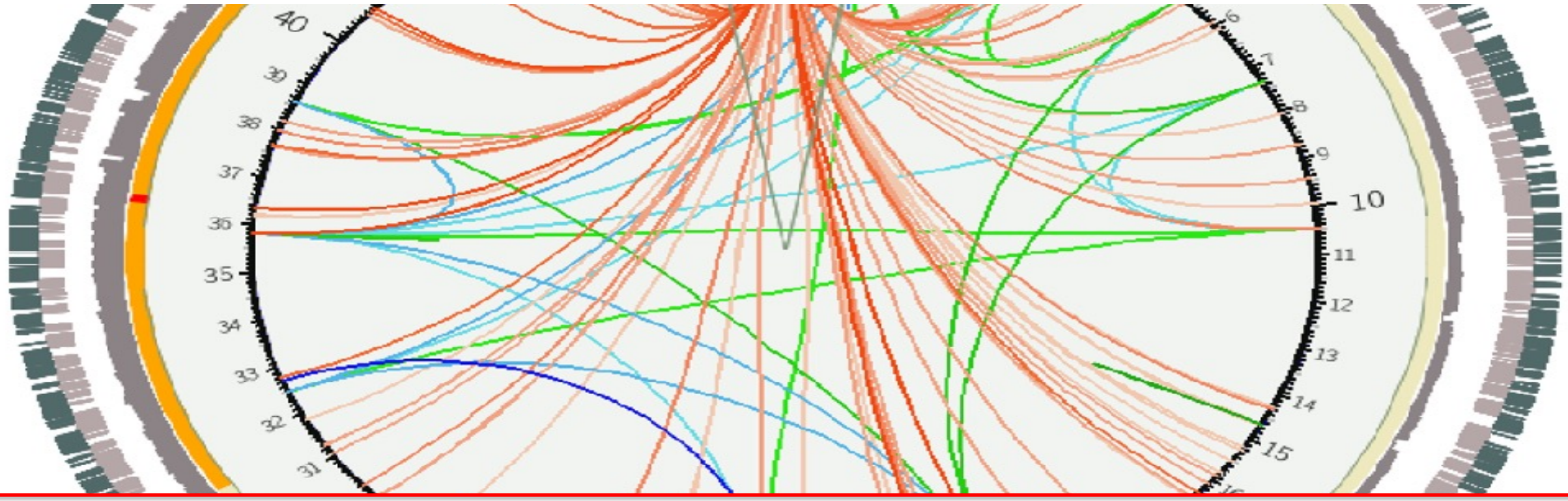


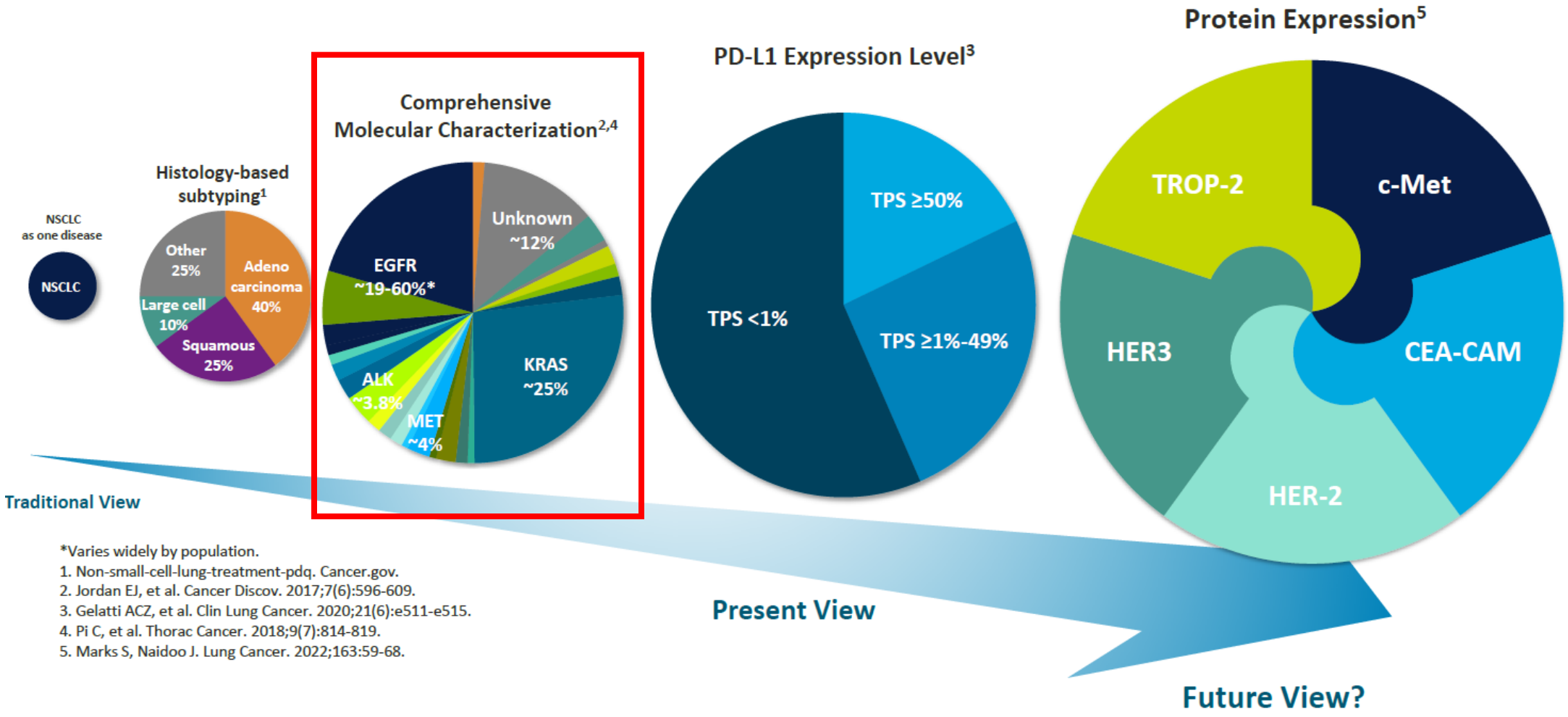
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Challenges in Pathology: Biomarkers

19th Annual New Orleans Summer Cancer Meeting, New Orleans, July 19th-21st, 2024

Ignacio I. Wistuba, M.D
Professor and Chair, Department of Translational Molecular Pathology
The University of Texas MD Anderson Cancer Center, Houston, TX

Paradigms in Lung Cancer Molecular Pathology - 2024

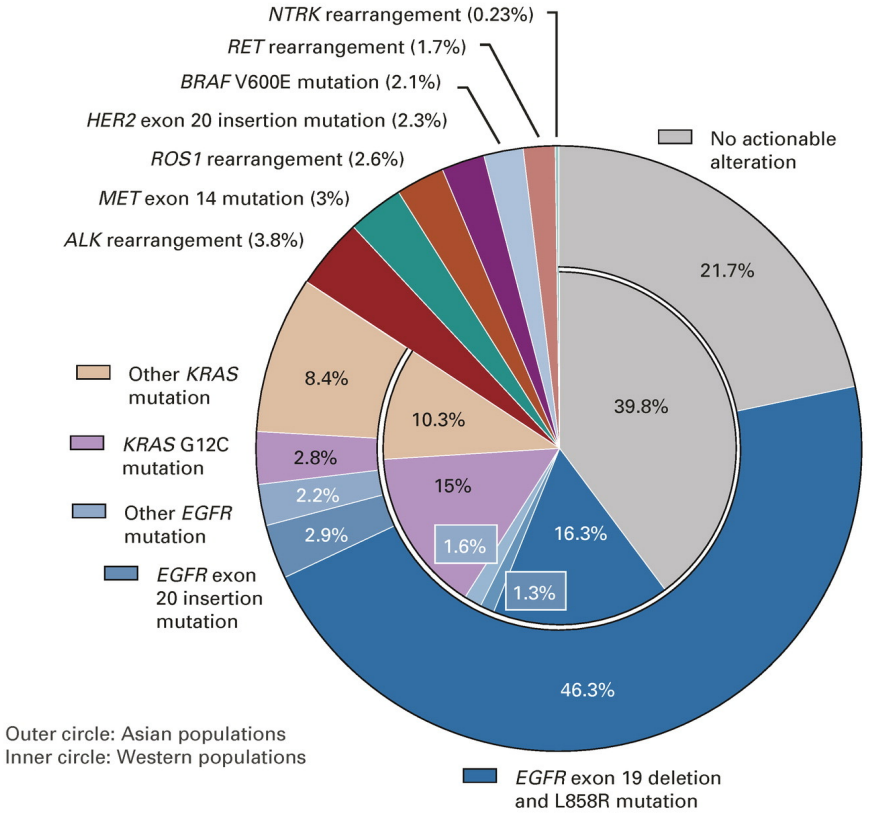


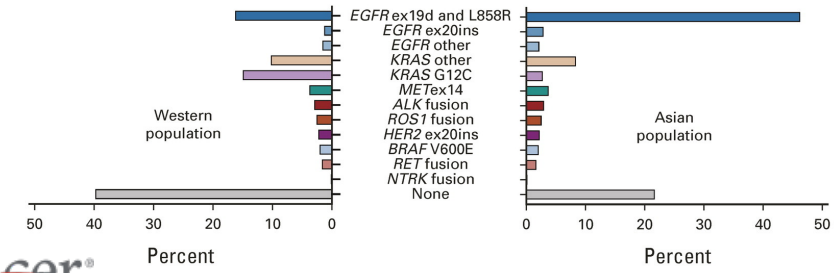
*Varies widely by population.

1. Non-small-cell-lung-treatment-pdq. Cancer.gov.
2. Jordan EJ, et al. Cancer Discov. 2017;7(6):596-609.
3. Gelatti ACZ, et al. Clin Lung Cancer. 2020;21(6):e511-e515.
4. Pi C, et al. Thorac Cancer. 2018;9(7):814-819.
5. Marks S, Naidoo J. Lung Cancer. 2022;163:59-68.

