Molecular Diagnostics in Cytology and Small Biopsy Specimens of Non-Small Cell Lung Cancer

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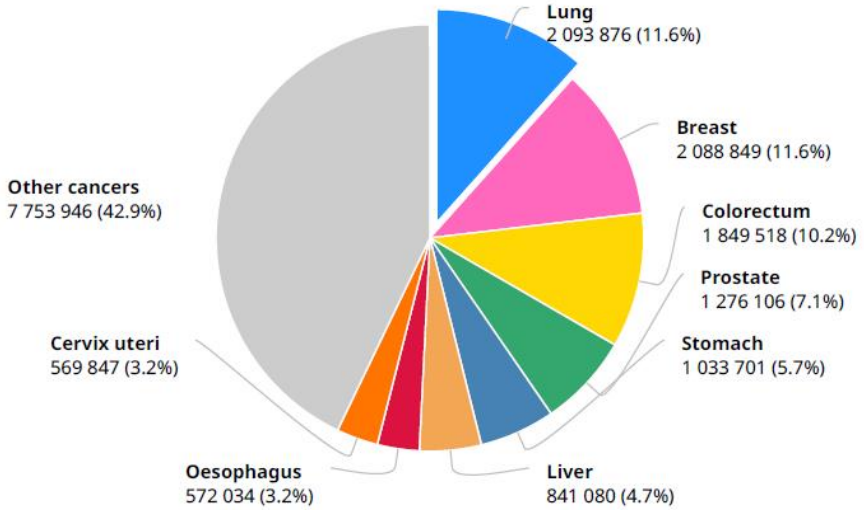
Lung Cancer: Epidemiology (Worldwide) WHO – Globocan 2018

International Agency for Research on Cancer
World Health Organization

Lung
Source: Globocan 2018

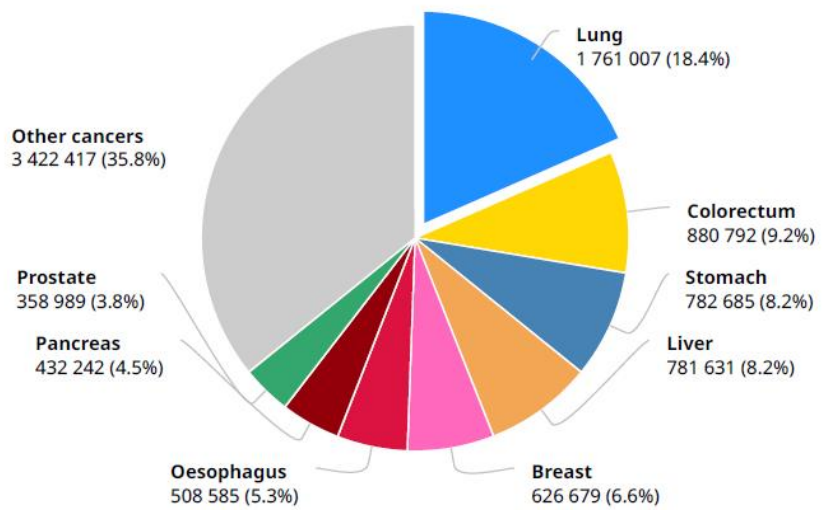
GCO

Number of new cases in 2018, both sexes, all ages



Total: 18 078 957 cases

Number of deaths in 2018, both sexes, all ages



Total: 9 555 027 deaths

Lung Cancer: Epidemiology (US)
NIH – 2019 SEER Database

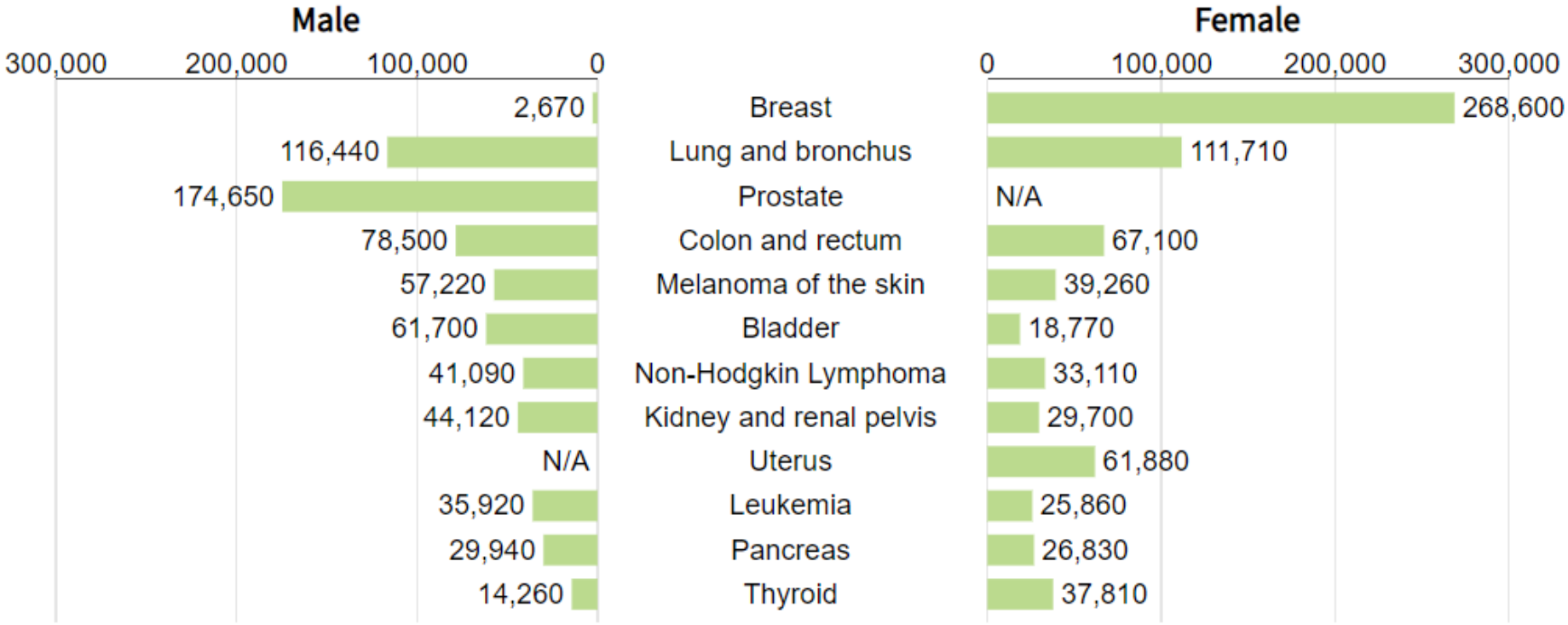
At a Glance

Estimated New Cases in 2019	228,150
% of All New Cancer Cases	12.9%

Estimated Deaths in 2019	142,670
% of All Cancer Deaths	23.5%

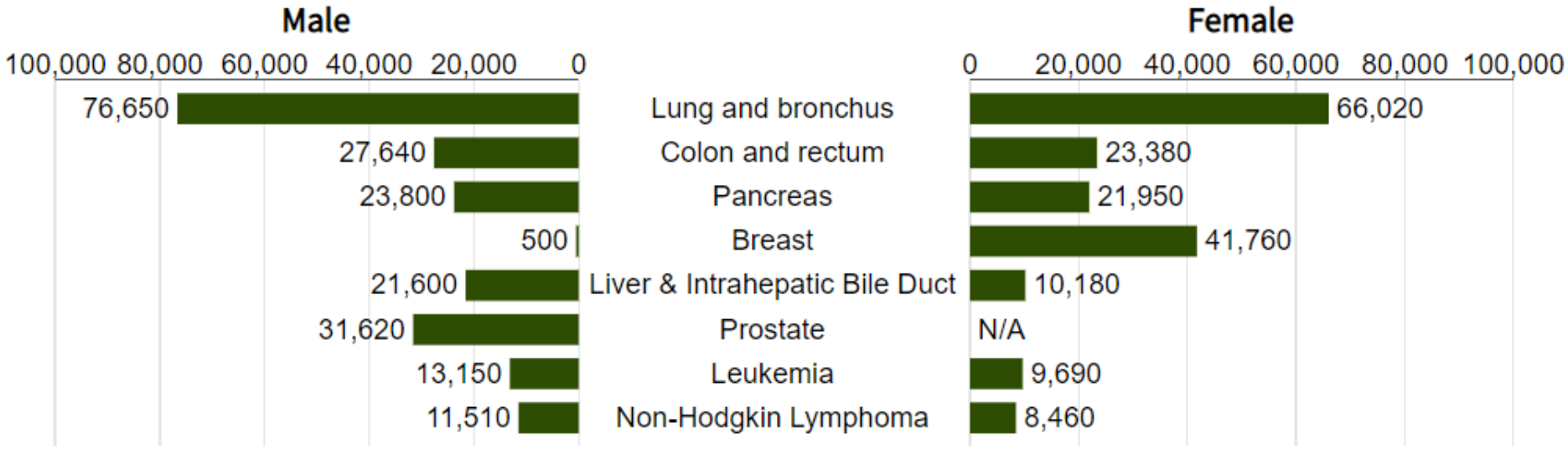
Percent Surviving 5 Years
19.4%
2009-2015

Lung Cancer: Epidemiology (US)
NIH – 2019 SEER Database



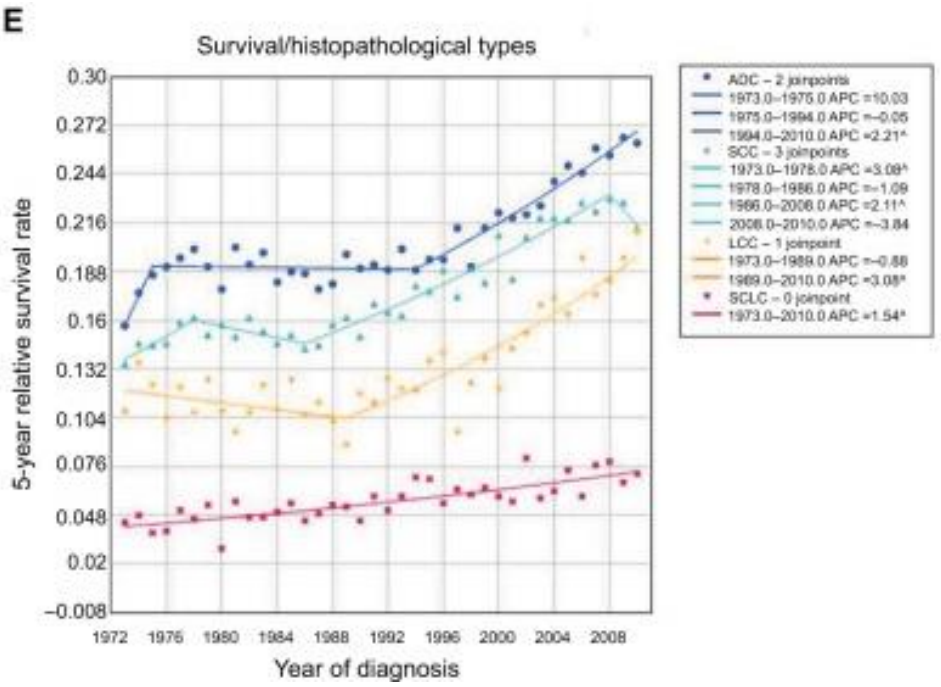
Source: Estimated New Cancer Cases and Deaths for 2019

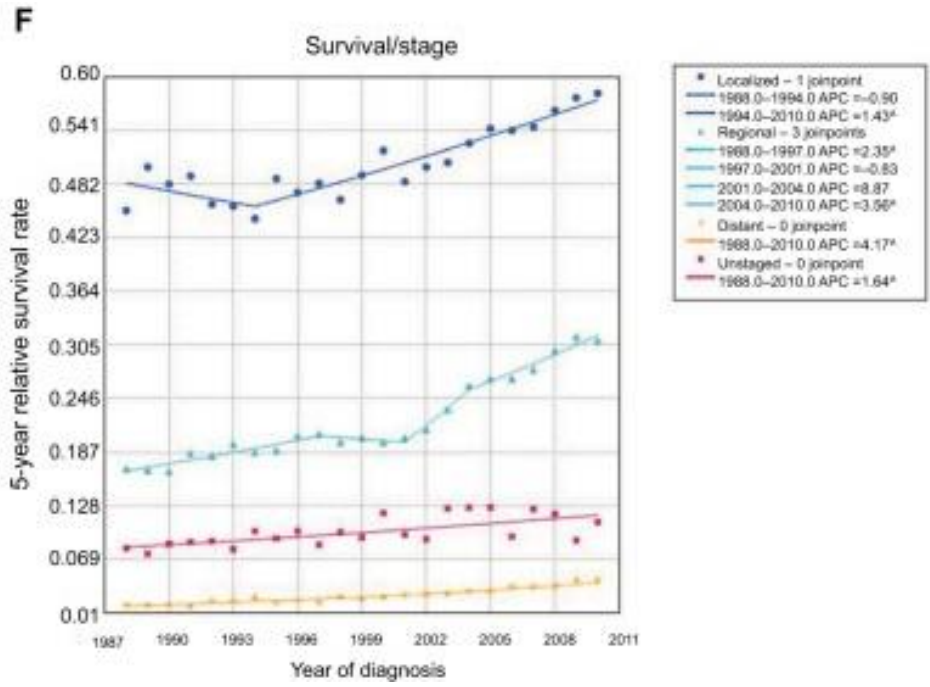
Lung Cancer: Epidemiology (US)
NIH – 2019 SEER Database



