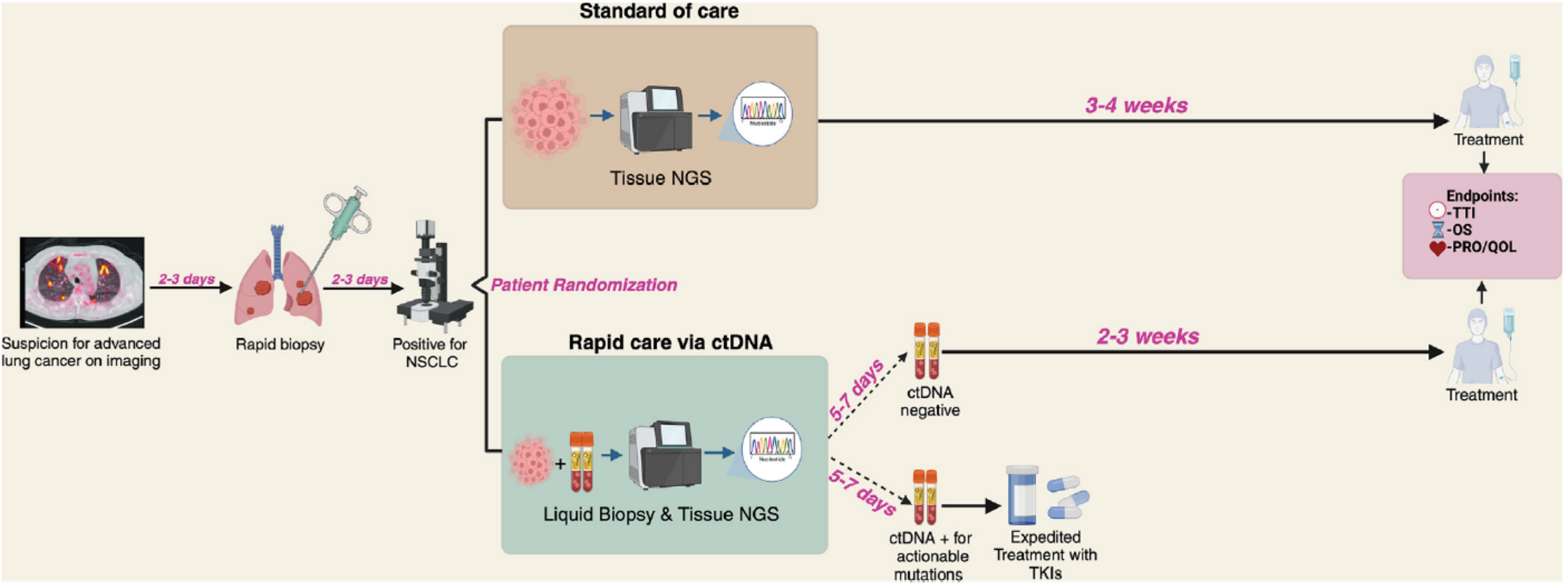
Malapelle U. Lung Cancer 2022

Liquid biopsy in NSCLC advanced stage



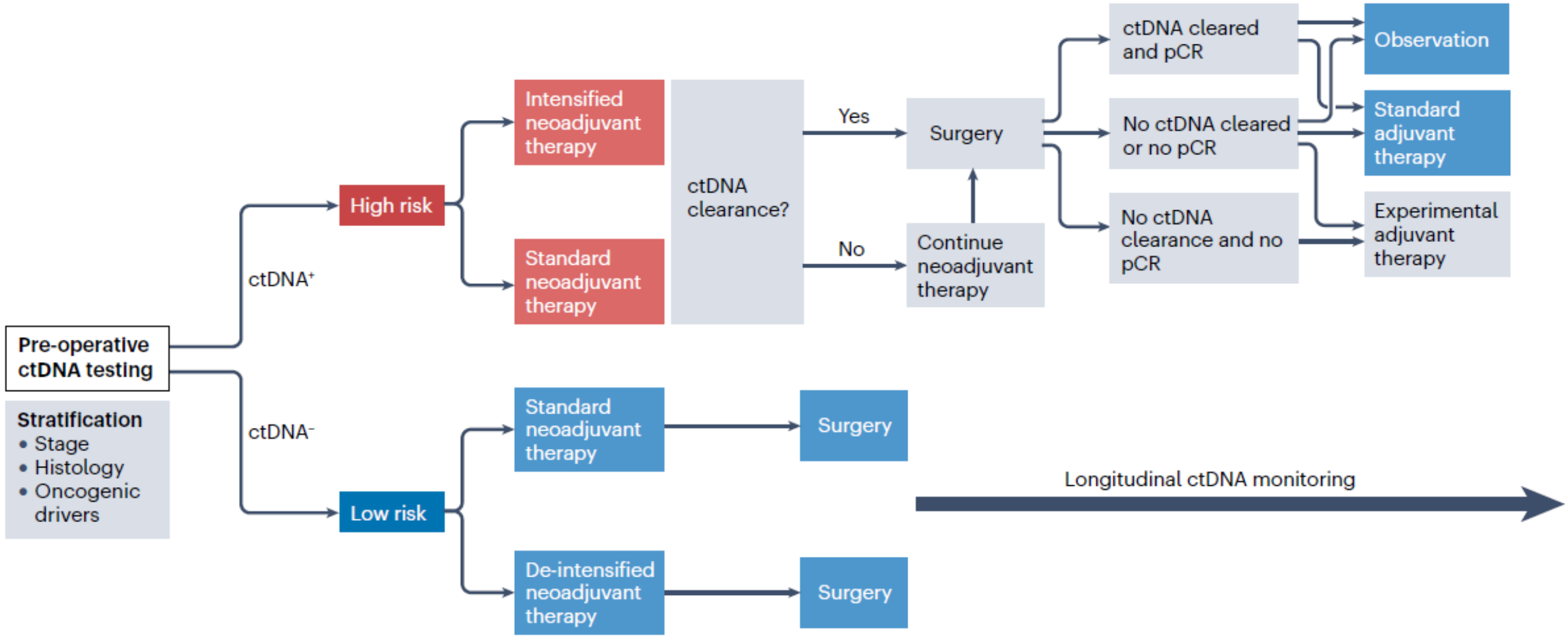
Hofman P. Cancers 2024