Slide (modified) courtesy of Dr. David Planchard, Institute Gustav Roussy

Genomic Abnormalities in Lung Adenocarcinoma



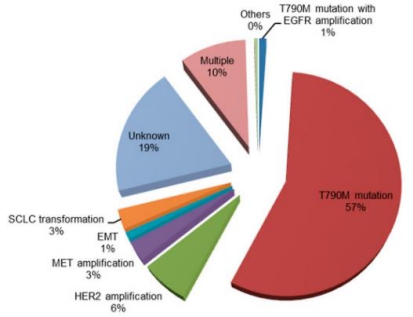


A. Tan, J Clin Oncol, 2022

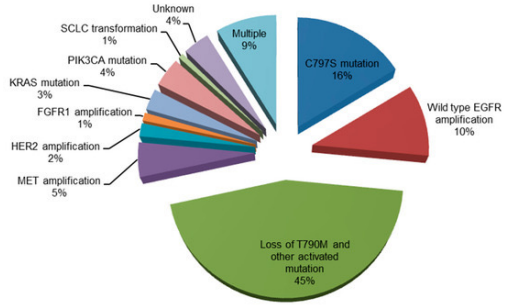
Mechanisms of Resistance

EGFR

First Generation TKIs

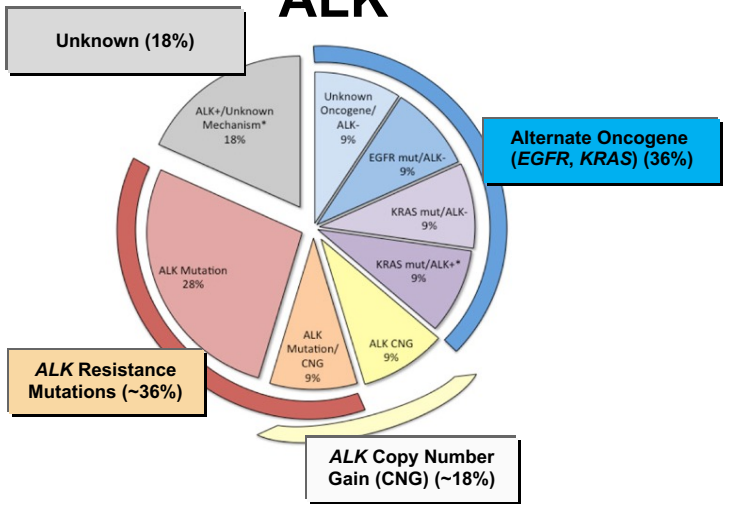


Second Generation TKIs



Nagano T, et al. Cells. 2018;7:212.

ALK



Doebele RC, et al. Clin Cancer Res. 2012;18:1472

Evolution and Expanding List of Guideline Recommendations for Genomic Testing in Advanced Stage NSCLC

"The NCCN NSCLC Guidelines Panel strongly endorses **broader molecular profiling** with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. **Broad molecular profiling** is a key component of the improvement of care of patients with NSCLC)."

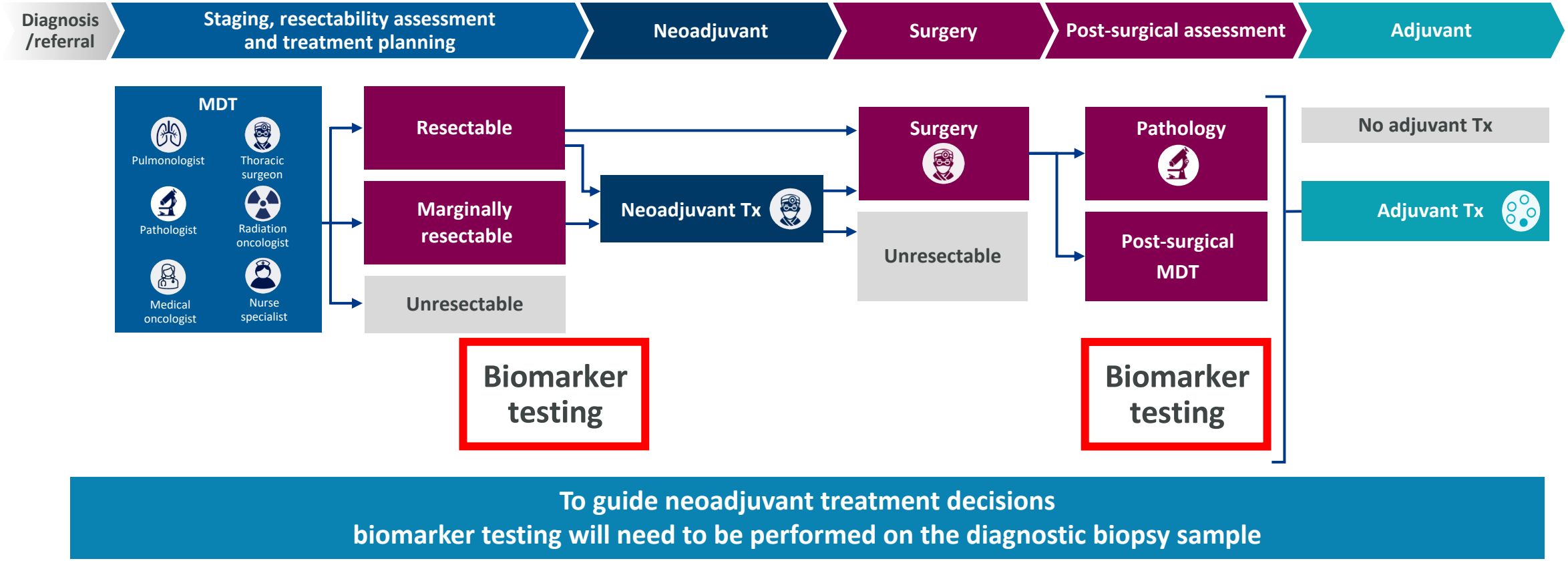
Genomic alteration (i.e., driver event)	Available targeted agents with activity against driver event in lung cancer
EGFR mutations	Osimertinib, erlotinib, gefitinib, afatinib, dacomitinib
ALK rearrangements	Alectinib, brigatinib, ceritinib, crizotinib, lorlatinib
ROS1 rearrangements	Crizotinib, ceritinib, entrectinib
BRAF V600E mutations	Dabrafenib + trametinib, vemurafenib
HER2 mutations	Ado-trastuzumab emtansine, afatinib, trastuzumab deruxtecan
MET amplification/mutation	Crizotinib, capmatinib
RET rearrangements	Cabozantinib, vandetanib, selpercatinib, pralsetinib
NTRK rearrangements	Entrectinib, larotrectinib,
EGFR Ex20ins	***Amivantamab***
KRAS G12C	***Sotorasib***

Cancer

NCCN Clinical Practice Guidelines. NSCLC. Version 5.2022.

Slide Courtesy of Dr. David Gandara

Biomarker Testing for Resectable NSCLC Helps to Inform Treatment Decisions



To guide neoadjuvant treatment decisions biomarker testing will need to be performed on the diagnostic biopsy sample

MDT, multidisciplinary team; NSCLC, non-small cell lung cancer; Tx, treatment

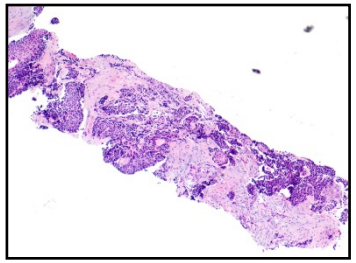
Remon J, et al. Ann Oncol 2021;32:1637–42

Cancer

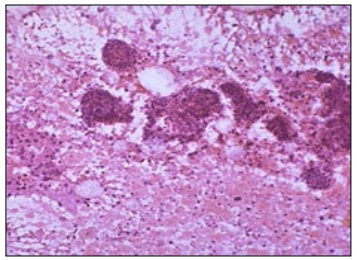
Diagnostic Algorithm for Lung Cancer Diagnosis 2024

cfDNA Testing
(Liquid Biopsy)

Blood

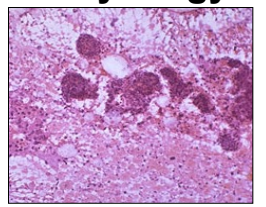


Biopsy

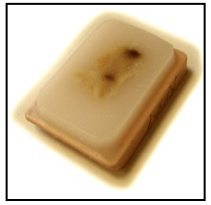


Cytology

Cytology: FNA, EBUS

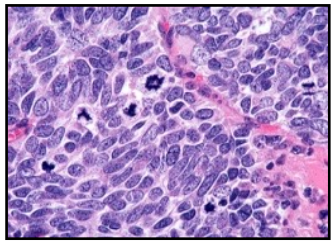
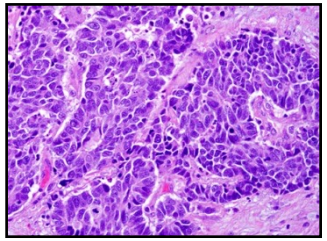
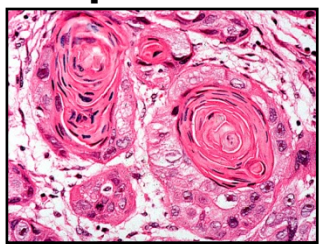
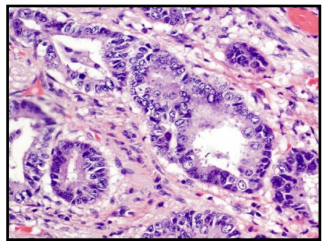
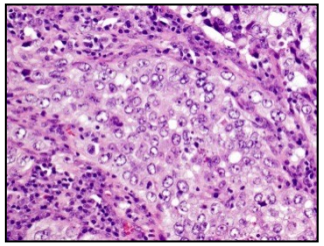