Source: Estimated New Cancer Cases and Deaths for 2019

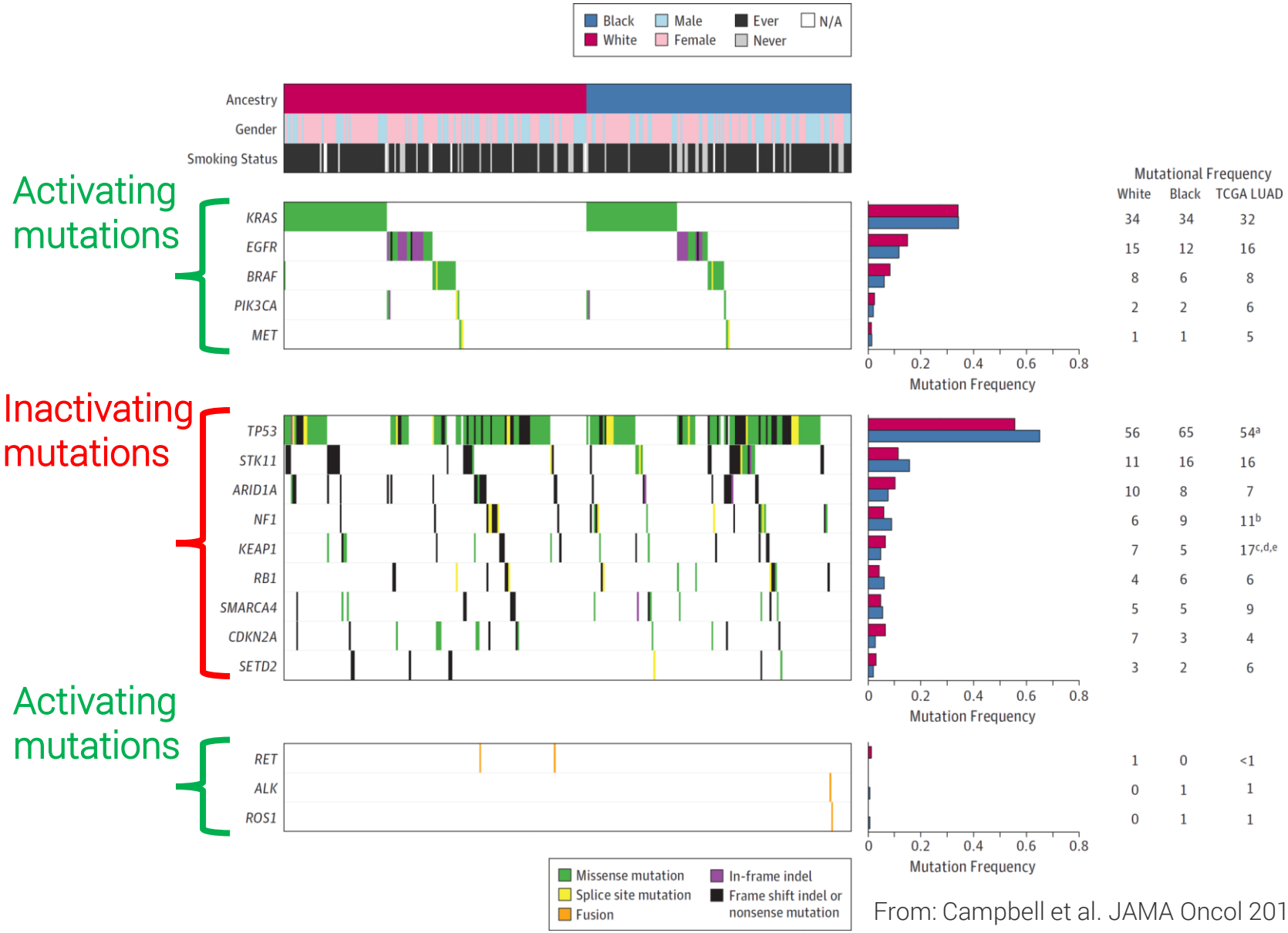
Lung Cancer: Epidemiology (US)
NIH – 2019 SEER Database
Mortality over Time





From: Lu et al. Cancer Manag Res. 2019;11:943–53.

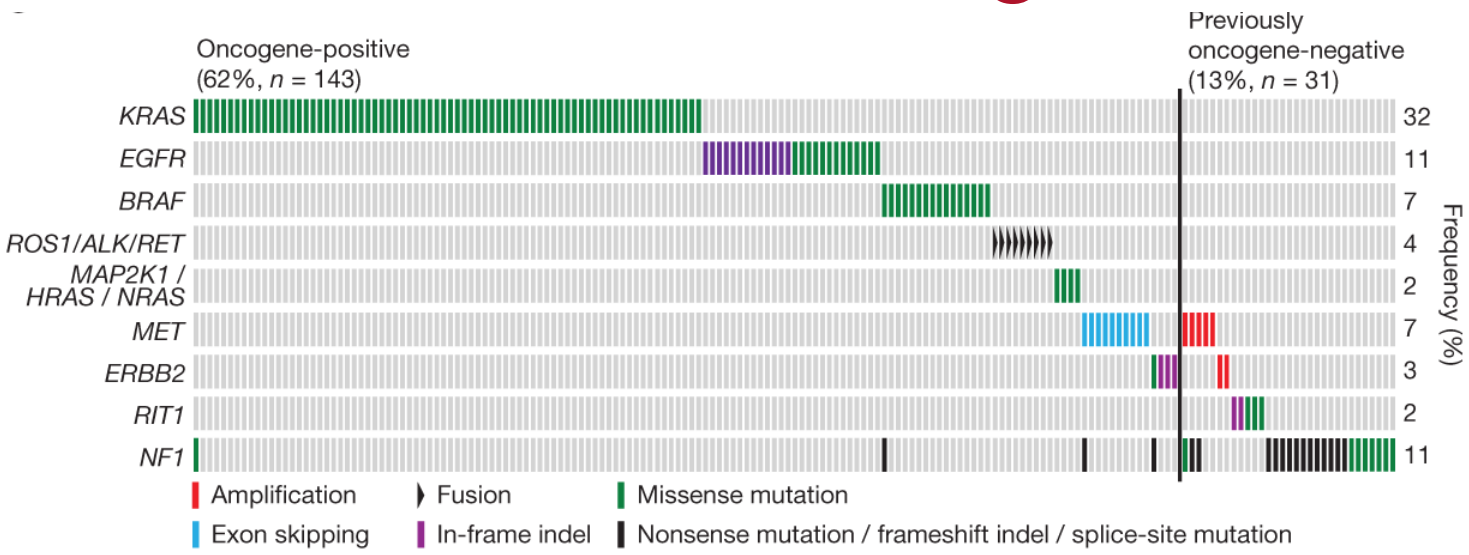
Landscape of Molecular Alterations in NSCLC

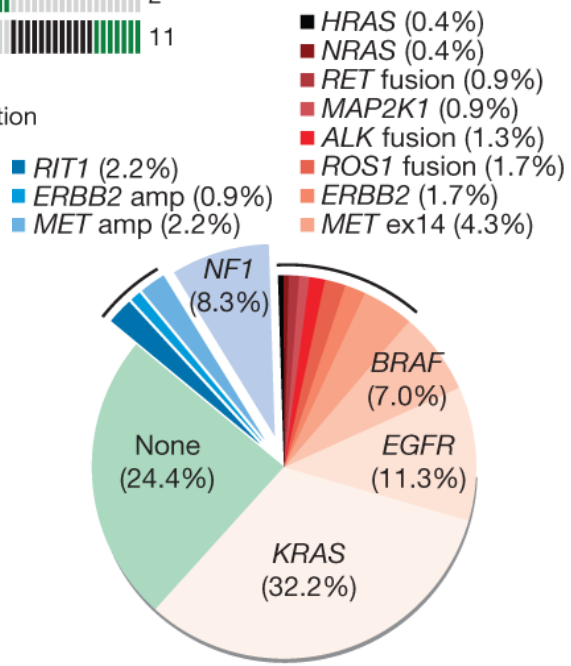


	Mutational Frequency		
	White	Black	TCGA LUAD
KRAS	34	34	32
EGFR	15	12	16
BRAF	8	6	8
PIK3CA	2	2	6
MET	1	1	5
TP53	56	65	54[a]
STK11	11	16	16
ARID1A	10	8	7
NF1	6	9	11[b]
KEAP1	7	5	17[c,d,e]
RB1	4	6	6
SMARCA4	5	5	9
CDKN2A	7	3	4
SETD2	3	2	6
RET	1	0	<1
ALK	0	1	1
ROS1	0	1	1

From: Campbell et al. JAMA Oncol 2017;3:801–9.

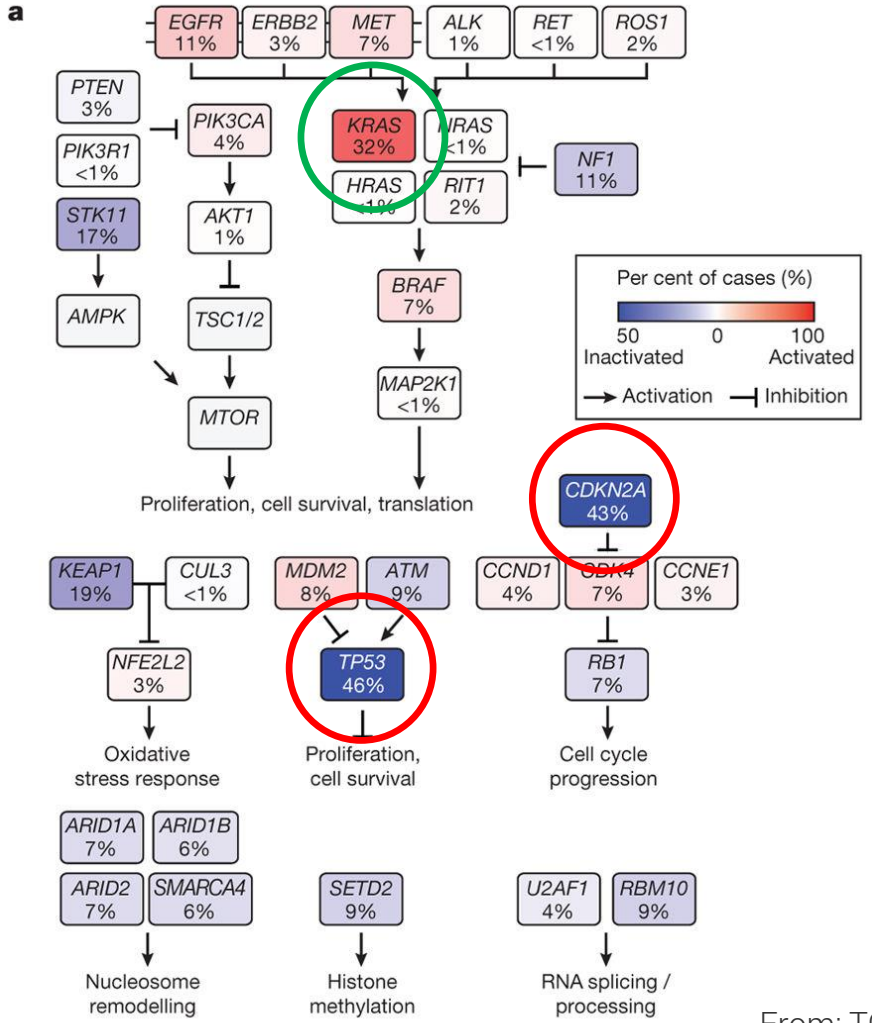
Landsape of Molecular Alterations in NSCLC – Activating Mutations





From: TCGA. Nature 2014;511:543–50.

Landsape of Molecular Alterations in NSCLC



From: TCGA. Nature 2014;511:543–50.

Ancillary Testing in NSCLC

- What specimens to test on?
- What to test for?
- What methods to use for testing?

Current Guidelines

CAP/IASLC/AMP Guidelines	NCCN Guidelines
2018	1.2020

Who/When to Test?

	NCCN Guidelines	CAP/IASLC/AMP Guidelines
Clinical Presentation	Advanced or Metastatic Disease	Advanced Stage Disease
	• Establish histologic subtype with adequate tissue for molecular testing (consider rebiopsy if appropriate)	• Each institution should set its own policy regarding patients with early stage disease
Histological Diagnosis	• Adenocarcinoma • Large cell • NSCLC, NOS • SCC: Consider testing in never-smokers, small biopsy or mixed histology	• Adenocarcinoma • Consider testing for other histologies when clinical features indicate higher probablility of driver mutation

What Specimens to Test?

- AMP/IASCL/CAP Guidelines specify that ANY cytology specimen can be used for molecular testing.
  - » Previous edition specified that small biopsies and cytology specimens where adenocarcinoma could not be excluded should be tested.
  - » No recommendations between testing the primary tumor vs. metastatic.

What Specimens to Test?

- NCCN 1.2020 Guidelines
- The purpose of the pathologic evaluation will vary depending on sample type:
  - » Biopsy or cytology specimen for initial diagnosis in a case of suspected NSCLC
  - » Resection specimen
  - » Obtained for molecular evaluation in the setting of established NSCLC diagnosis

Small Biopsies and Cytology Specimens

- Primary purpose is to:
  - » Make an accurate diagnosis based on the WHO 2015 classification.
  - » Preserve the tissue for molecular studies, especially in advanced/metastatic disease.
- In small biopsies/cytology specimens with poorly differentiated carcinoma, the terms “Non-small cell carcinoma” (NSCC) or “Non-small cell carcinoma – not otherwise specified” (NSCC-NOS) should be used should be used as little as possible and only when more specific diagnosis is not possible.
- “NSCC-favor adenocarcinoma” and “NSCC-favor squamous cell carcinoma” are acceptable.
- Preservation of material for molecular testing is critical. Effort should be undertaken to minimize block reorientation and the number of IHC stains for cases that cannot be classified on histologic examination alone.

Small Biopsiesand Cytology Specimens

- In small biopsies and cytology specimens obtained for molecular testing in the context of and established diagnosis after progression on targeted therapies, the primary purpose is to:
  - » Confirm the original pathologic type with minimal use of tissue for IHC only in suspected small cell carcinoma transformation or different histology.
  - » Preserve material for molecular analysis.

- FFPE material is suitable for most molecular analyses, except bone biopsies previously treated with acid decalcifying solutions.
  - » Non-acid decalcification approached may be successful for subsequent molecular testing.