Shorter time to treatment with liquid biopsy



Parikh K. JTO 2025

ctDNA in neoadjuvant setting

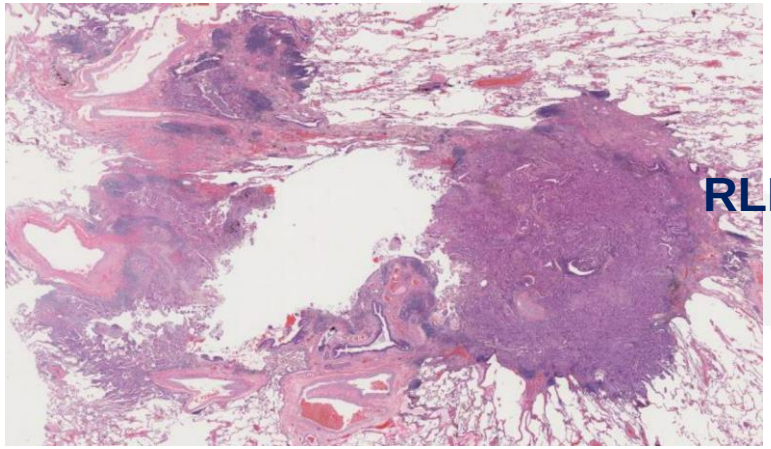


Normanno N. Nature Reviews Clin Oncol 2025

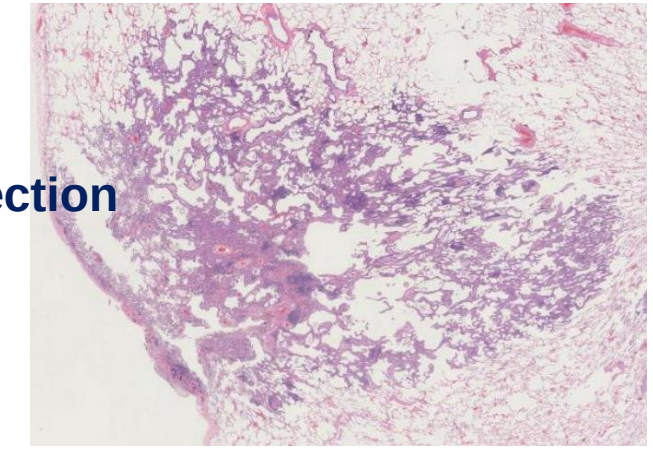