Smears



Cell Block

NSCLC

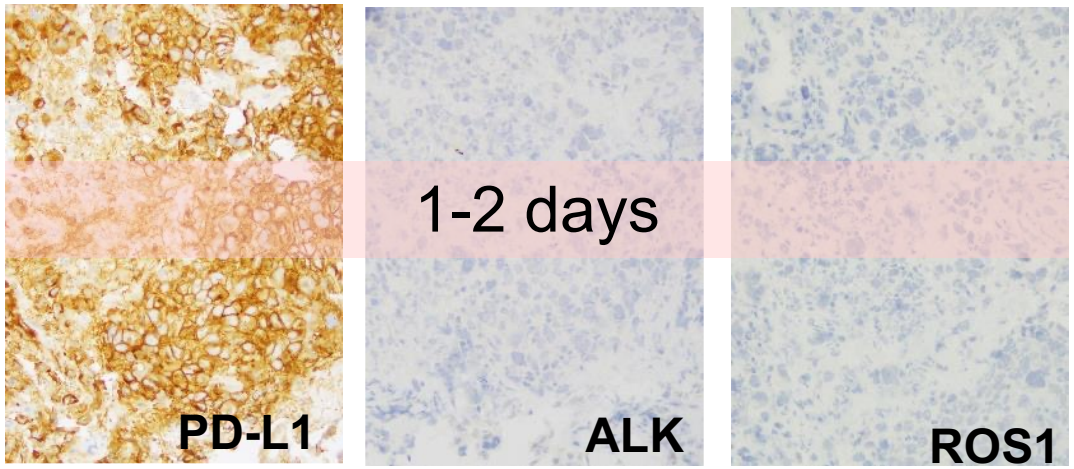
SCLC	LCNEC	Squamous	Adenoca	NSCLC-NOS
				
Morphology	Morphology IHC NE (+)	Morphology IHC p63/p40 (+)	Morphology IHC TTF1 (+)	Morphology IHC (-)

EGFR (incl. Exon 20), BRAF, KRASG12C, and
HER2 mutations; MET ex14 splicing mutations*;*
ALK, ROS1, NTRK, RET and *NRG1* fusions

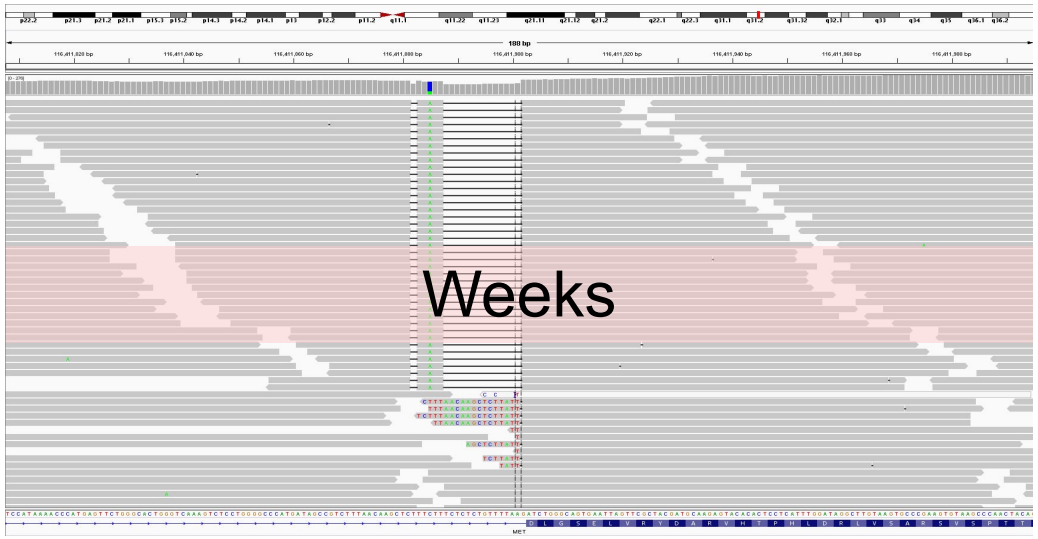
PD-L1 IHC

NSCLC Biomarker Testing → Tricky Timing

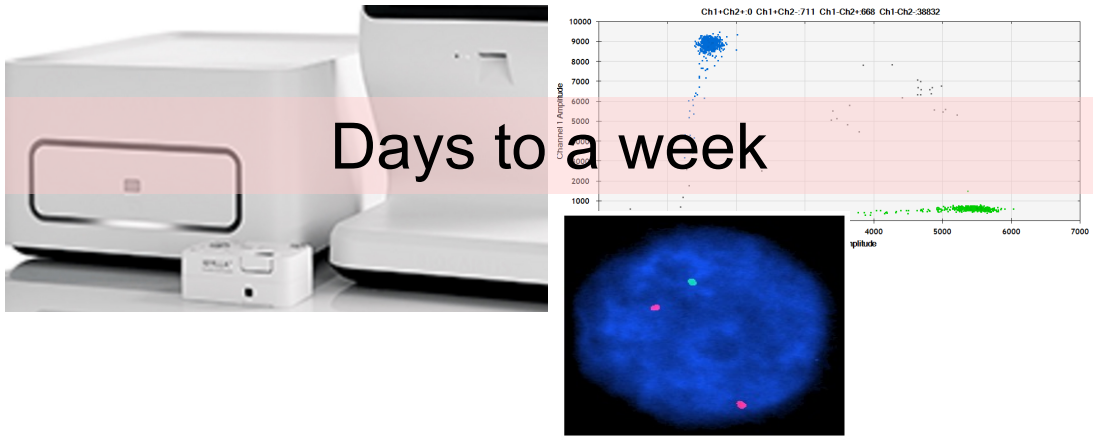
Immunohistochemistry

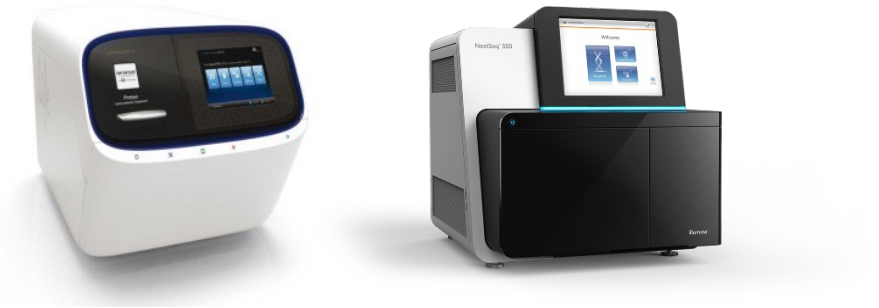


Next Generation of Sequencing (NGS)



PCR-based Assay and FISH



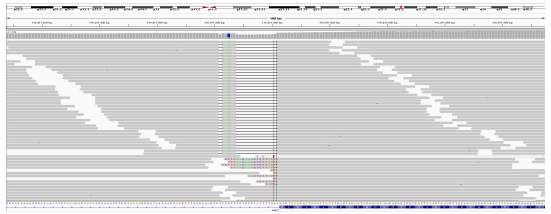


Slide (modified) Courtesy of Lynette Sholl, MD, Harvard Medical School and Brigham And Women's Hospital

Gene Fusion Detection: DNA vs RNA NGS

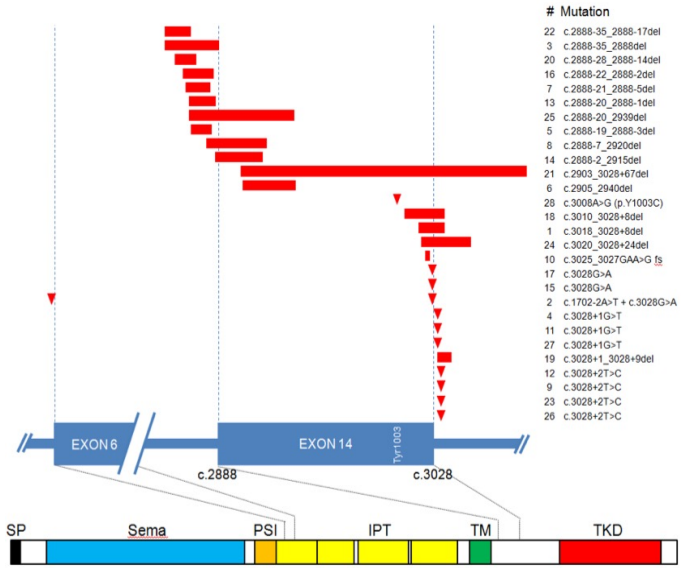
MET Exon 14 Skipping

Next Generation of Sequencing (NGS) on DNA

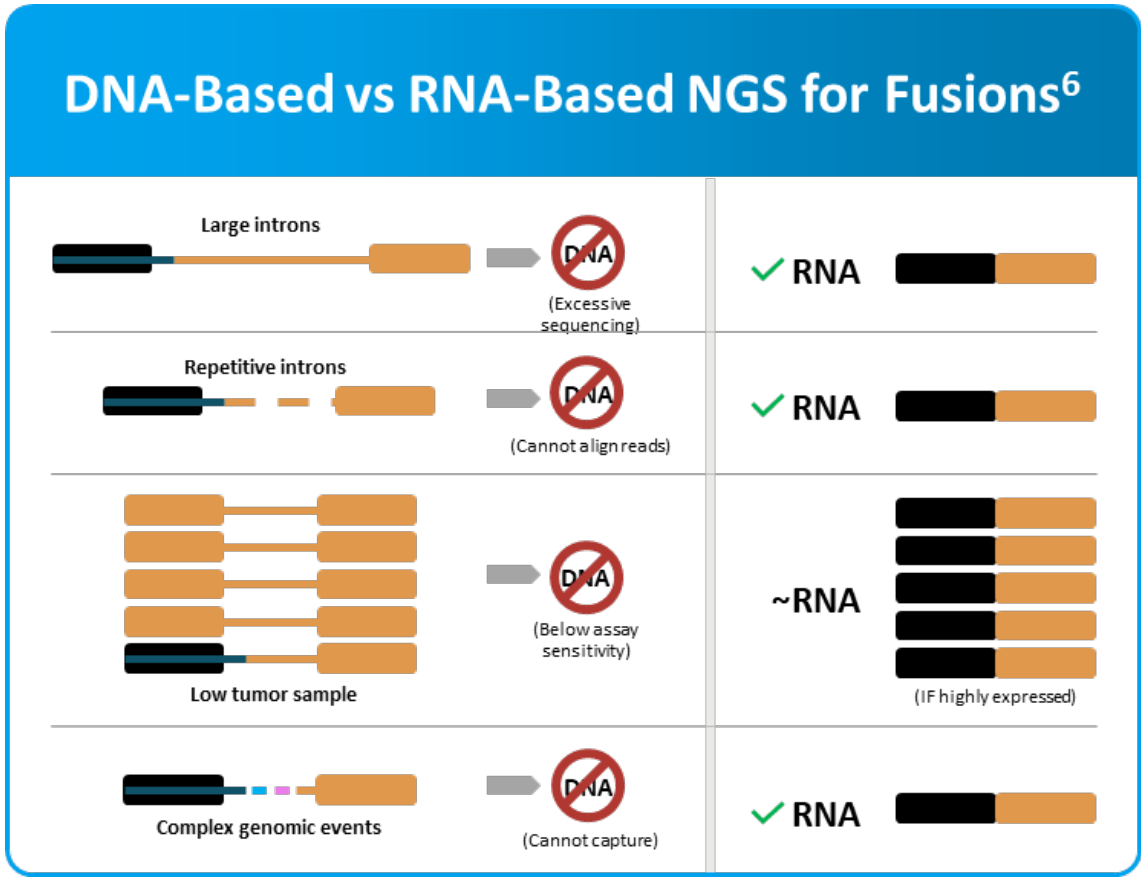


MET p.D963_splice

Additional Fusion Variants Detected Using RNA-based NGS



RNA-based NGS can detect fusion missed by DNA-based NGS: *ALK, ROS1, RET, NTRK, NRG1*



Awad MM, et al. J Clin Oncol. 2016;34(7):721-30. Benayed R, et al. Clin Cancer Res. 2019;25(15):4712-4722. Drilon A, et al. J Clin Oncol. 2021;39(25):2791-2802.