Small Biopsies and Cytology Specimens – NCCN 1.2020

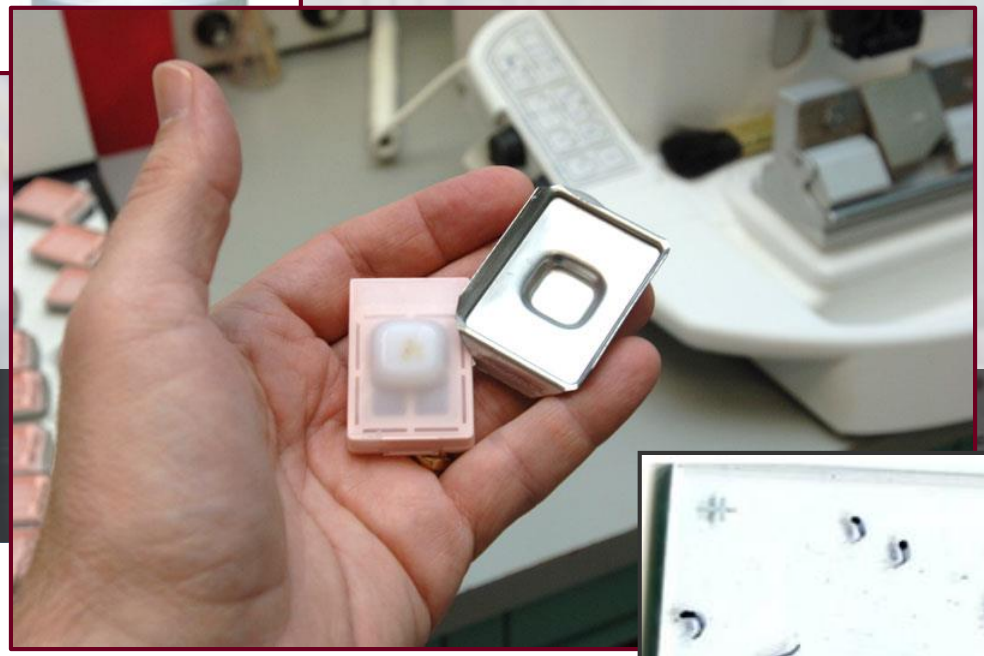
- While many molecular pathology laboratories currently also accept cytopathology specimens such as cell blocks, direct smears or touch preparations, laboratories that do not are strongly encouraged to identify approaches to testing of non-FFPE cytopathology specimens.

Immunohistochemistry

Immunohistochemistry

- Judicious use of IHC is recommended to preserve tissue for molecular testing, most notably in small specimens.
- In small specimens, a limited number of immunostains with 1 adenocarcinoma marker (TTF-1, napsin-A) and one squamous (p40, p63) should suffice for most diagnostic problems. Virtually all tumors that lack squamous morphology and express p63 and TTF-1 are preferably classified as adenocarcinoma. A simple panel of TTF-1 and p40 may be sufficient to classify most NSCC-NOS cases.
- Testing for NUT expression by IHC should be considered in all poorly differentiated carcinomas that lack glandular differentiation or specific etiology, particularly in non-smokers or in patients presenting at a young age, for consideration of a pulmonary NUT carcinoma.
- IHC should be used to differentiate primary lung adenocarcinoma, SCC, large cell NE carcinoma and mesothelioma.

Cytology Specimen types and molecular testing



Fine Needle Aspiration (FNA)

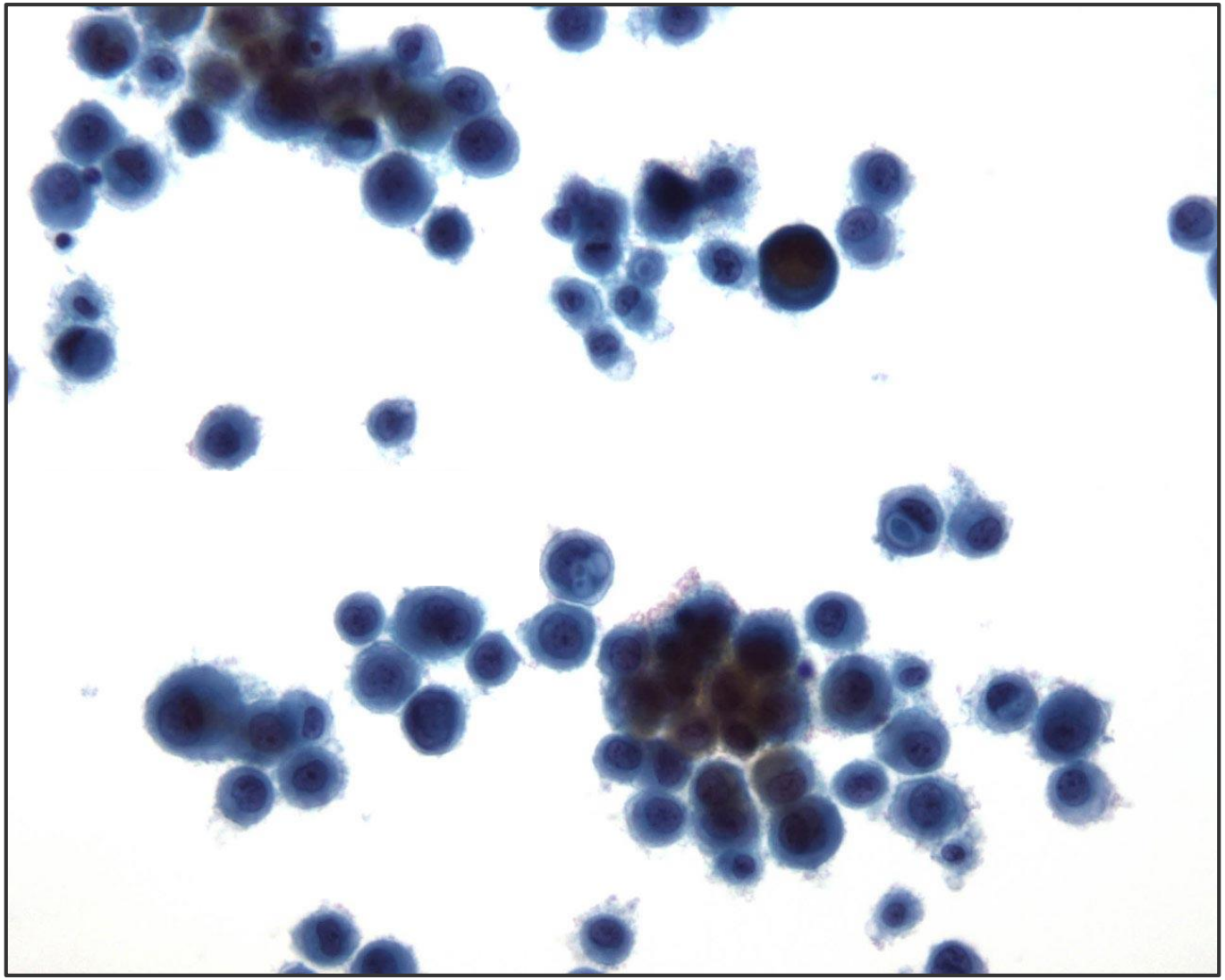
- Advantage of targeting a specific lesion and can be performed with minimal invasion

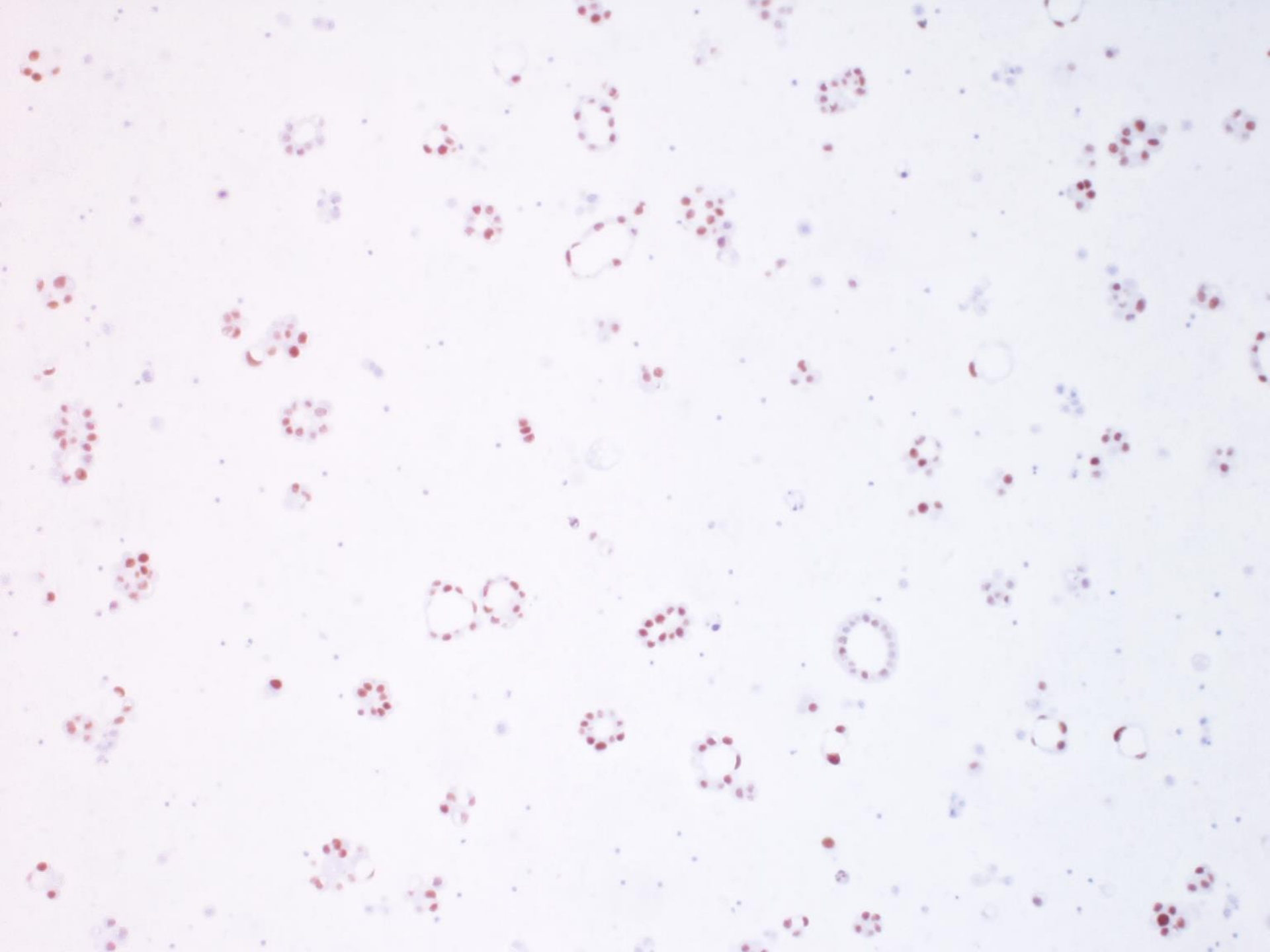
- Advantage of having a relatively pure population of lesional cells

Exfoliative Cytology

- Testing for high-risk HPV is standard of care in cervical screening and is used to clinically guide treatment
- Urovysion FISH for urine cytology specimens

Effusion Cytology





Correct estimate of % of tumor cells (tumor burden) is important for both:

- Adequacy assessment

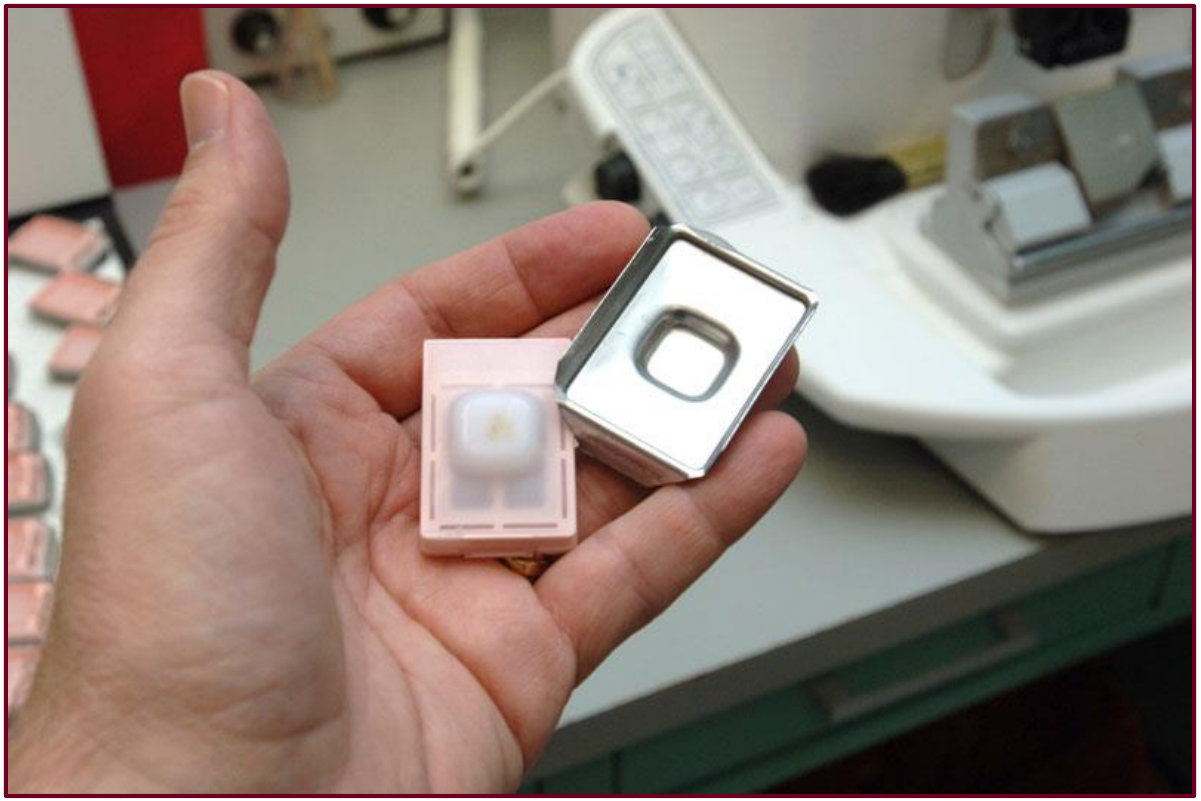
- Correlation with mutant allele frequency (MAF)
    - Primary clone or subclone
    - Somatic vs germline mutation

Specimen types and preparation

Liquid-Based Collection

- Advantages:
  - » Technical skills not necessary for slide preparation
  - » Preservative solution designed for DNA(RNA) preservation
- Disadvantages:
  - » Inability to perform immediate assessment
  - » Potential solution the evaluation of 1 stained preparation from sample to be tested