Multiple lung tumors

- D = 15 mm
- ADC G2
- Point mutation RAF1, p.S259T, VAF 5,1 %

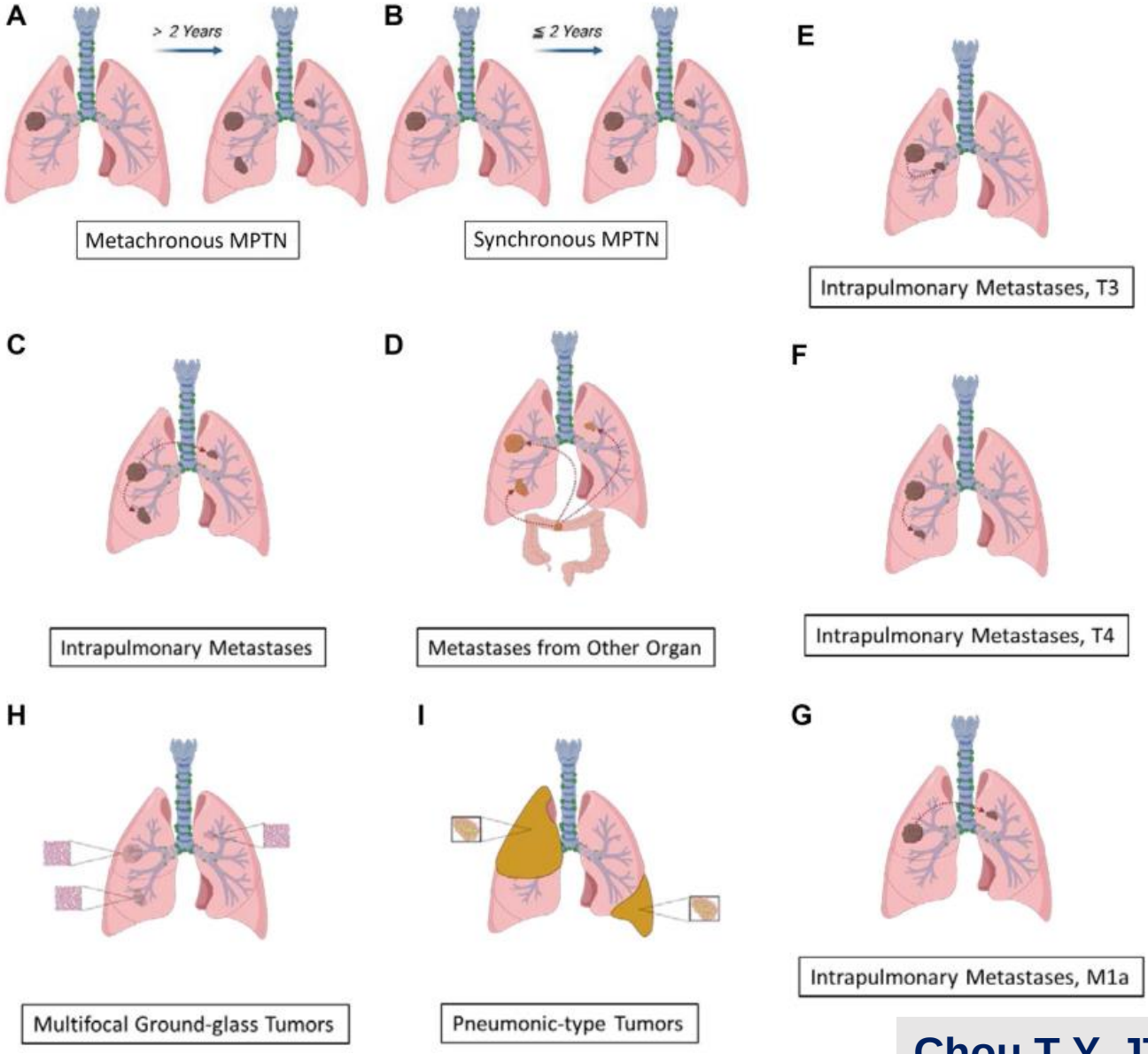
- D = 14 mm
- MIA
- Point mutation KRAS, p.G12V, VAF 15,5 %