Practical Points for Lung Cancer Biomarker Testing

- **Type of sample**: tissue, cytology (FNA), blood
- **Stage of the disease**
- **Molecular testing assays**:
  - IHC: PDL-1 and ALK/ROS1 (surrogates)
  - FISH: *ALK*, *ROS1*, and *NTRK* fusions
  - NGS or d/qPCR panels: *EGFR*, *MET ex14*, and *BRAF mutations, pluse ALK*, *ROS1*, and *NTRK* fusions.
- **Tissue turn around times** (TATs):
  - TAT1: Biopsy collection to pathology diagnosis (~2 days)
  - TAT2: Pathology diagnosis to molecular diagnostic lab (~1 – 7 days)
  - TAT3: Molecular diagnostic lab to molecular report (NGS panels, 10 days)
- **Blood TATs**:
  - TAT1: Blood collection to molecular diagnostic lab (~1 – 2 days)
  - TAT2: Molecular diagnostic lab to molecular report (NGS panels, 10 days)

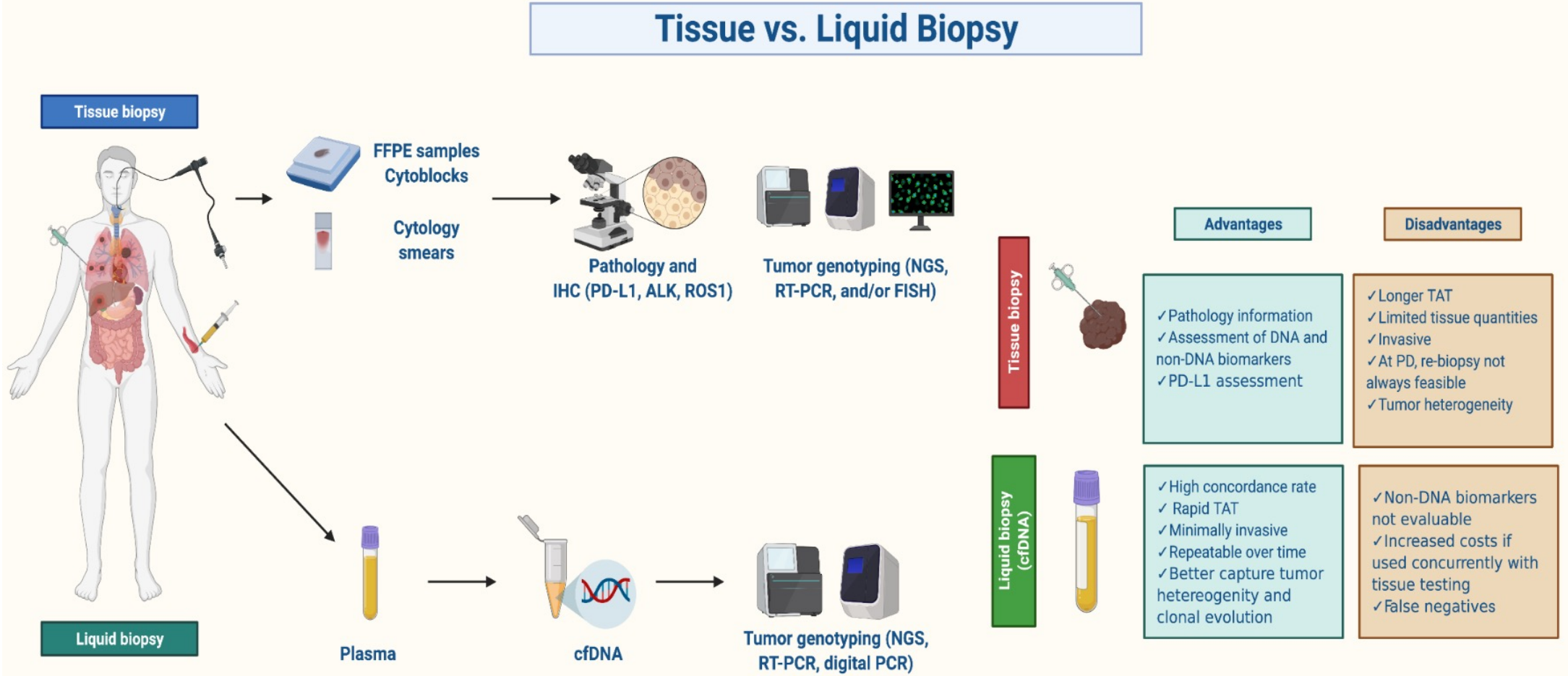
Key Quality Metrics to Guide Quality Improvement on NSCLC Biomarker Testing

Proposed Quality Metric	90% Compliance Goal
Pathology diagnostic TAT (i.e., time from specimen received in pathology to final pathologic diagnosis)	≤ 3 working days
Biomarker Test Order TAT (i.e., time from final pathologic diagnosis to biomarker test ordered)	≤ 2 working days
Pathology biomarker TAT (i.e., time from final pathologic diagnosis and/or biomarker test ordered to specimen sent to molecular lab) for eligible patients	≤ 3 working days
Molecular biomarker TAT (i.e., time from specimen received in molecular testing laboratory to reporting of all biomarker results) for eligible patients	≤ 10 working days
Overall biomarker TAT (i.e., time from final pathologic diagnosis rendered to reporting of all biomarker results) for eligible patients	≤ 14 working days

Cancer®

S. Roy-Chowdhuri, Cancer, in press

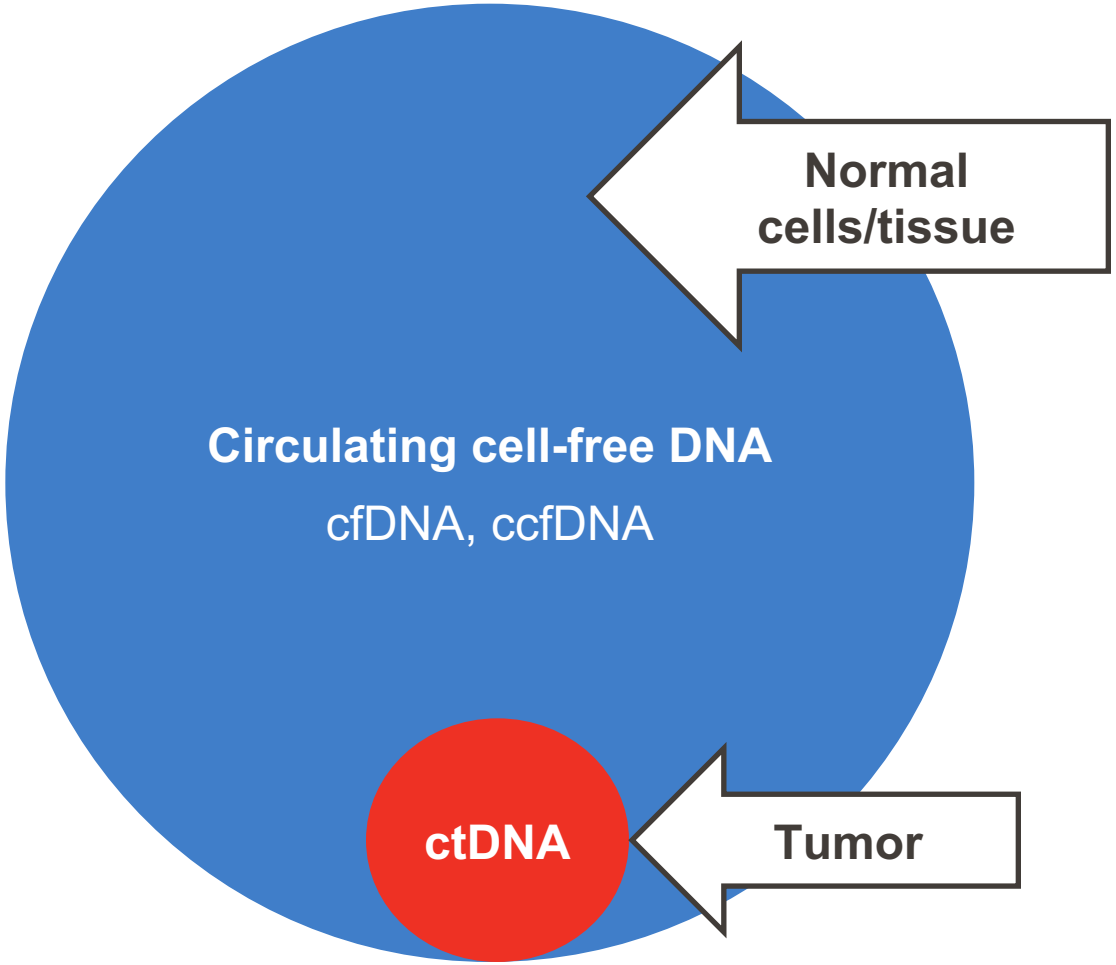
Tissue vs. Liquid Biopsy for Molecular Profiling