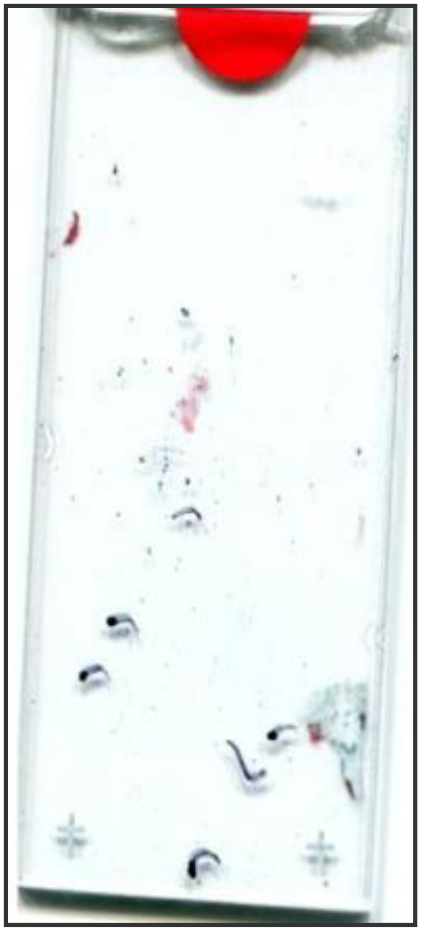
Cell Blocks



- Best understood cytopathology specimen regarding extraction of DNA/in situ methods
- No need for separate validation from FFPE samples (in most cases)
- Applies to FNA, exfoliative and effusion cytology

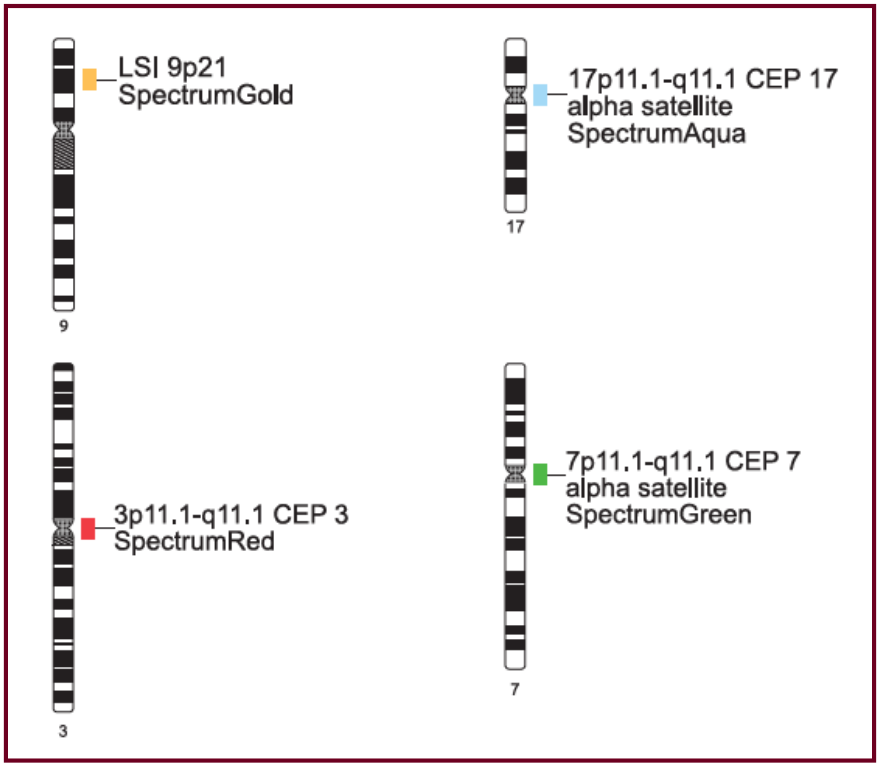
Direct Smears

- High quality of nucleic acids extracted with the common staining techniques, (Papanicolaou, Romanowsky/Diff-Quik)
- Great resource for thyroid FNAs
- Alcohol rather than formalin-based fixation
- Ease of immediate assessment
- Disadvantage:
  - » The slide with lesional material must be sacrificed for molecular testing and is lost from the diagnostic archive
  - » Slide scanning or photographic archive
  - » Partial scraping and re-coverslipping

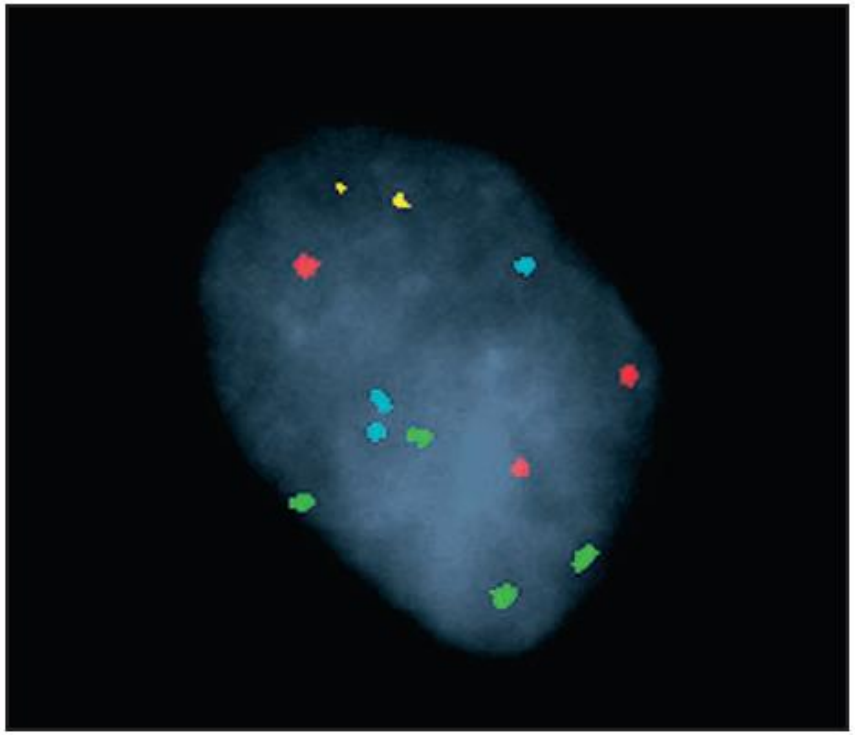


FISH in Cytology Specimens - Urovysion

- Loss of 9p21 and chromosome 3, 7 and 17 aneuploidy correlates with urothelial carcinoma

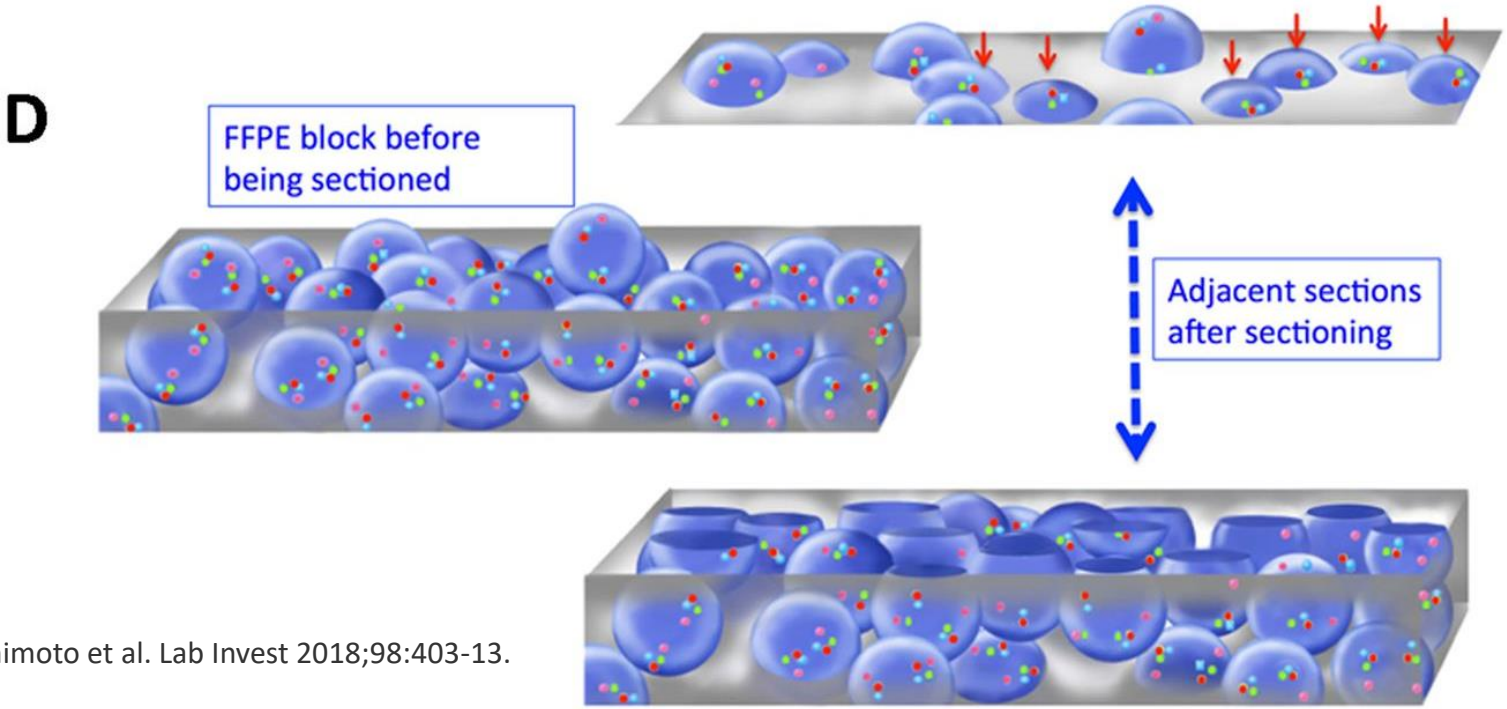


Abbott Molecular, Des Plaines, IL



From: Fritcher et al., 2011.

Truncation Artifact Present in FFPE Slides



From: Yoshimoto et al. Lab Invest 2018;98:403-13.

- Specific validation needed for cytology (smear, single layer) specimens.

What to Test For? – NCCN 1.2020

Pathology Dx	Testing
• Adenocarcinoma • Large cell NE carcinoma • NSCLC-NOS	• EGFR • ALK • ROS1 • BRAF • Testing should be conducted as part of broad molecular profiling • PD-L1
• Squamous cell carcinoma	• Consider EGFR and ALK testing in never smokers or small biopsy specimens or mixed histology • Consider ROS1 and BRAF testing in small biopsy specimens or mixed histology • Testing should be conducted as part of broad molecular profiling • PD-L1

What to Test For? – CAP/IASLC/AMP

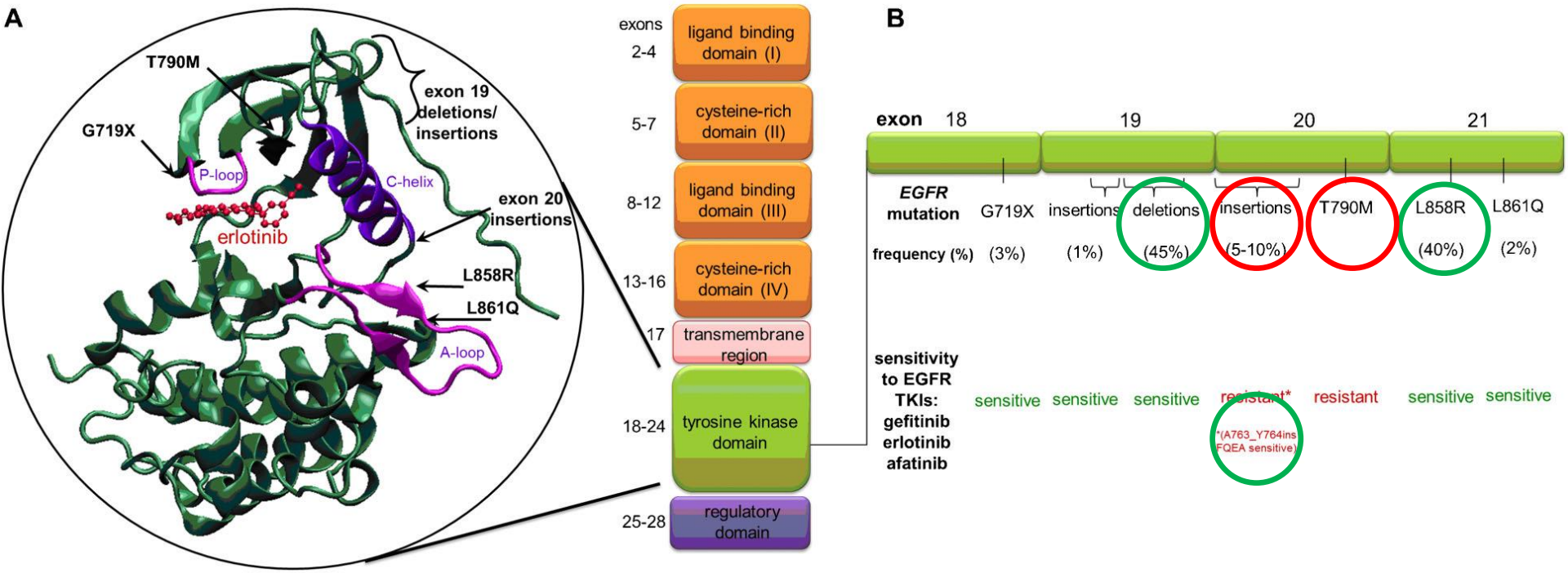
Pathology Dx	Testing
• Adenocarcinoma	• EGFR • ALK • ROS1
• Other histologies	• May test when clinical features indicate a higher probability of an oncogenic mutation

NCCN 1.2020
Sensitizing EGFR Mutation Positive
First Line Therapy

		Preferred	Alternative
Sensitizing EGFR Mutation Positive	EGFR mutation discovered prior to first-line systemic therapy	• Osimertinib • Erlotinib	• Afatinib • Gefitinib • Dacomitinib
	EGFR mutation discovered during first-line therapy	Complete planned systemic therapy, including maintenance therapy, or interrupt, followed by Osimertinib	• Erlotinib • Afatinib • Gefitinib • Dacomitinib

Most Common EGFR Mutations in NSCLC

From: Jorge et al. Braz J Med Biol Res 2014;47:929–39.



How to test?
2018 CAP/IASLC/AMP Guidelines

- Analytic methods must be able to detect mutation in a sample with 20% or more malignant cell content.
- Plaftorms such as unmodified Sanger sequencing with a sensitivity limit of 50% tumor cellularity are not sufficient in practice because many lung cancer samples are small and comprise a majority of benign stromal cells. PCR-based methods are more sensitive by comparison.
  - » It is no longer appropriate to offer a low-sensitivity test that cannot test tumors with 20% to 50% tumor content and requires patients to undergo more procedures, and potentially more invasive procedures, solely to procure a tissue sample with high tumor content.
- It is not appropriate to use IHC for EGFR mutation testing.
  - » Same goes for EGFR FISH.