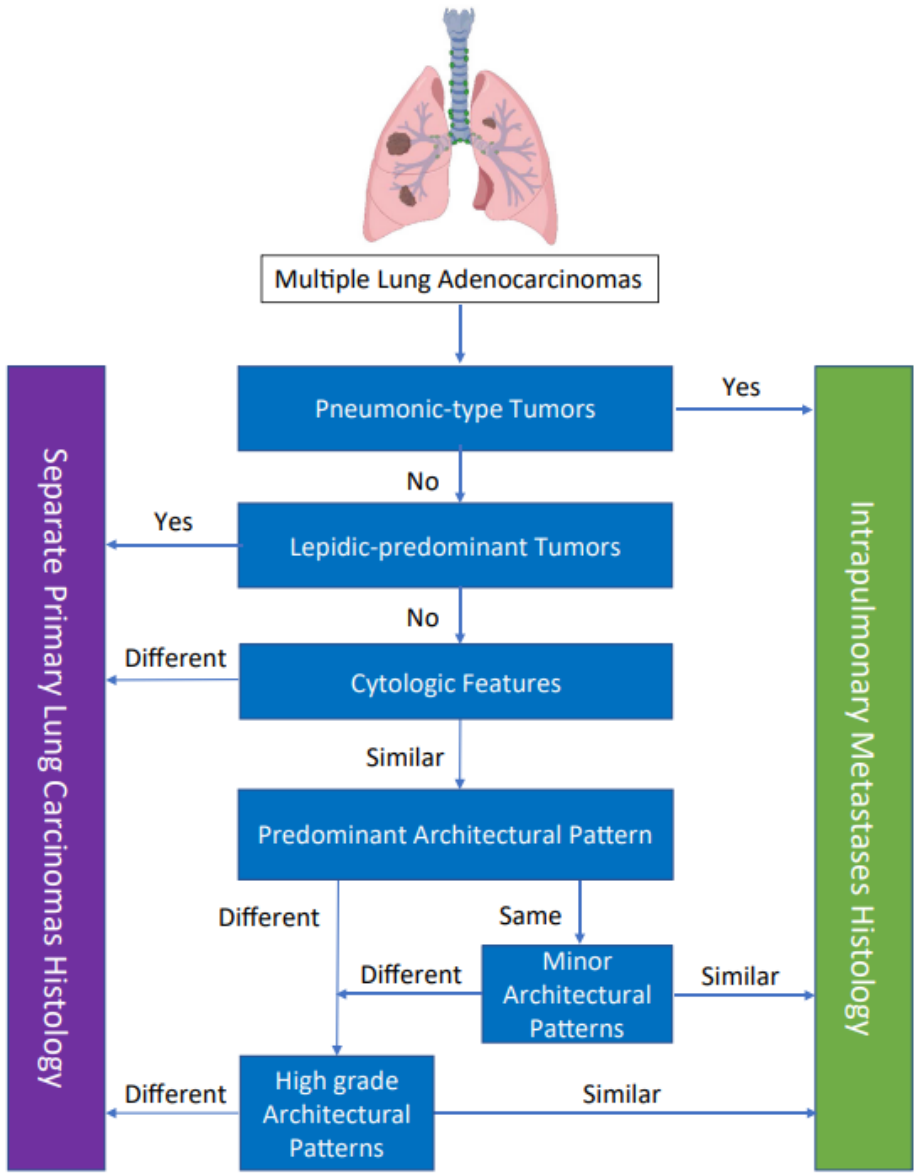


RLL lobectomy + RUL wedge resection



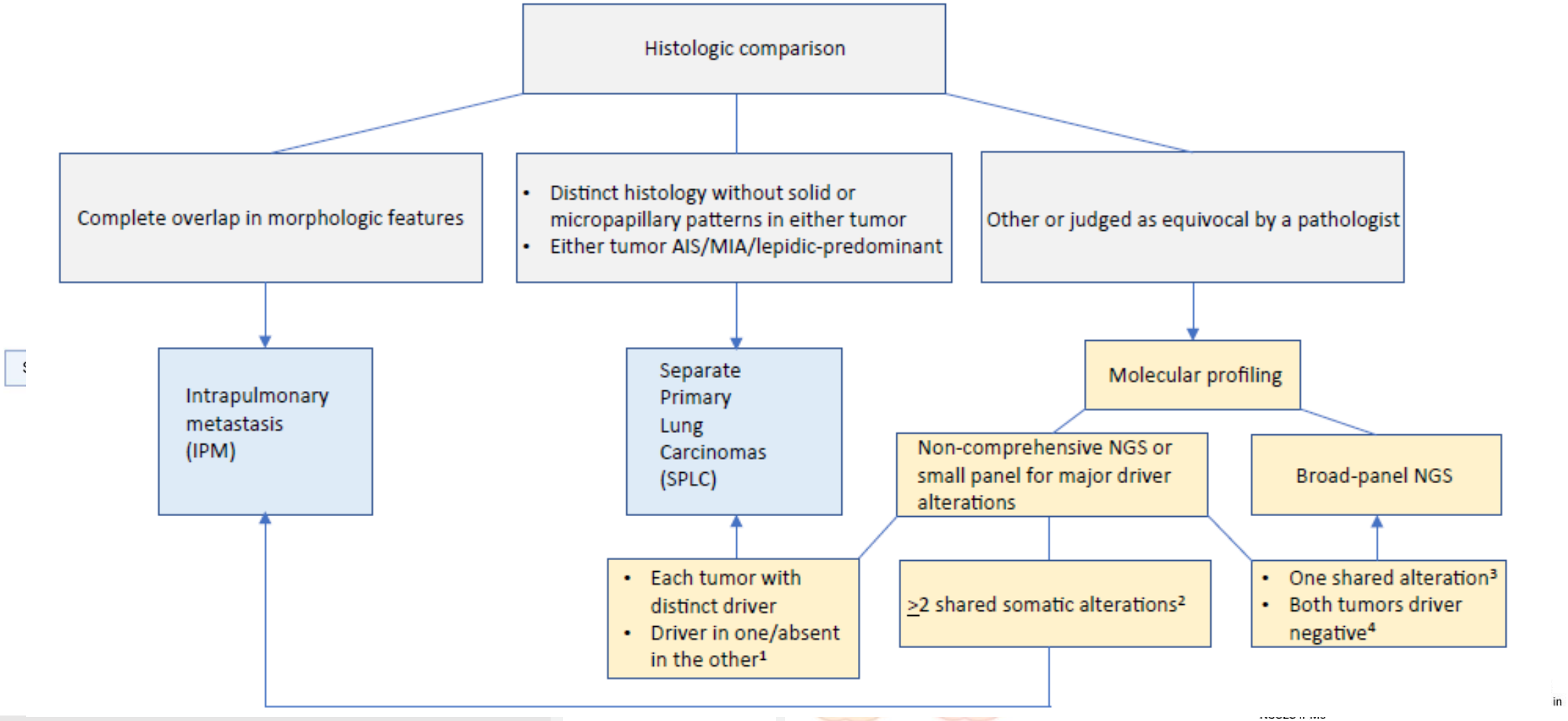
Primary LADC vs IPM





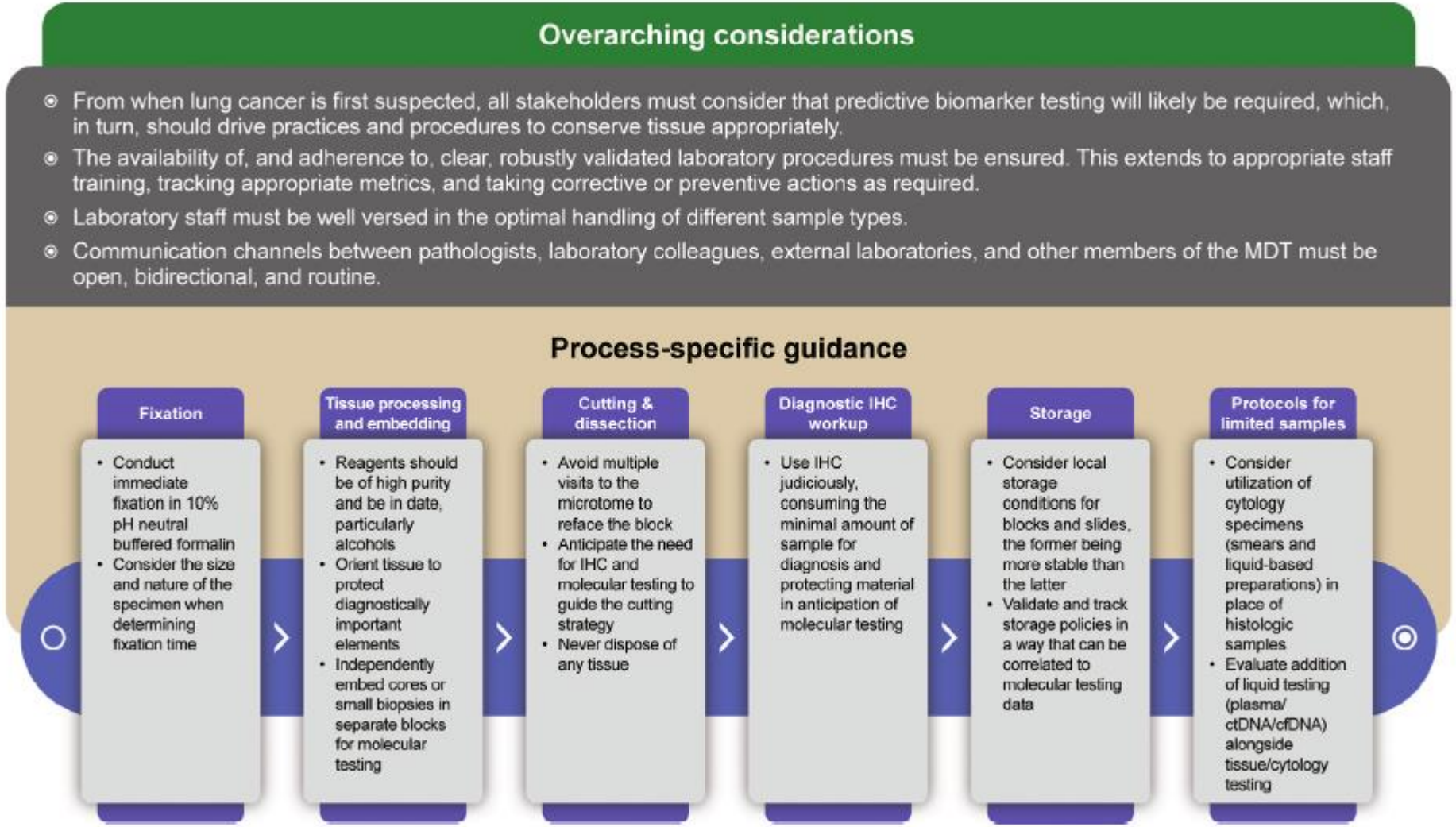
Chou T-Y. JTO 2024

Multiple NSCLC



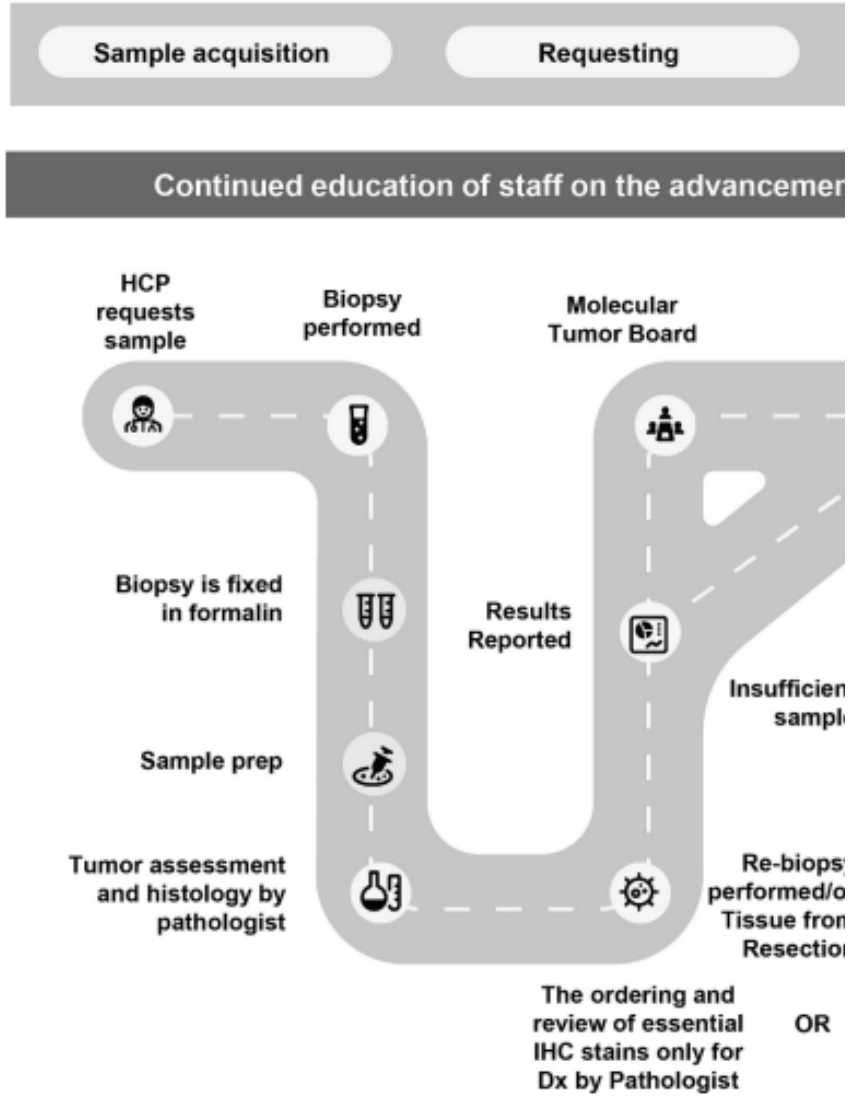
Olteanu G-E. Adv Anat Pathol 2024
Chang J. Mod Pathol 2024

Optimizing tissue path



Kerr K. Histopathology 2024

Predictive biomarker testing implementation



Challenge	Recommendations
Biomarker testing in operable NSCLC is not routinely requested	- More frequent revision and harmonization of guidelines across various markets and regions. - Education of pathologists and physicians and laboratories to ensure they are aware of newly available therapies and associated testing. - Regulations to ensure that reimbursement for pertinent biomarker testing becomes available simultaneously with the approval of targeted drugs.
Insufficient tissue for biomarker testing from the initial diagnostic biopsy	- Ensuring that tissue conserving practices are followed at diagnosis to allow sufficient material for downstream biomarker testing. - Utilisation of alternative sample types such as cytology or liquid biopsies when tissue is not available.
Biomarkers are tested for individually instead of in within a multigene NGS panel	- Implementation of frameworks (upscaling of capacity, quality assurance, availability of reimbursement) to allow access to NGS testing. - Formation of multi-disciplinary virtual molecular tumour boards (MTBs) for interpreting complex data to guide treatment decisions.