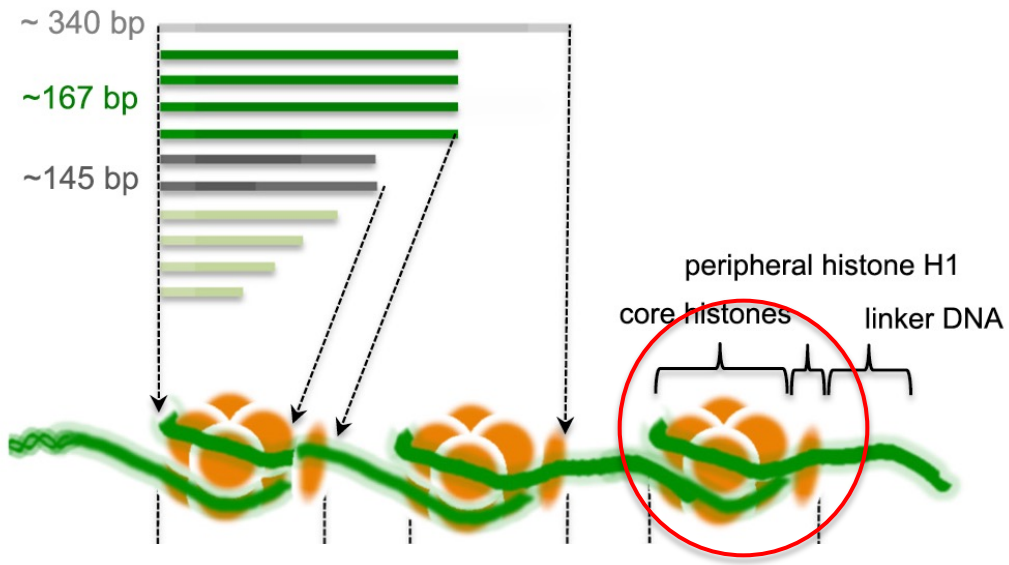
Cancer

C. Rolfo et al, J Thorac Oncol, 2021

Characteristics and Terminology for Circulating Tumor DNA (ctDNA)



167 bp fragments of DNA, a nucleosome



The linker DNA between nucleosomes is cleaved leaving 167 bp cell-free DNA fragments (145 bp plus a ~20 bp segment wrapping histone H1). Originally described by Wyllie in 1980.

Cancer

Chandrananda et al. 2015 BMC Medical Genomics.
Wyllie 1980 Nature.; Slide from Rick Lanman

cfDNA Genotyping Analysis

Pre-analytical Issues

Plasma

- Amount of blood/plasma
- Type of tubes
- Time for processing

Next Generation of Sequencing (NGS)

Large/ Intermediate Panels

- **1% VAF: ~100** tumor genomic equivalents (typical detection limit of most ctDNA assays)
- **0.01% VAF: ~1** tumor genomic equivalent

VAF = variant allele frequency

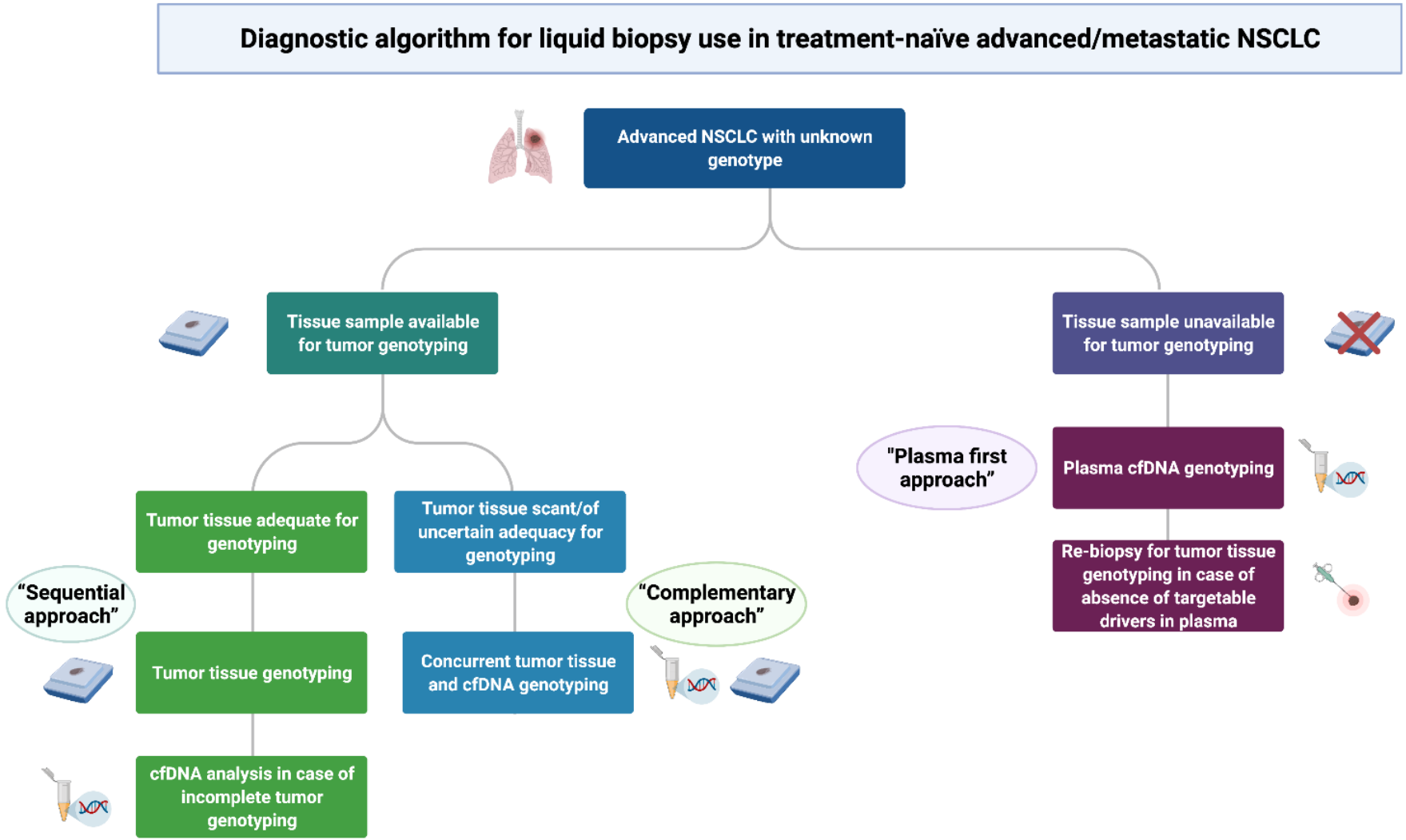
PCR-base Methods

Digital Droplet (dd)PCR

qPCR

Small Panels/ Single Genes

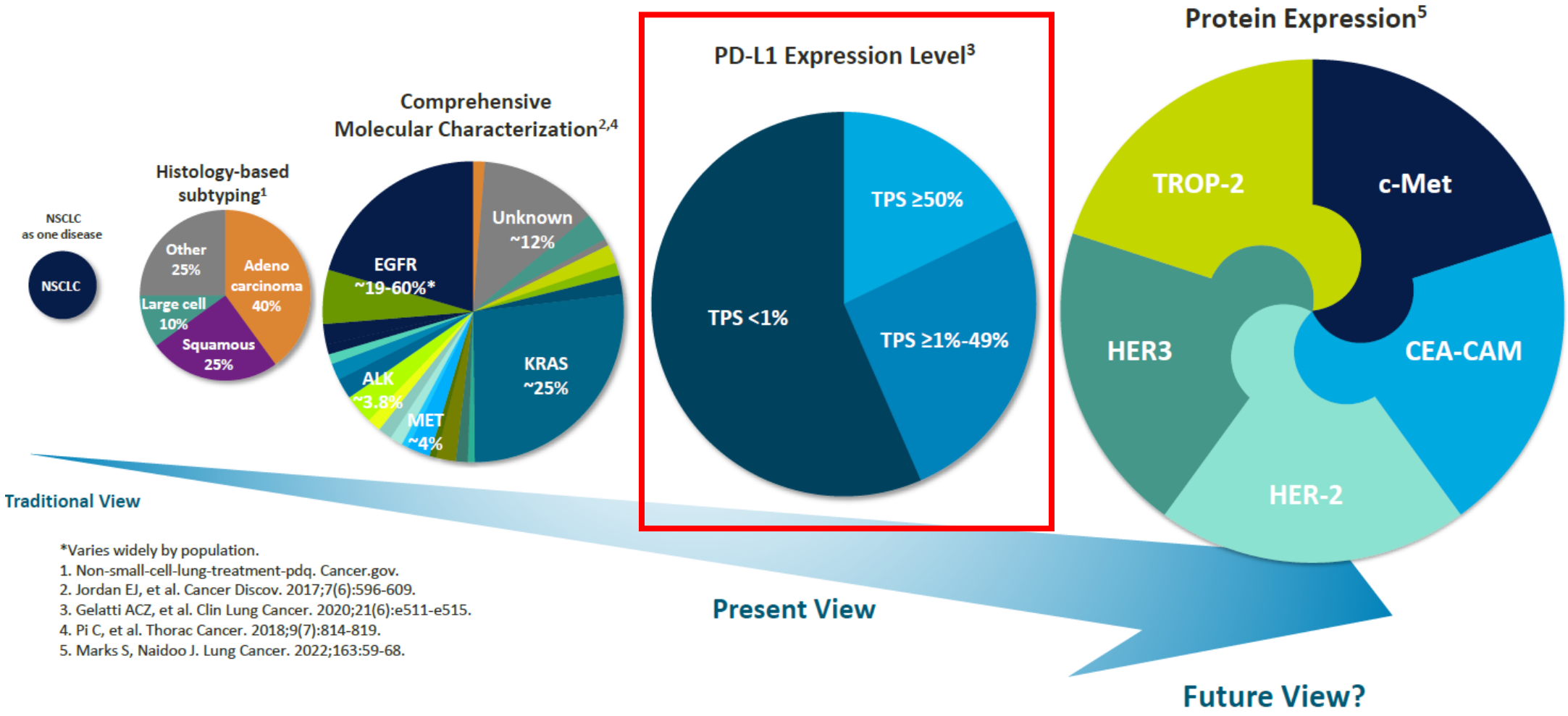
Tissue vs. Liquid Biopsy for Molecular Profiling



Cancer

C. Rolfo et al, J Thorac Oncol, 2021

Paradigms in Lung Cancer Molecular Pathology - 2024



*Varies widely by population.