NCCN 1.2020
Sensitizing EGFR Mutation Positive Progression on Osimertinib

			Subsequent Therapy
Asymptomatic			• Consider definitive local therapy for limited lesions • Continue Osimertinib
Symptomatic	Brain		• Consider definitive local therapy for limited lesions • Continue Osimertinib
	Systemic	Isolated Lesion	• Consider definitive local therapy for limited lesions • Continue Osimertinib
		Multiple Lesions	See initial systemic therapy options

NCCN 1.2020
Sensitizing EGFR Mutation Positive
Progression on Erlotinib/Afatinib/Gefitinib/Dacomitinib

T790M Testing			Subsequent Therapy
Asymptomatic			• Consider definitive local therapy for limited lesions • Osimertinib for T790M+ • Continue E/A/G/D
Symptomatic	Brain		• Consider definitive local therapy for limited lesions • Osimertinib for T790M+ • Continue E/A/G/D • NCCN Guidelines for CNS tumors
	Systemic	Isolated Lesion	• Consider definitive local therapy for limited lesions • Osimertinib for T790M+ • Continue E/A/G/D
		Multiple Lesions	T790M+: Osimertinib (if not previously given)
			T790M-: Initial systemic therapy options

How to Test for Resistance?

NCCN Guidelines 1.2020	2018 CAP/IASLC/AMP Guidelines
For patients with an underlying EGFR sensitizing mutation who have been treated with EGFR TKI, minimum appropriate testing includes high sensitivity evaluation for p.T790M. Assays for the detection of T790M should be designed to have an analytic sensitivity of 5% allelic fraction.	Recommendation: Laboratories testing for EGFR T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting EGFR T790M mutations in as little as 5% of EGFR alleles.
When there is no evidence of T790M, testing for alternate mechanisms of resistance (MET amplification, ERBB2 amplification) may be used.	• A second acquired resistance mutation, C797S, can arise in tumors that have progressed after Osimertinib treatment for T790M • Testing for C797S is not recommended for routine management at this time.

Other Specific Treatments

Genetic Alteration	First Line Treatment	
	Preferred	**Alternative**
ALK Rearrangement	• Alectinib • Brigantinib	• Ceritinib • Crizotinib
ROS1 Rearrangement	• Crizotinib	• Entrectinib • Ceritinib
BRAF V600E Mutation	• Dabrafenib + Trametinib • Vemurafenib	• Dabrafenib
NTRK1/2/3 Rearrangement	• Larotrectinib	• Entrectinib
PD-L1 Expression Positive	• Pembrolizumab	

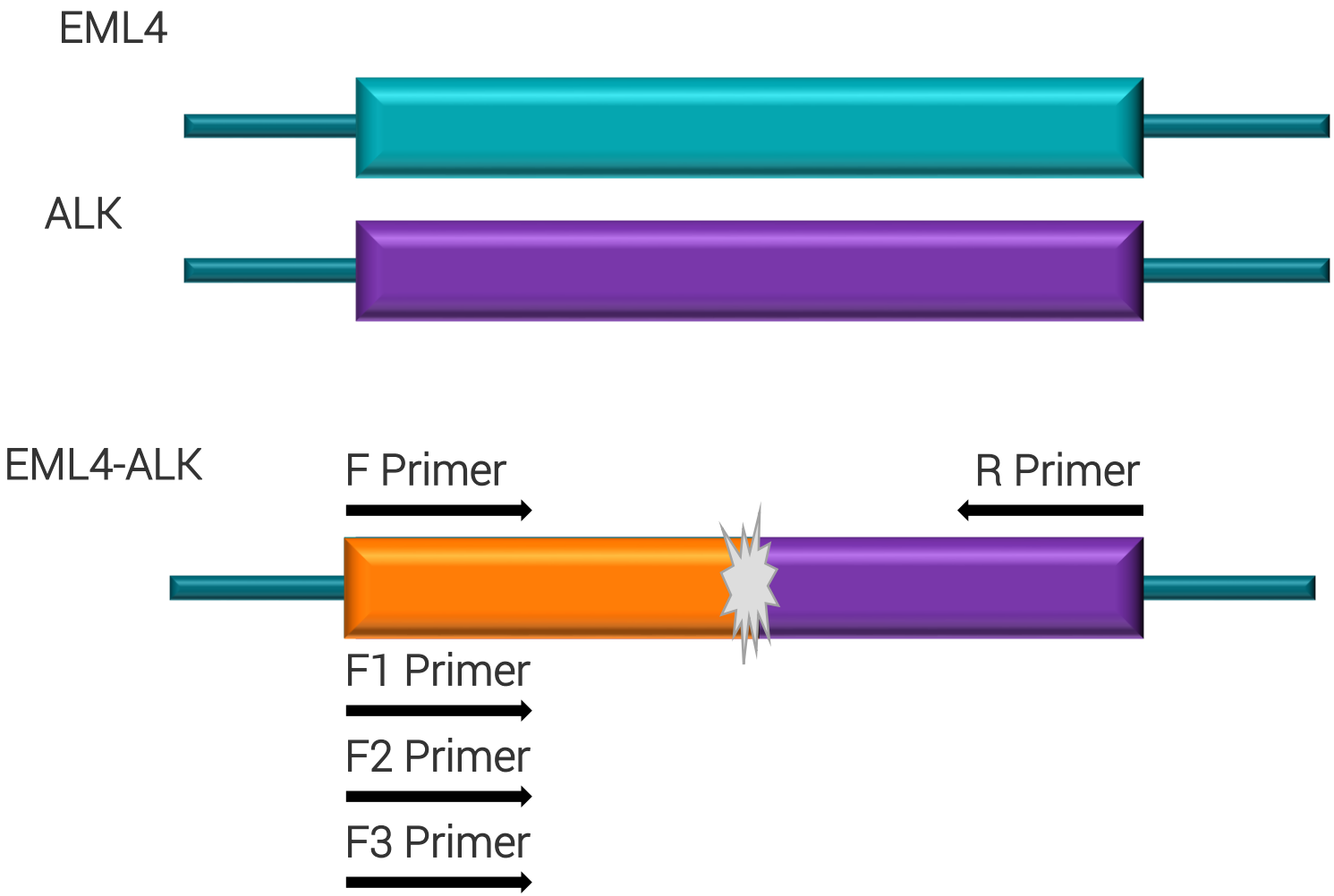
How to Test for Other Genetic Alterations

Genetic Alteration	Recommendations	
	NCCN 1.2020	**2018 CAP/IASLC/AMP**
ALK Gene Rearrangements	• FISH • IHC can be deployed as an alternative strategy • FDA approved IHC (ALK D5F3) can be utilized as a stand-alone test • NGS methodologies can detect ALK fusions • Targeted real-time PCR are used in some settings	• IHC is an equivalent alternative to FISH for ALK testing • RT-PCR and NGS have shown comparable performance with IHC when designed to detect the majority of fusions
ROS1 Gene Rearrangements	• FISH can be deployed (it may underdetect FIG-ROS1 variant • IHC can be deployed; however needs confirmation. Screening modality • NGS can detect, although DNA-based NGS can undertetect ROS1 fusions • PCR unlikely to detect fusions with novel partners	• ROS1 IHC may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method

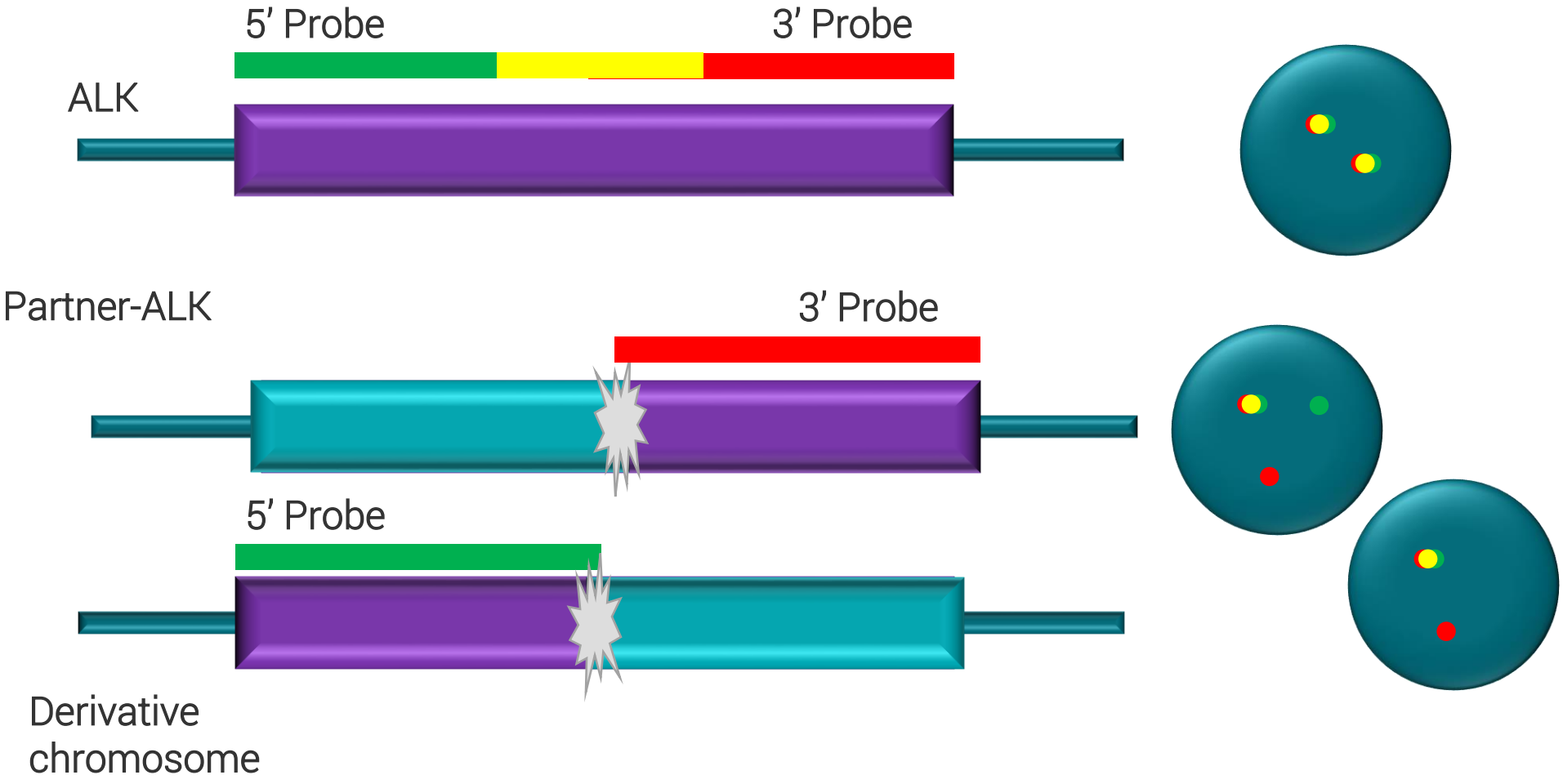
How to Test for Other Genetic Alterations

Genetic Alteration	Recommendations	
	NCCN 1.2020	2018 CAP/IASLC/AMP
BRAF Point Mutations	• Real-time PCR, Sanger sequencing (paired with tumor enrichment) and NGS employed methodologies • IHC only after extensive validation	• Not indicated as a routine stand-alone assay outside the context of a clinical trial • Appropriate to include as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative
KRAS Point Mutations	• No recommendations	• Not indicated as a routine stand-alone assay outside the context of a clinical trial • Appropriate to include as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative
NTRK (NTRK1/2/3) Gene Fusions	• FISH, IHC PCR, NGS can be used • IHC is complicated by baseline expression in some cases • FISH may require 3 probe sets • DNA-based NGS may underdetect NTRK1 and NTRK3 fusions	• No recommendations