Hofman P. Lung Cancer 2025

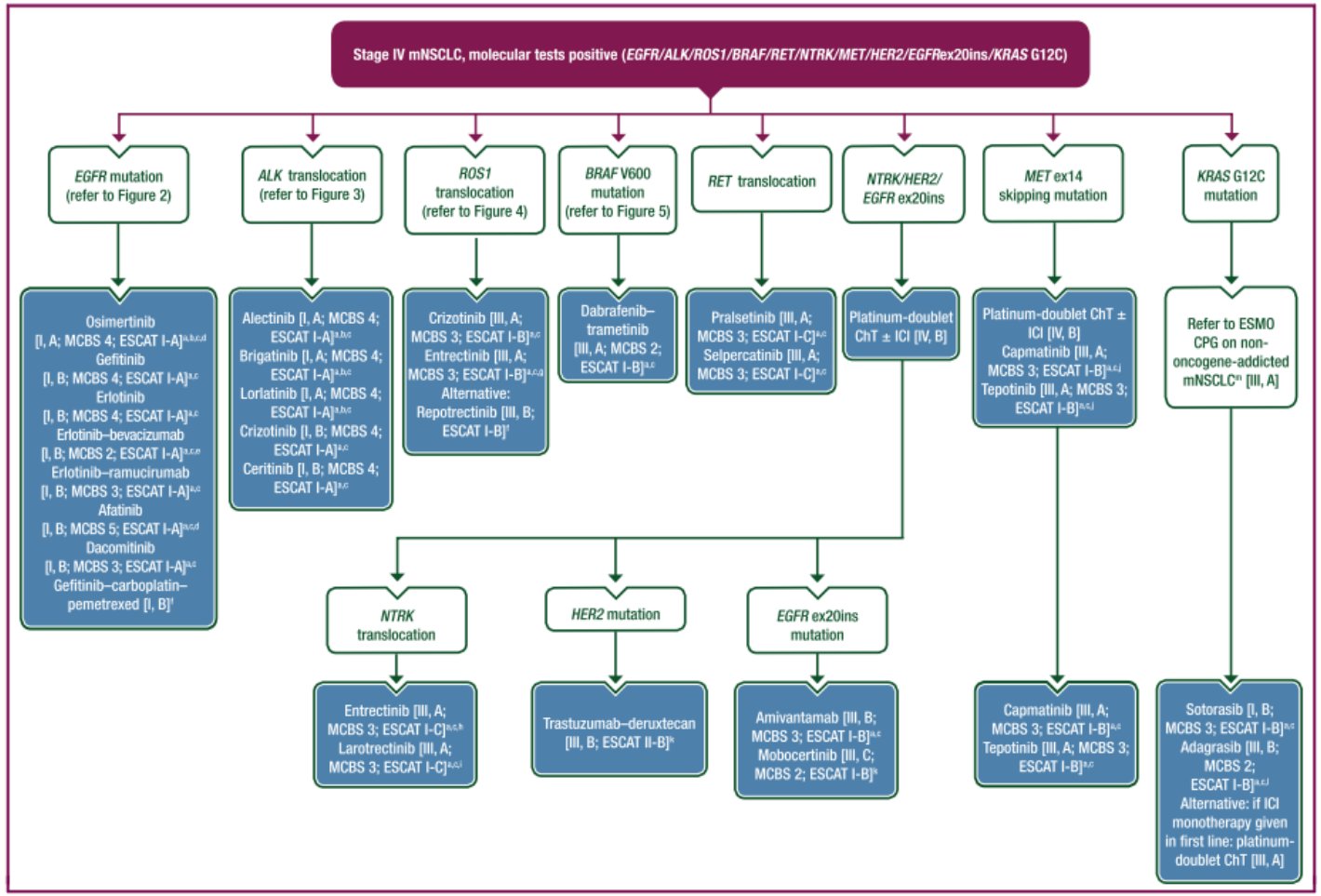
Guidelines & recommendations - molecular