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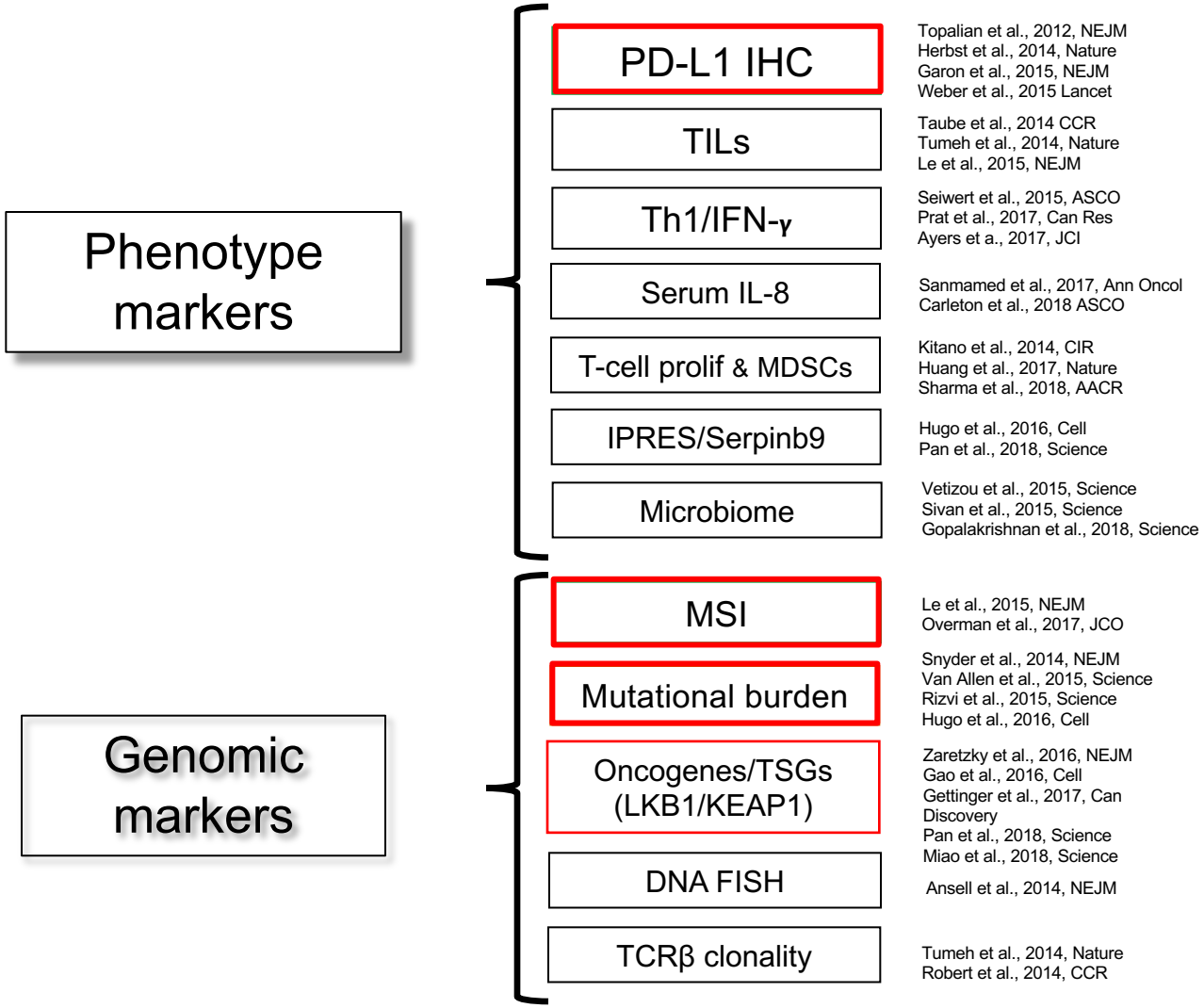
5. Marks S, Naidoo J. Lung Cancer. 2022;163:59-68.

Slide (modified) courtesy of Dr. David Planchard, Institute Gustav Roussy

Developing Markers for Immunotherapy

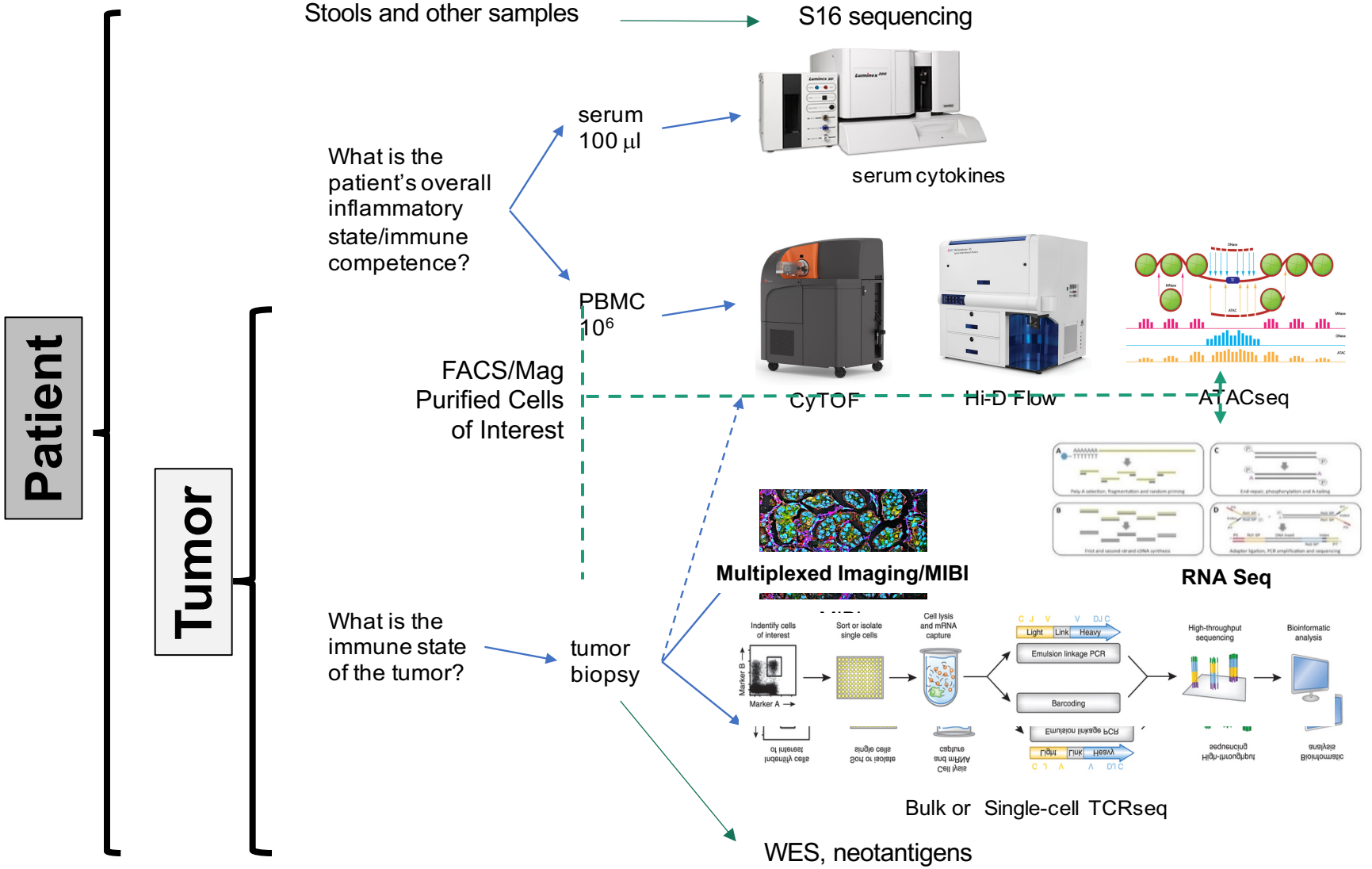


Regulatory approved as biomarkers by the FDA

Cancer

Slide (modified)Courtesy of Dr. Kurt Schalper, Yale Cancer Center.

Immune-profiling Workflows

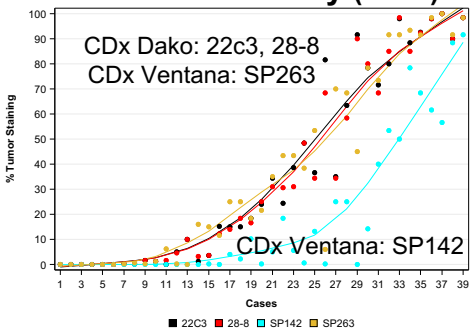


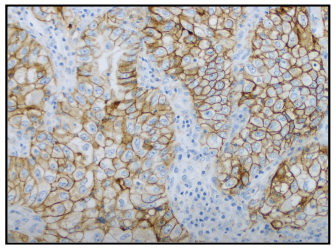
Modified from slide courtesy Holden Maecker, Stanford University CIMAC

IMT Biomarkers in Lung Cancer: PDL-1 IHC

Several Antibodies

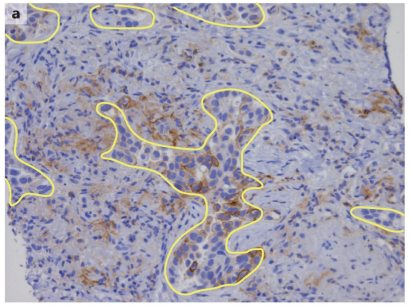
Blue-Print Study (n=40)





22C3 PharmDx

Several Scoring Systems



$$\text{TPS (\%)} = \frac{\text{Number of PD-L1-stained tumour cells}}{\text{Total number of viable tumour cells}} \times 100\% \text{ (for 22C3 or SP263)}$$

$$\text{TC (\%)} = \frac{\text{Number of PD-L1-stained tumour cells}}{\text{Total number of viable tumour cells}} \times 100\% \text{ (for SP142)}$$