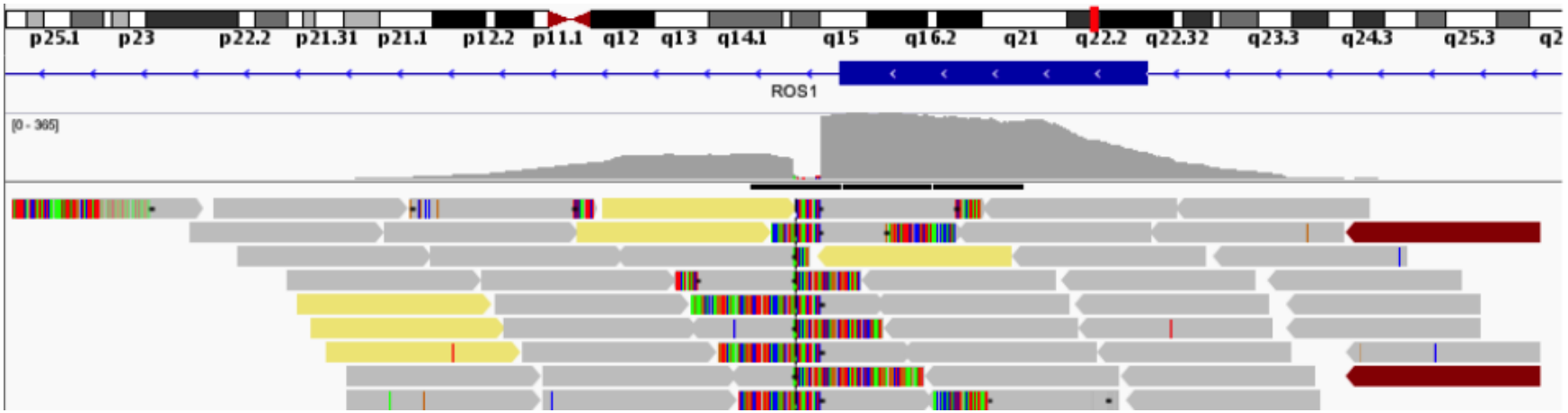
PCR for Gene Rearrangements



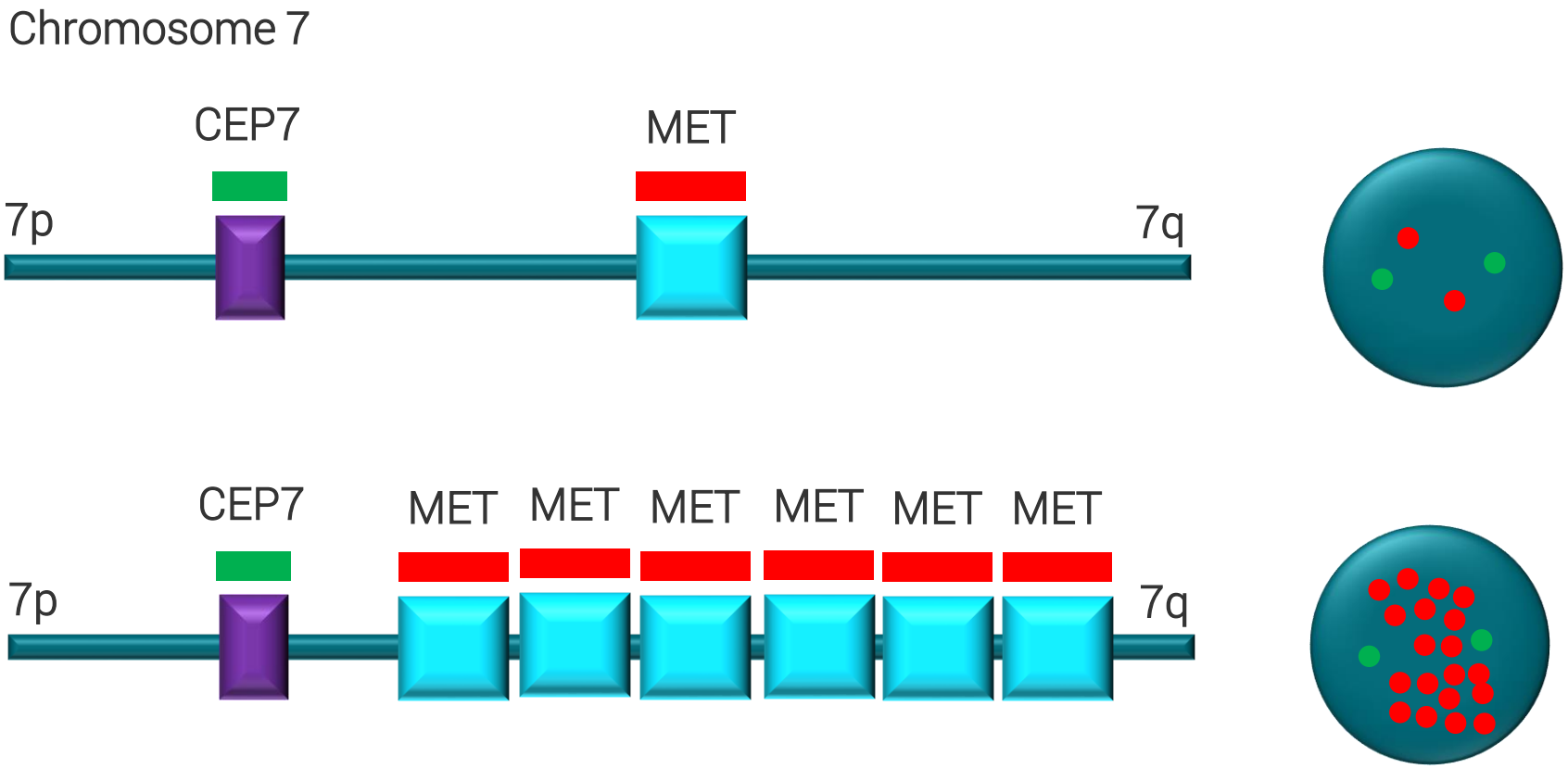
FISH for Gene Rearrangements
Break-apart Probes



Gene Rearrangement Detection by NGS



FISH for Gene Amplification

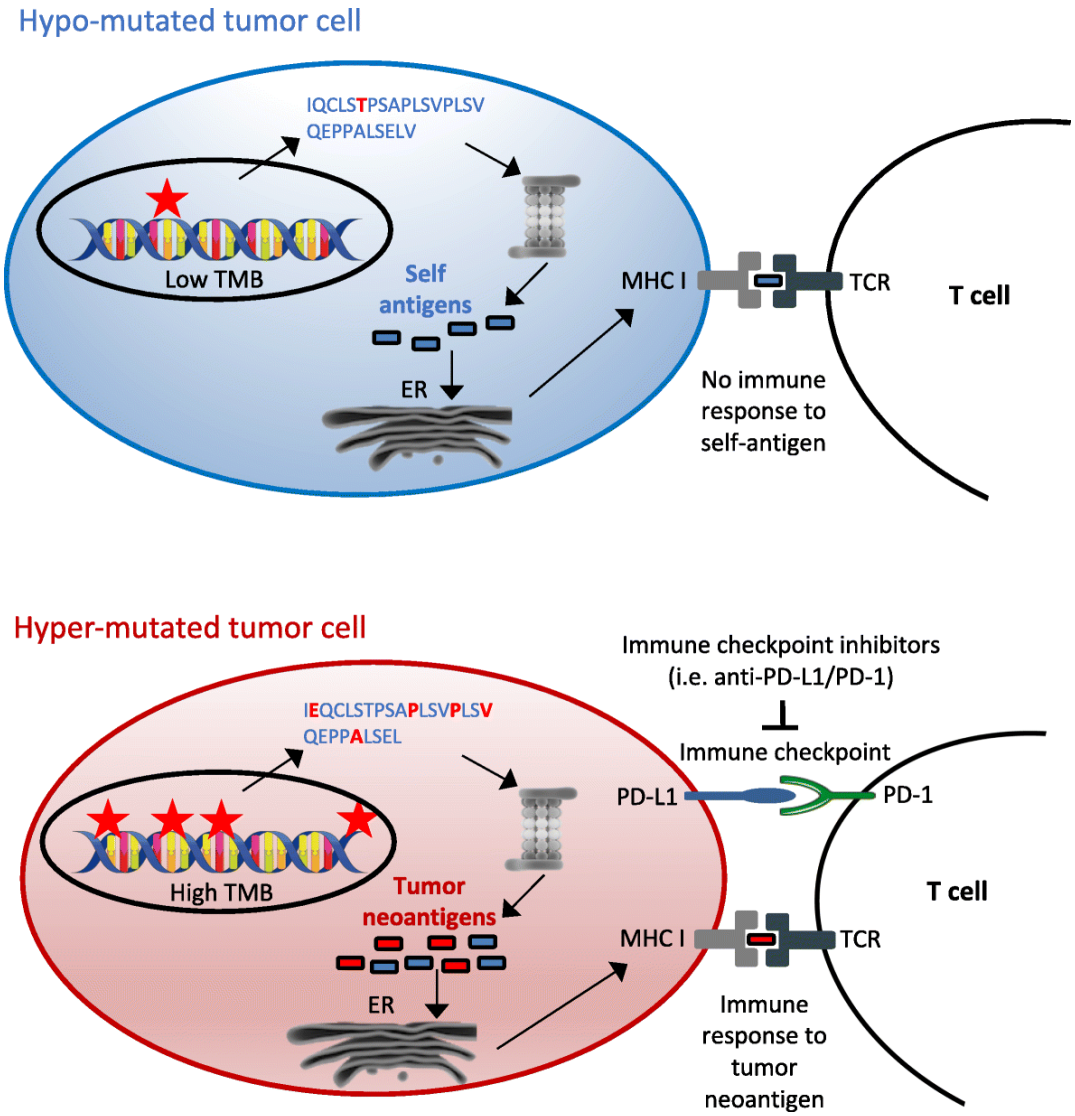


NCCN 1.2020 Emerging Biomarkers

Genetic Alteration	Available Targeted Agents	2018 CAP/IASLC/AMP Guidelines
High-level MET Amplification OR MET Exon 14-skipping Mutation	• Crizotinib	• Not indicated as a routine stand-alone assay outside the context of a clinical trial • Appropriate to include as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative
RET Rearrangements	• Cabozanitnib • Vandetanib	
ERBB2 (HER2) Mutations	• Ado-trastuzumab emtasine	
Tumor Mutational Burden (TMB)	• Nivolumab + Ipilibumab • Nivolumab	• No mention

Mutational Tumor Burden (TMB)

- NOT to be confused with Tumor Burden of a specimen: Percentage of tumor cells over total cells.
- TMB is defined as the total number of mutations, including both base substitutions and short insertions/deletions, per coding area of a tumor genome.
  - » Usually expressed as number of mutations per Megabase (Mb; 1 million base pairs)
- Initially calculated based on exome studies, currently consensus is that targeted NGS panels with at least 1.5 Mb coverage have similar findings to those of an exome.
- TMB varies significantly between different cancer types
  - » Melanoma has some of the highest number of mutations
  - » GI cancers, such as pancreatic cancer and MMR–proficient colorectal cancer, having some the lowest.
- NSCLC spans a range in TMB, with a relatively higher TMB seen in smoking-related lung cancer, whereas lower tumors in never-smokers



From: Fancello et al. J Immunother Cancer 2019;7:183.

Mutational Tumor Burden (TMB) in NSCLC

- Relation between higher TMB and response to checkpoint inhibition has been suggested by several studies.

- Rizvi et al. tested pembrolizumab in lung tumors with high nonsynonymous mutational and neoantigen levels and found that this was associated with longer PFS and improved durable clinical benefit

Checkmate 227

- Open-label, randomized Phase 3 trial
- Nivolumab, or Nivolumab + Ipilimumab, or Nivolumab + Platinum Doublet Chemotherapy vs. Platinum Doublet Chemotherapy
- Patients with chemotherapy-naive Stage IV or recurrent NSCLC
- Demonstrated superior PFS in patients with high TMB (≥10 mutations per Mb), irrespective of PD-L1 expression or histology, who received combination immunotherapy instead of chemotherapy in the first-line metastatic setting (hazard ratio [HR], 0.58; 95% CI, 0.41-0.81)

- However…

Checkmate 227

- Subsequent OS data have revealed a statistically nonsignificant benefit of ipilimumab + nivolumab in patients with high TMB (HR, 0.77; 95% CI, 0.56-1.06)

- Comparable survival benefit was seen in patients with TMB <10 mut/Mb (HR, 0.78; 95% CI, 0.61-1.00)

- The supplemental biologics license application seeking frontline FDA approval of ipilimumab with nivolumab for advanced NSCLC with TMB ≥10 mut/Mb was withdrawn pending final data from part 1a of Checkmate 227

Checkmate 227 – Latest Update (12/2019)

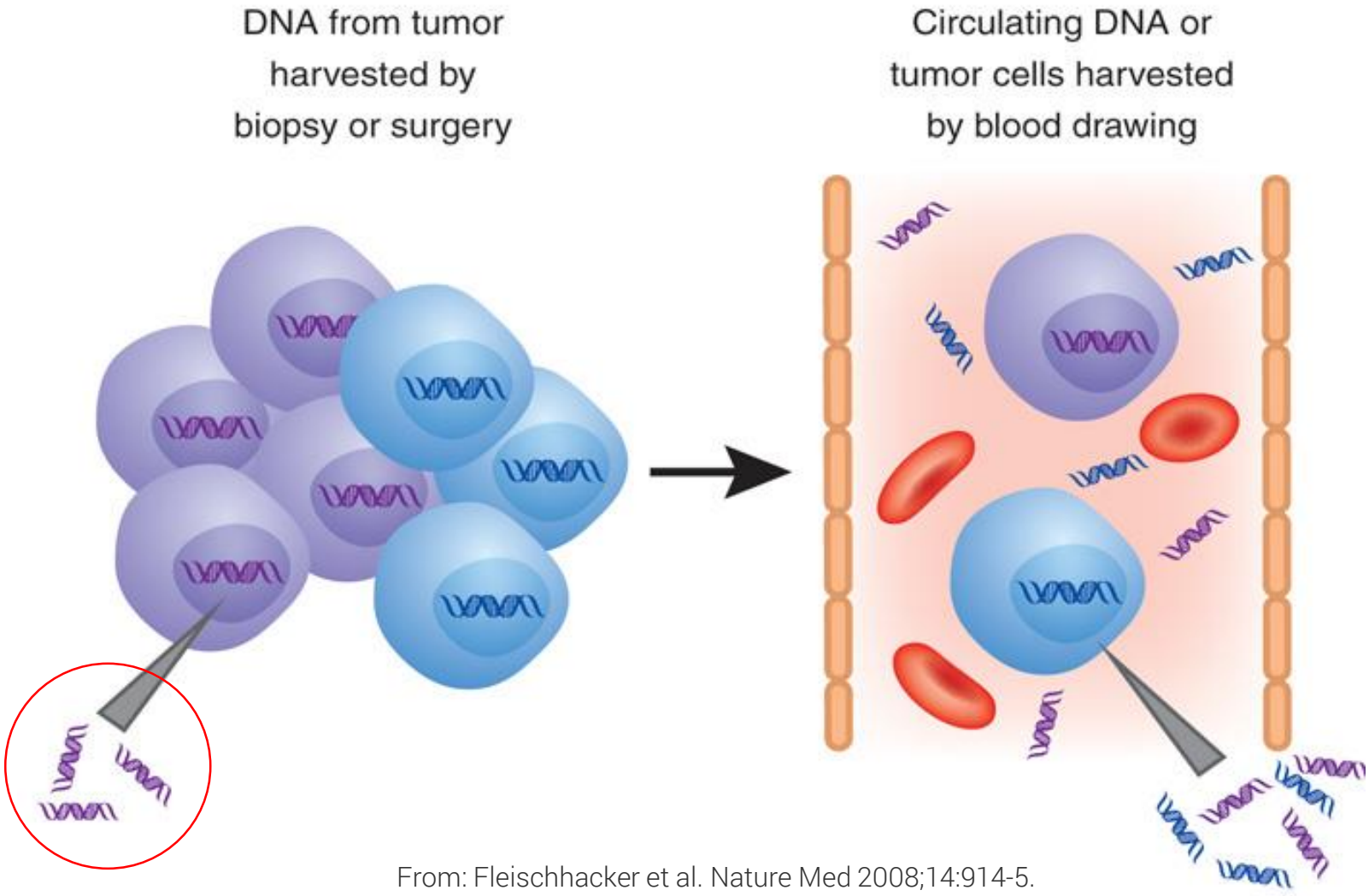
- Hellmann et al. N Engl J Med. 2019 Nov 21;381(21):2020-2031.
- An overall survival benefit with nivolumab + ipilimumab, as compared with chemotherapy, was observed regardless of the subgroup of PD-L1 expression level.
- Among the 679 patients (58.2%) in whom the TMB was evaluated, a similar degree of overall survival benefit was observed in patients who received nivolumab + ipilimumab, regardless of TMB status (10 mut/Mb cutoff), despite the previous observation of improved PFS in patients with high TMB.
- Combining the two key biomarkers (PD-L1 and TMB) did not identify a subgroup that had an increased magnitude of benefit with nivolumab + ipilimumab over chemotherapy, although the sample sizes become more modest in these analyses.