IASLC
Conquering Thoracic Cancers Worldwide

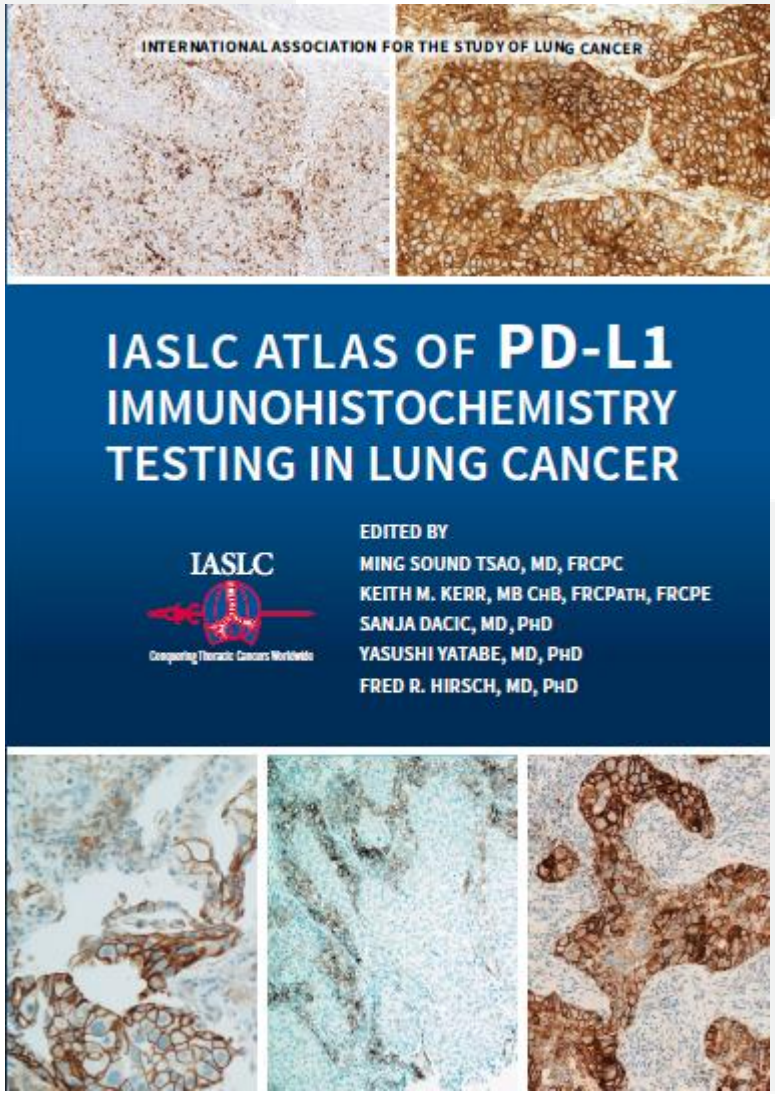
IASLC ATLAS OF MOLECULAR TESTING FOR TARGETED THERAPY IN LUNG CANCER

ESMO guidelines 2023

Newly diagnosed patients	*EGFR*	Any validated method to cover mutations in exon 18-21 (DNA NGS preferred)
	ALK	RNA NGS; IHC ± molecular confirmation (NGS, FISH)
	ROS1	RNA NGS; IHC screening, molecular confirmation essential (NGS, FISH)
	RET, *MET*, *NTRK*, *ERBB2* (*HER2*), *KRAS*, *BRAF*	DNA/RNA NGS panel testing
	PD-L1	IHC
	EGFR T790M, *MET* (as appropriate) (cfDNA/tissue DNA)	PCR/NGS/ISH
Relapsed patients on targeted therapy	*EGFR* (category 1)	Broad molecular profiling (NGS)[c]



Guidelines & recommendations – PD-L1



- PD-L1 IHC should be systematically determined in advanced NSCLC [I, A]

PD-L1 expression	IHC to identify PD-L1 expression at the appropriate level and on the appropriate cell population(s) as determined by the intended drug and line of therapy. Only specific trial assays are validated. Internal and external quality assurance are essential	To enrich for those patients more likely to benefit from anti-PD-1 or anti-PD-L1 therapy. For pembrolizumab, testing is a companion diagnostic for nivolumab and atezolizumab, testing is complementary	I, A

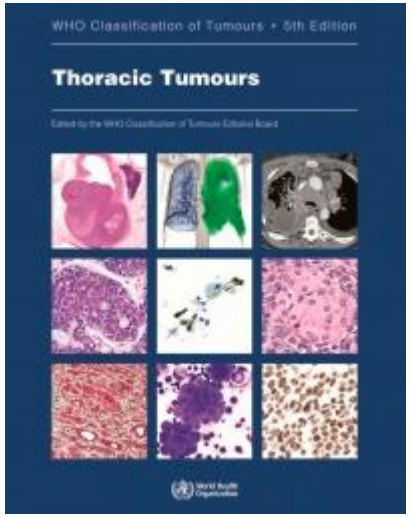
ESMO guidelines 2019

REVIEW ARTICLE

PD-L1 Testing for Lung Cancer in 2019: Perspective From the IASLC Pathology Committee

JTO 2019

testing for PDL1 is now recommended
for advanced-stage non-neuroendocrine carcinomas



Never ending story

Variable	ctDNA [91–93]	miRNA [94–96]	bTMB [54,81,97,98]	Immunological Markers [20,99,100]
Type of Biomarker	Genetic (circulating DNA)	Genetic (non-coding RNA)	Genetic (mutational burden)	Protein (immune proteins)
Detection Method	NGS, digital PCR	Real-time PCR, microarrays	NGS, digital PCR	IHC, flow cytometry
Clinical Utility	Diagnosis, prognosis, monitoring	Prognosis, monitoring	Prognostic, predictive	Diagnostic, predictive
Prognostic and Predictive Aspects	High sensitivity for early detection	Correlates with immunotherapy response	Predicts response to specific immunotherapies	Expression correlated with survival and response
Variability Factors	Influenced by tumor burden, detection techniques	Influenced by sample conditions	Requires standardization in measurement	Sensitive to detection methods and immune status
Advantages	Non-invasive, high sensitivity	Non-invasive, easily quantifiable	Information on tumor heterogeneity	Directly related to mechanisms of action of therapies
Limitations	Cost, need for sequencing	Inter- and intra-individual variability	Influenced by technical and biological factors	Requires validation for specific interpretation
Cost-Effectiveness	Moderate–high	Low–moderate	High due to sequencing technologies	Moderate, depends on the marker and method
Usage Recommendations	Widely recommended in clinical guidelines	In research, some clinical applications	Recommended in specific contexts	Emerging use, supported by recent studies
Recent Innovations	Advances in digital PCR technology	New miRNAs associated with NSCLC	Improvements in accuracy and cost of NGS	New predictive markers of response to PD-1/PD-L1

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