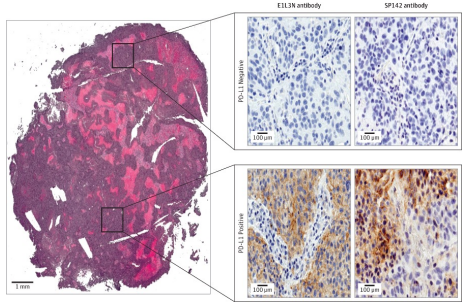
Malignant Cells

$$\text{IC (\%)} = \frac{\text{Area of tumour infiltrated by PD-L1-stained immune cells}}{\text{Total tumour area}} \times 100\% \text{ (for SP142)}$$

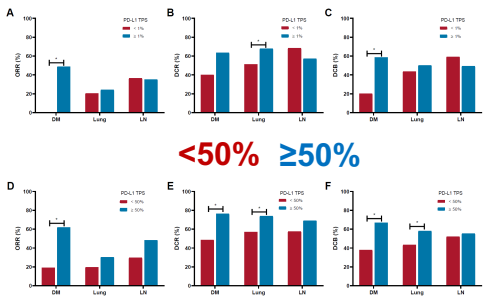
Immune Cells

Heterogeneity

Intra-tumoral



Inter-tumoral



<50% ≥50%

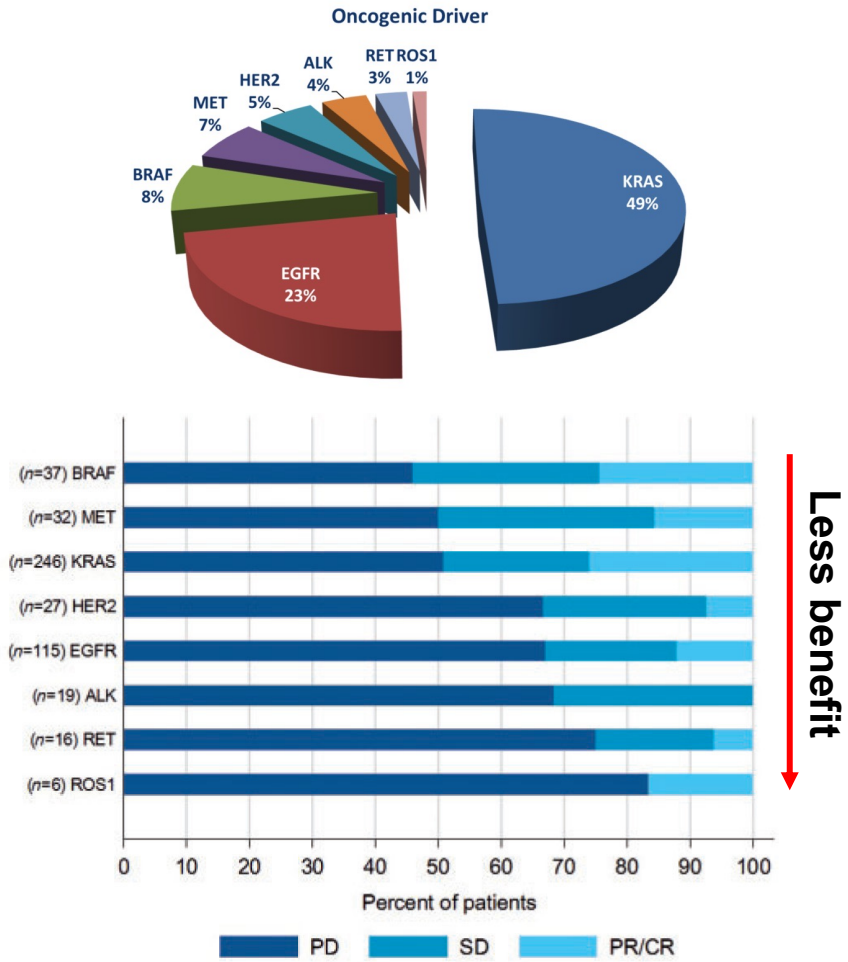
It is an imperfect biomarker, but not too bad after all:

- Standard test for advanced metastatic NSCLC treated with single agent pembrolizumab (TPS ≥50%), atezolizumab (TC >50% or IC >10%), and cemiplimab (TPS ≥50%).
  - For lower expressors: single agent anti-PD-L1/PD-1 treatment is less effective.
  - In tumors with TPS ≥50%: it seems that chemotherapy does not add benefit (pooled analysis form FDA 12
- Combination therapy: Ipilimumab + nivolumab in patients with TPS ≥1%
- Good turn around time!
- Being explored as biomarker for IO combinations (anti-PD-L1/PD-1 +TIGT3, +LAG-3)

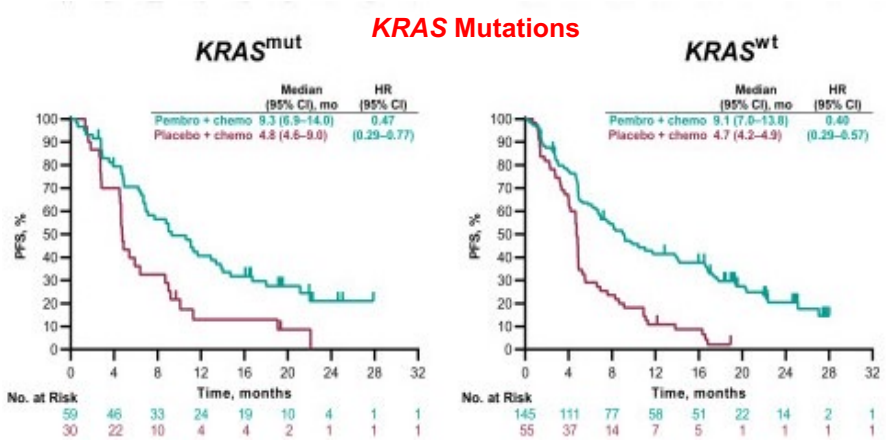
L. Pai-Scherf et al, Oncologist, 2017
R. Herbst et al, N Engl J Med, 2020
M.D. Hellmann et al, N Engl J Med, 2019
O. Akinboro et al, J Clin Oncol Suppl, 2021
A. Sezer et al, Lancet, 2021
H. Safa et al, Targeted Oncology, 2023

Hirsch FR, et al. J Thorac Oncol. 2017;12(2):208-222 McLaughlin J, et al. JAMA Oncol. 2016;2(1):46-54. Hong L, et al. J Thorac Oncol. 2020;15(9):1449-1459. Doroshow DB, et al. Nat Rev Clin Oncol. 2021;18(6):345-362.

IMT Biomarkers in Lung Cancer: Oncogenes



Anti-PDL-1 (Pembro) + Chemotherapy (Keynote 189-407)



Immune checkpoint inhibitors and Oncogene Status:

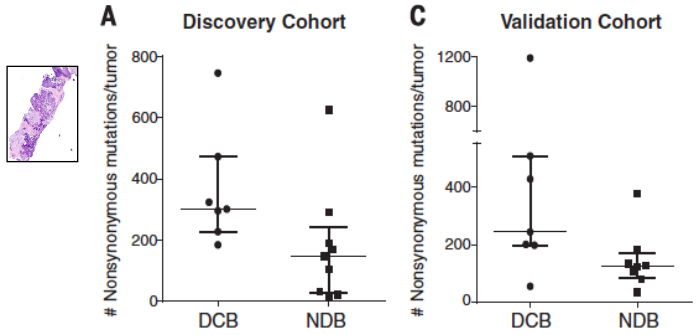
- No benefit in *EGFR* and *ALK* mutant tumors.
- Benefit on patients with some degree of smoking history and with *BRAF*, *KRAS* and *MET ex14* mutations.
- The association between *KRAS* mutations and survival outcomes becomes lost in the context of chemo + ICI therapy (Keynote 189 and 407).
- *KRAS* mutant tumors typically correlate with inflammatory phenotype (TILs, TMB, PD-L1 IHC)
- Single-gene biomarkers *STK11* and *KEAP1* associate with worse outcomes in ICI…*but not in all cohorts.*

Mazieres J, et al. Ann Oncol. 2019;30(8):1321-1328. G Garassino MC, et al. J Thorac Oncol. 2022;17(12):1415-1427.

IMT Biomarkers in Lung Cancer: TMB

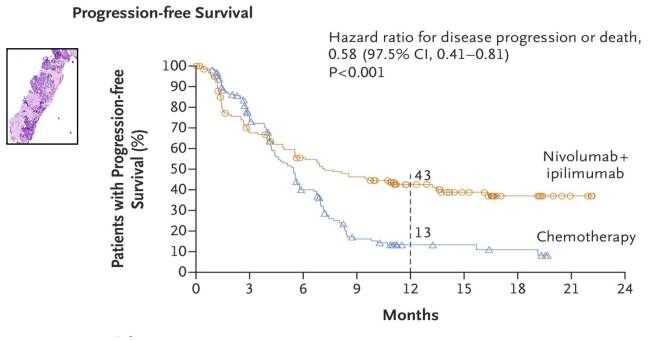
Anti-PD-1 (Nivolumab)

WES, cut off ≥178 mutations



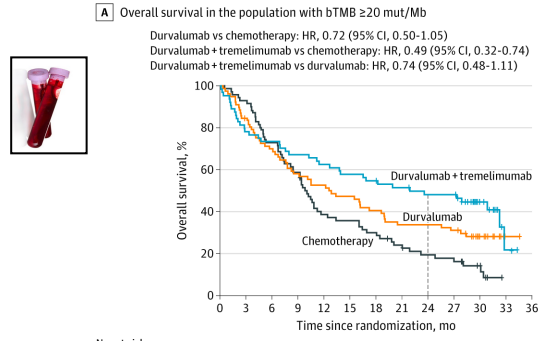
Anti-PD-1+CTLA-4
(Nivolumab + Ipilimumab; CheckMate 227)