BUSTER WAS CAUGHT BARKING UP THE WRONG TREE AGAIN.

Challenges with TMB

- TMB as a biomarker has other limitations
  - » Lack of standardization between the testing platforms used
  - » Lack of an identified, fixed TMB threshold defining a tumor as having “high” TMB
  - » Various thresholds of TMB have been used by different studies
  - » Possible algorithmic approach
- TMB harmonization project

Circulating Tumor Cells and Cell-Free Tumor DNA



From: Fleischhacker et al. Nature Med 2008;14:914-5.

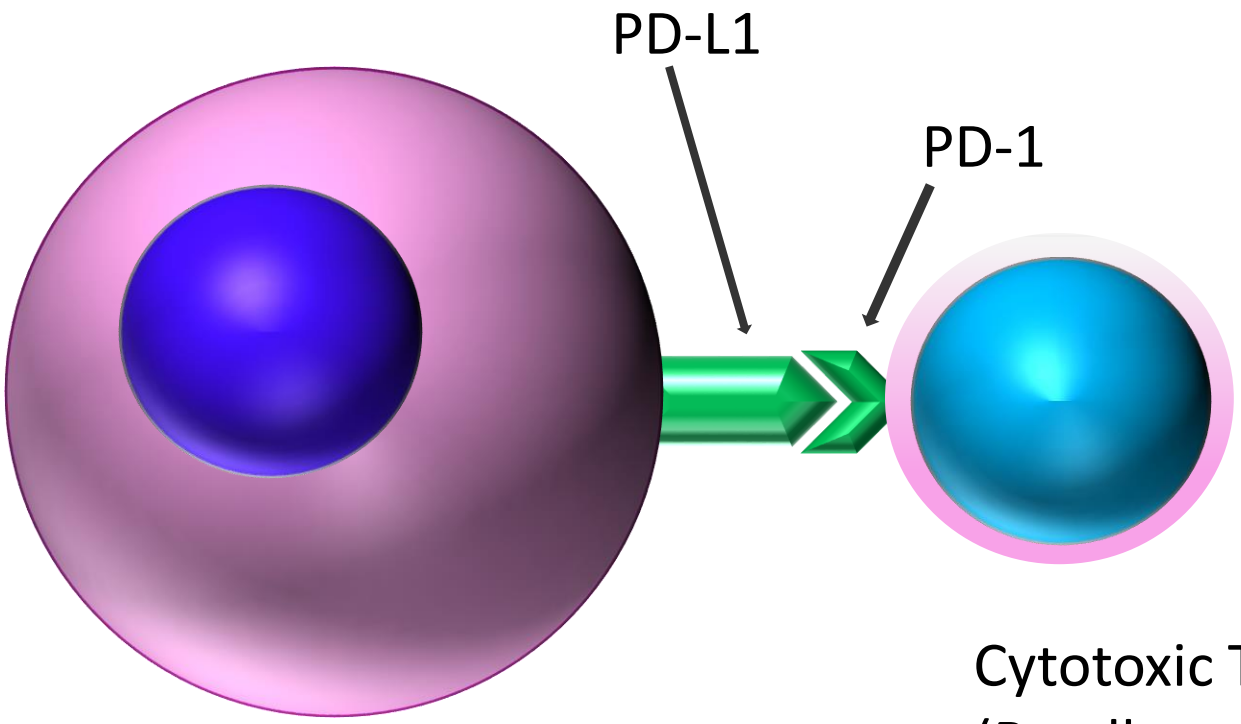
Circulating Cell-Free Tumor DNA

NCCN 1.2020	2018 CAP/IASLC/AMP
Cell-free/circulating tumor DNA (cfDNA) should not be used in lieu of tissue diagnosis.	There is currently insufficient evidence to support the use of circulating cell-free plasma DNA molecular methods for the diagnosis of primary lung adenocarcinoma.
Standards and guidelines for cfDNA testing for genetic alterations have not been established.	
There is a 30% false negative rate, and alterations can be detected that are not related to the tumor (e.g. IDH1, KRAS, TP53 mutations of CHIP)	
cfDNA testing can be used in specific circumstances: • Patient not medically fit for tissue sampling • Insufficient tissue for molecular analysis and follow up tissue analysis will be done if an oncogenic driver is not identified	

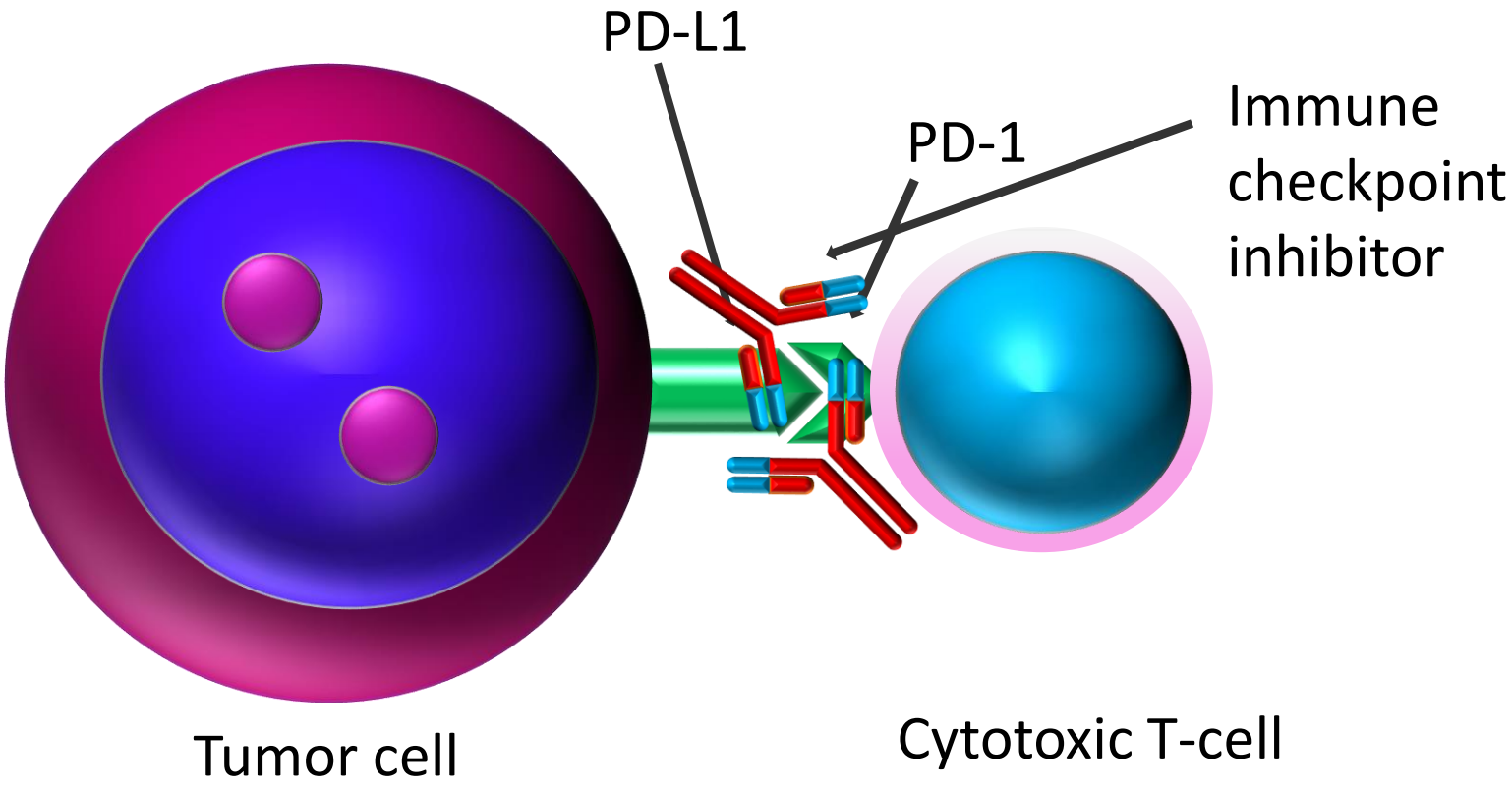
Circulating Cell-Free Tumor DNA

NCCN 1.2020	2018 CAP/IASLC/AMP
cfDNA may be considered at progression instead of tissue biopsy to detect whether patients have T790M. • However, if liquid biopsy is negative, then tissue biopsy is recommended.* • *Same can be applied to CNS involvement.	• In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA assay to identify EGFR mutations. • Physicians may use cell-free plasma DNA methods to identify EGFR T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted TKIs; testing of the tumor sample is recommended if the plasma result is negative. • There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of EGFR T790M mutations at the time of EGFR TKI resistance.

PD-1/PD-L1 Interaction in Normal Immunomodulation



PD-1/PD-L1 Interaction in Cancer



PD-L1 Major Updates in the Last Year - NSCLC

- Changes in pembrolizumab (Keytruda®) approval as first line monotherapy in non-small cell lung cancer (NSCLC)
  - » 22C3 companion diagnostic tumor proportion score (TPS) cutoff of 1% (no more 50%)
  - » Different algorithms for ≥50% vs. 1-49% TPS in NCCN 1.2020 Guidelines

Dako 22C3 PharmDx - pembrolizumab (Keytruda®) NSCLC – APRIL 2019 UPDATE

Indication	Comment
NSCLC 1st line **MONOTHERAPY** treatment • EGFR/ALK non-mutant NSCLC AND • Stage III, non-candidates for surgery/definitive chemoradiation • Metastatic	FDA approved with PD-L1 22C3 • ≥1% tumor proportion score (TPS) • APRIL 2019 UPDATE
NSCLC 2nd line **MONOTHERAPY** treatment • EGFR/ALK non-mutated NSCLC • EGFR/ALK mutant NSCLC with progression on EGFR or ALK specific, FDA approved therapy	FDA approved with PD-L1 22C3 • ≥1% tumor proportion score (TPS)
NSCLC 1st treatment, in **COMBINATION** with chemotherapy • EGFR/ALK non-mutated metastatic non-squamous NSCLC • Metastatic squamous NSCLC	FDA approved • NO 22C3 IHC TESTING REQUIRED

Indications for pembrolizumab (Keytruda®) treatment

Indication	Comment
SITE AGNOSTIC dMMR/MSI tumors 2nd line treatment	FDA approved • NO 22C3 IHC TESTING REQUIRED • dMMR IHC or MSI TESTING REQUIRED

Dako 28-8 pharmDx - nivolumab (Opdivo®) - NSCLC

Clone	**28-8 rabbit anti-PD-L1 monoclonal antibody**
Platform	• EnVision FLEX visualization system • Autostainer Link 48
NSCLC 2nd line treatment (squamous and non-squamous) Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving OPDIVO.	FDA approved (**COMPLEMENTARY**) for treatment with nivolumab (Opdivo®, Bristol-Myers Squibb, New York, NY) • ≥1% tumor proportion score (TPS) in **NON-SQUAMOUS NSCLC** • 28-8 IHC OPTIONAL FOR NON-SQUAMOUS NSCLC • NO TESTING FOR SQUAMOUS CELL CARCINOMA

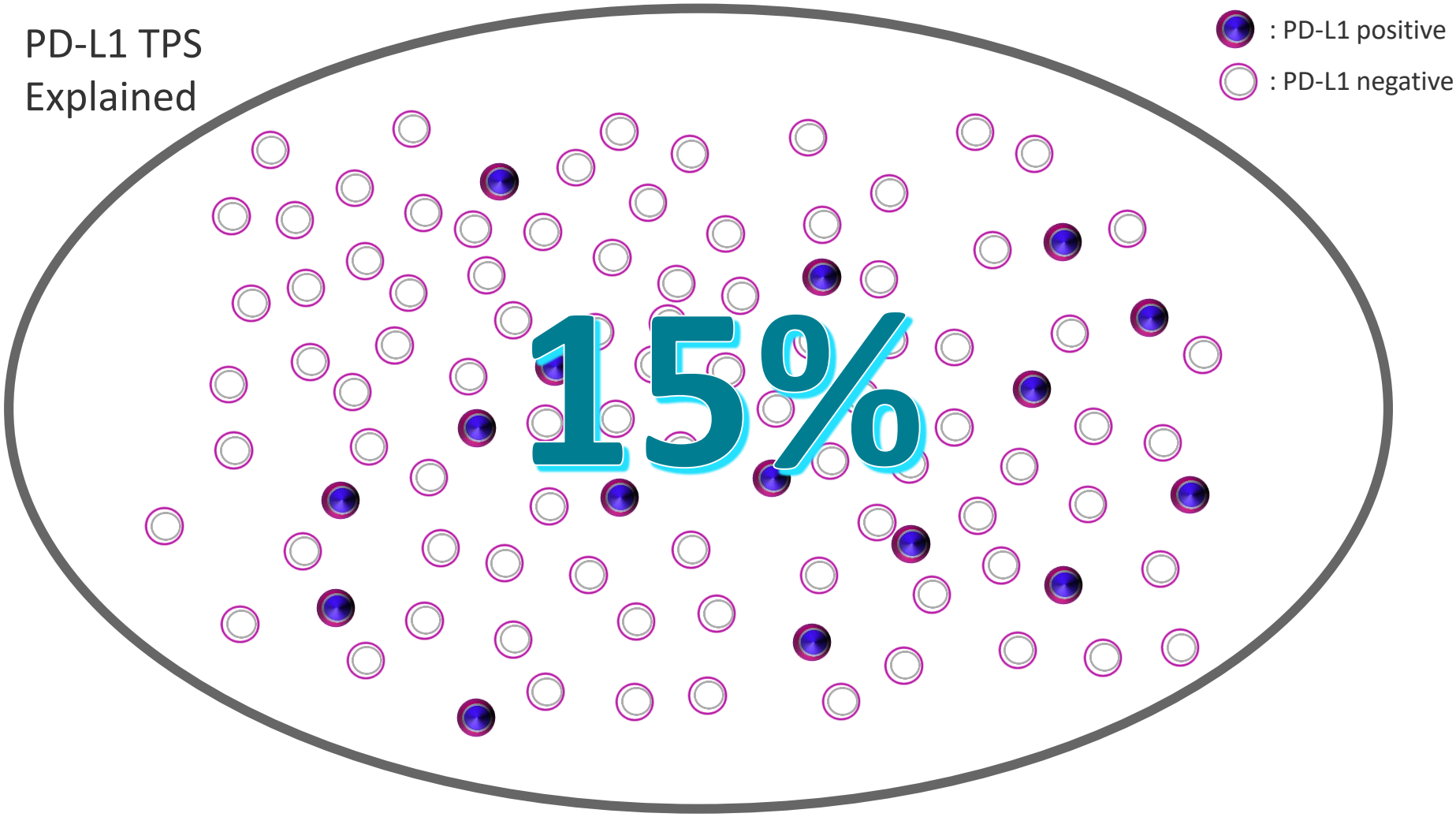
PD-L1 22C3 (NSCLC) and 28-8 Scoring: Tumor Proportion Score (TPS)

$$TPS = \frac{\#\ of\ PD{-}L1\ positive\ tumor\ cells}{Total\ \#\ of\ PD{-}L1\ positive\ and\ PD{-}L1\ negative\ tumor\ cells} \times 100\%$$