Tissue NGS panel, cut off >10 mutations



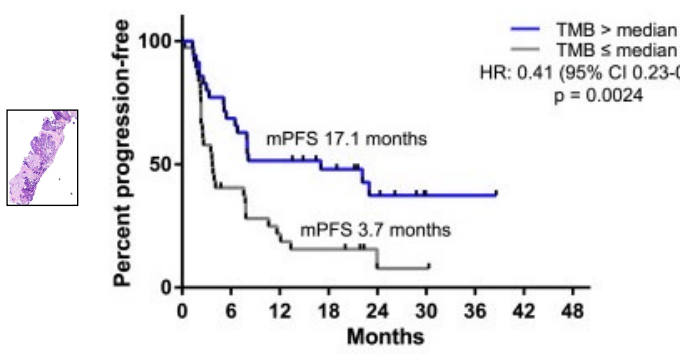
Anti-PDL-1+CTLA-4
(Durvalumab + Tremelimumab; MYSTIC)

Blood NGS panel, cut off ≥20 mutations



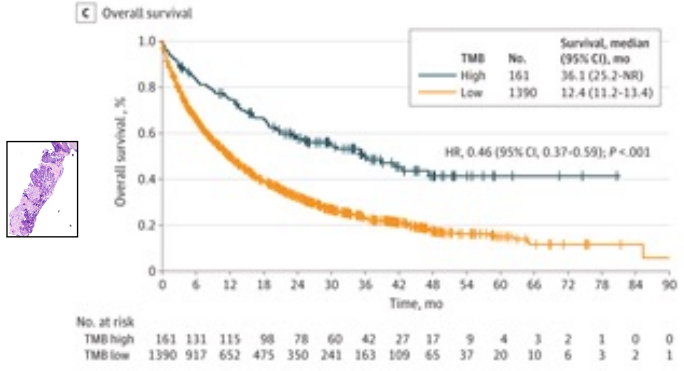
Anti-PD-1+CTLA-4
(Nivolumab + Ipilimumab; CheckMate 012)

WES, cut off ≥158 mutations (median)



Anti-PD-1/PD-L1
(Multicenter)

Tissues NGS panels, >19 mutations

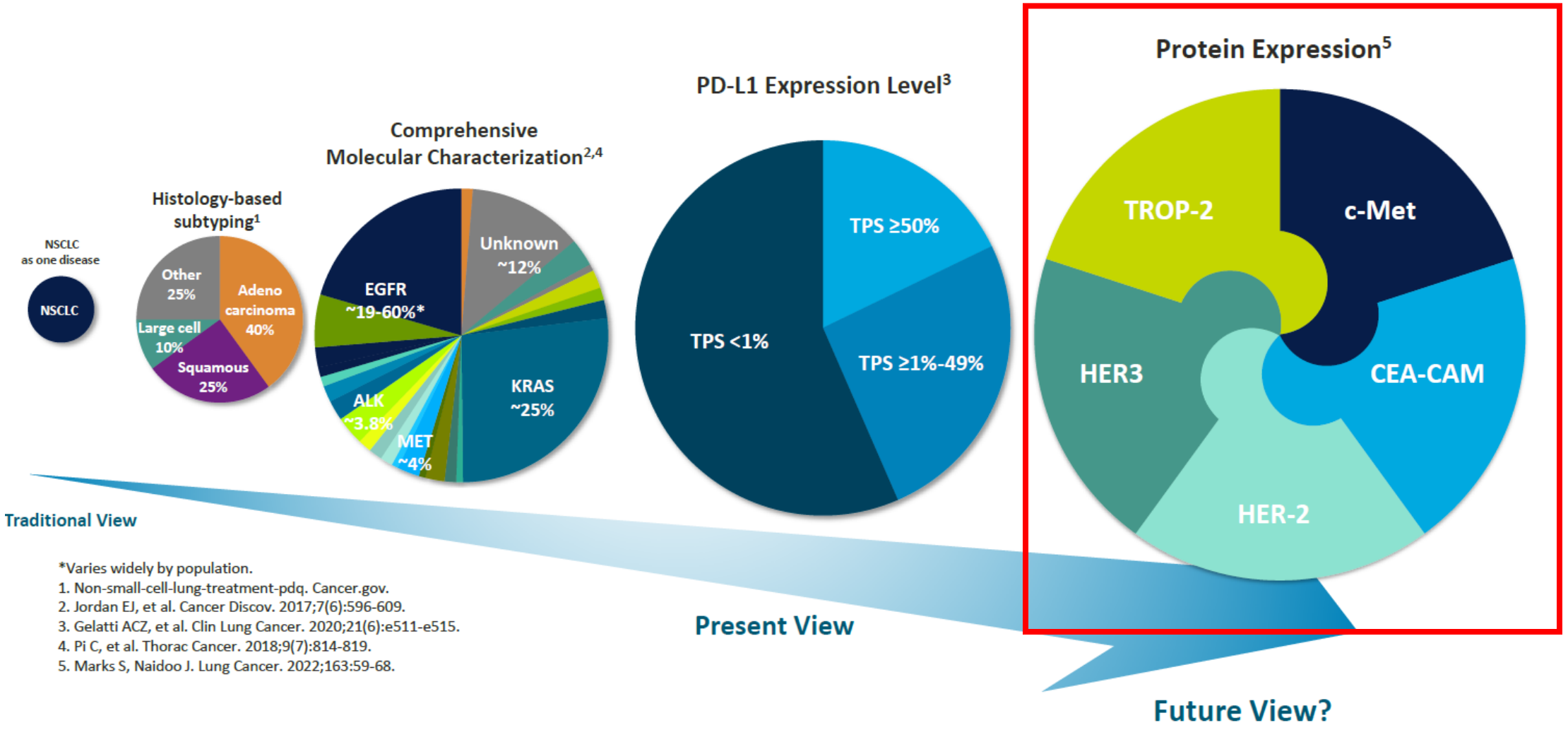


It is another (more) imperfect biomarker:

- **Several methodologies and samples:**
  - Tissue: WES (≥ 243, 178, 158, 75 mts); NGS panels >10, >19 mts).
  - Blood: NGS panels (≥20., 16 mts).
- **Tissue TMB:**
  - No predictive value in patients treated with ICI + chemo.
- **Blood TMB:**
  - Not predictive value on single anti-PDL-1 (B-FAST, B-FIRST).
  - It has been suggested to be a prognostic biomarkers.
- **FDA approved tissue TMB (2021) for solid tumors**
  (Keynote 158; no NSCLC case was included)

Rizvi NA, et al. Science. 2015;348(6230):124-8. M. Hellmann MD, et al. N Engl J Med. 2018;378(22):2093-2104. Rizvi NA, et al. JAMA Oncol. 2020;6(5):661-674. Hellmann MD, et al. Cancer Cell. 2018;33(5):853-861.e4. Ricciuti B, et al. JAMA Oncol. 2022;8(8):1160-1168.

Paradigms in Lung Cancer Molecular Pathology - 2024



*Varies widely by population.

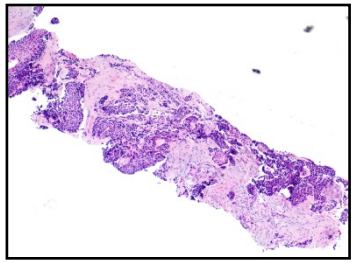
1. Non-small-cell-lung-treatment-pdq. Cancer.gov.
2. Jordan EJ, et al. Cancer Discov. 2017;7(6):596-609.
3. Gelatti ACZ, et al. Clin Lung Cancer. 2020;21(6):e511-e515.
4. Pi C, et al. Thorac Cancer. 2018;9(7):814-819.
5. Marks S, Naidoo J. Lung Cancer. 2022;163:59-68.

Slide (modified) courtesy of Dr. David Planchard, Institute Gustav Roussy

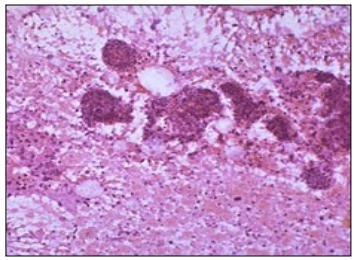
Diagnostic Algorithm for Lung Cancer Diagnosis

cfDNA Testing (Liquid Biopsy)

Blood

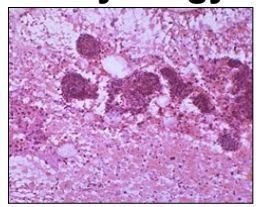


Biopsy

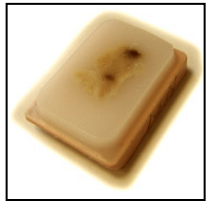


Cytology

Cytology: FNA, EBUS



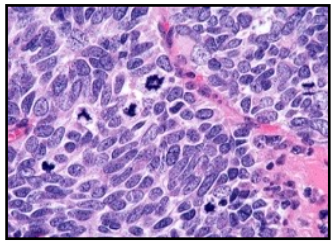
Smears



Cell Block

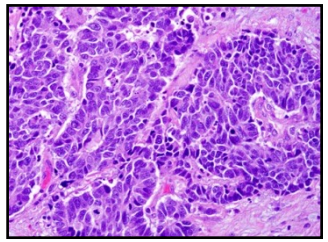
NSCLC

SCLC



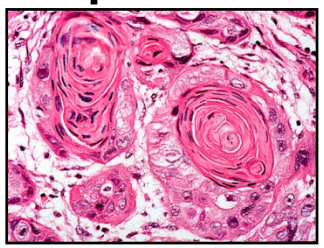
Morphology

LCNEC



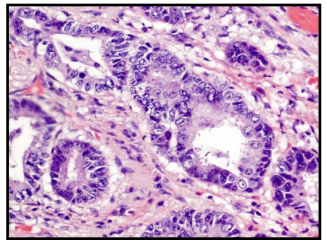
Morphology
IHC NE (+)

Squamous



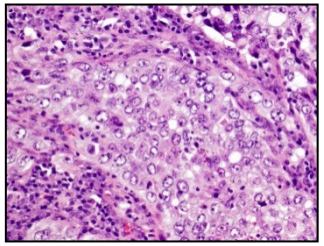
Morphology
IHC p63/p40 (+)

Adenoca



Morphology
IHC TTF1 (+)

NSCLC-NOS



Morphology
IHC (-)

EGFR (incl. Exon 20), BRAF, KRASG12C, and *HER2 mutations; MET ex14* splicing mutations*; ALK, ROS1, NTRK, RET* and *NRG1* fusions

Yes: DNA and RNA NGS

Yes: Multiplex IHC

PD-L1 IHC

Can we add more markers?

Cancer

Thanks