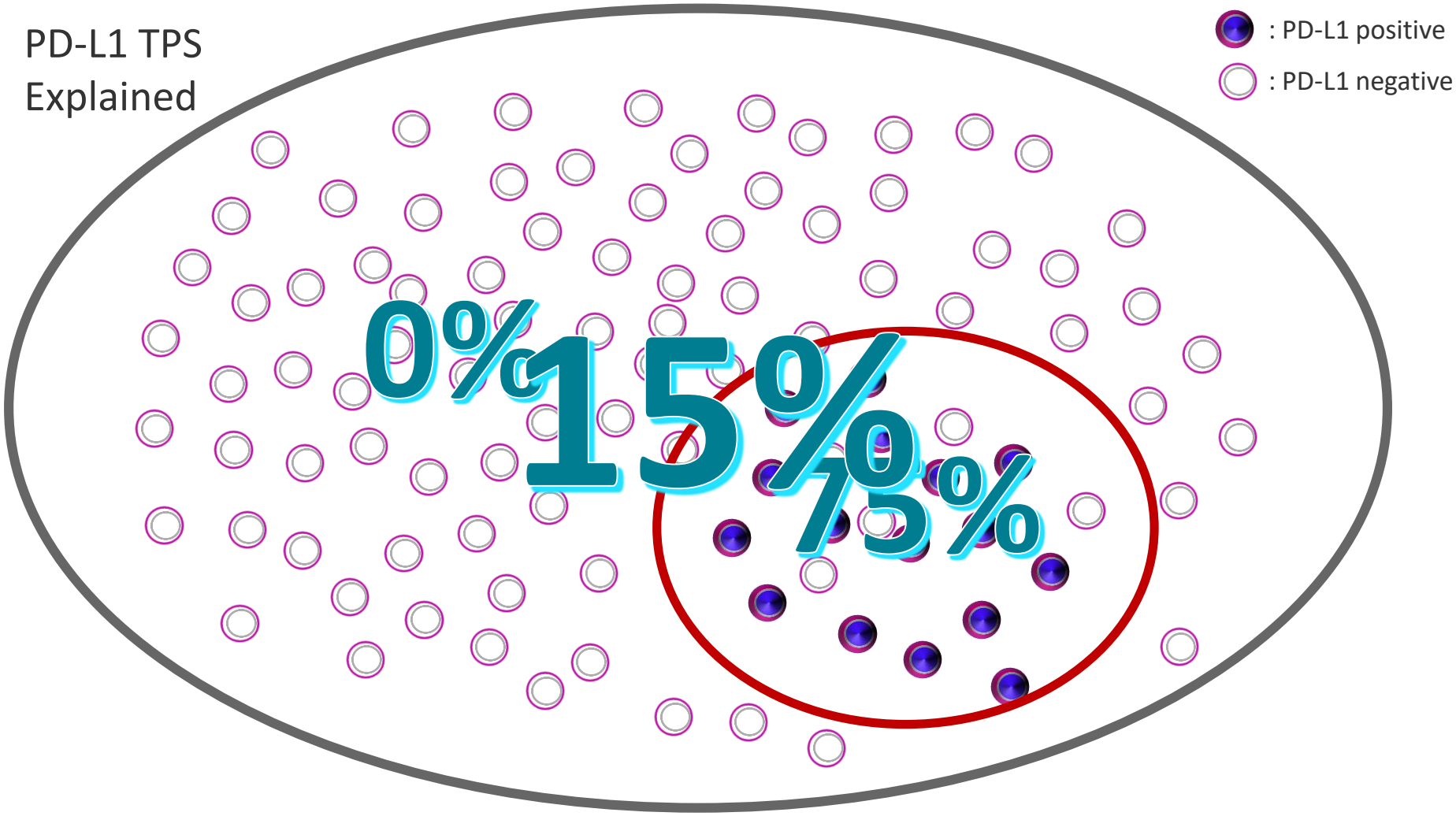
What to score?

- Score partial or complete cell membrane staining.
  - Exclude cytoplasmic staining from scoring.
- Score only viable tumor cells
  - Exclude infiltrating immune cells, normal cells, necrotic cells, debris.
- Staining intensity not important.

PD-L1 TPS Explained



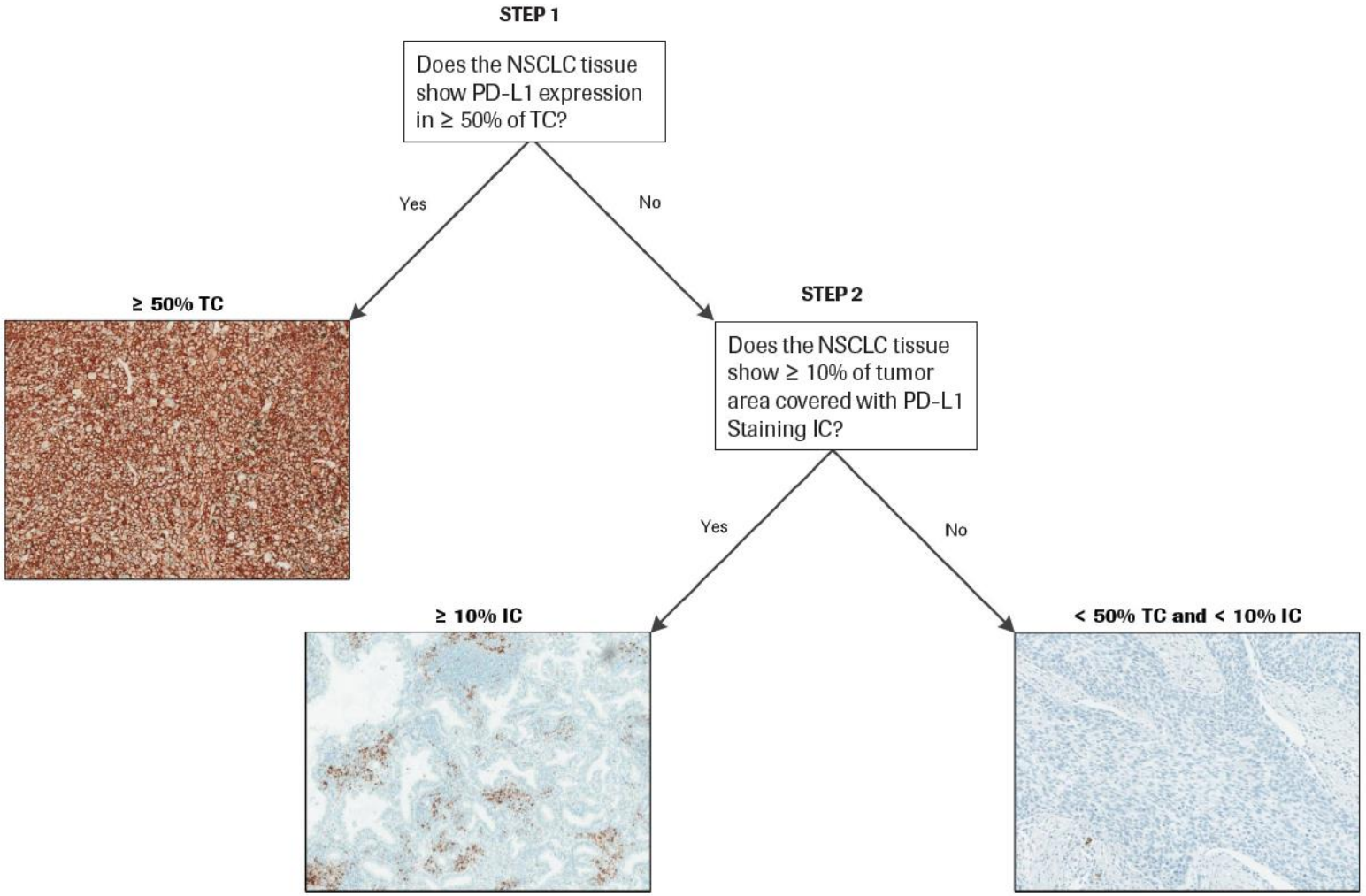
PD-L1 TPS Explained



Ventana PD-L1 SP142 - atezolizumab (Tecentriq®)

Clone	SP142 rabbit monoclonal anti-PD-L1 antibody
Platform	• OptiView DAB IHC Detection Kit • OptiView Amplification Kit • VENTANA BenchMark ULTRA instrument
NSCLC 2nd line treatment (metastatic)	FDA approved (**COMPLEMENTARY**) for atezolizumab (Tecentriq®, Roche Genentech, South San Francisco, CA)
Non-squamous NSCLC **1st line COMBINATION** therapy (metastatic)	FDA approved (**COMPLEMENTARY**) for atezolizumab (Tecentriq®, Roche Genentech, South San Francisco, CA) DECEMBER 2018 UPDATE
Small cell lung carcinoma (SCLC) 1st line combination therapy	NO PD-L1 TESTING NEEDED MARCH 2019 UPDATE

SP142 Interpretation - NSCLC



Ventana PD-L1 SP263 - durvalumab (Imfinzi®)

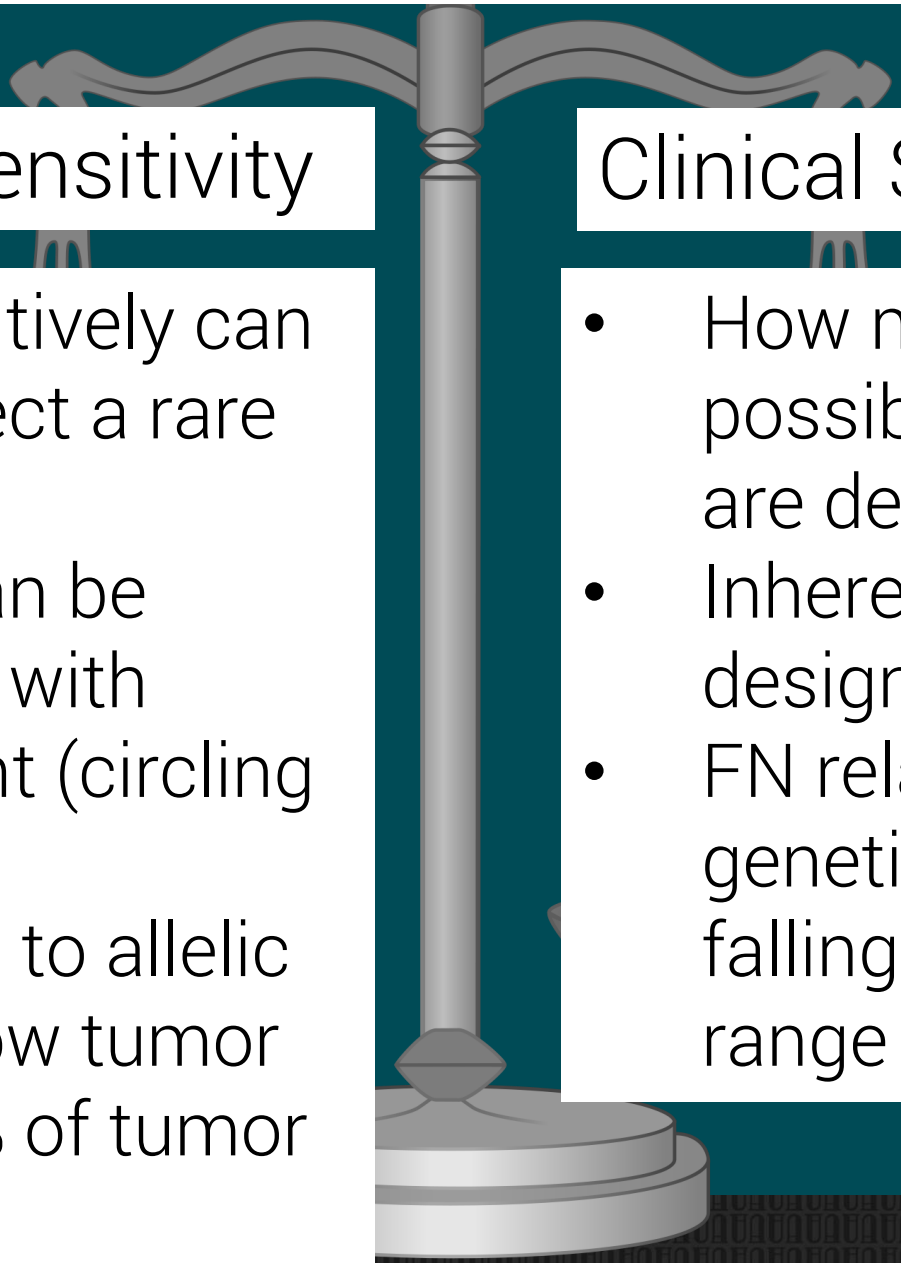
Clone	SP263 rabbit monoclonal anti-PD-L1 SP263 antibody
Platform	• OptiView DAB IHC Detection Kit • OptiView Amplification Kit • VENTANA BenchMark ULTRA instrument
Non small cell lung cancer 2nd line treatment • Unresectable/stage III AND • Progression after platinum therapy and radiotherapy	• NO IHC TESTING REQUIRED

Immune Checkpoint Inhibitor Treatment in NSCLC

- Generally or tumors that DO NOT harbor
  - » EGFR mutations
  - » ALK rearrangements
- Patients with either one of the above generally do not respond as well to ICI treatment, irrespective of PD-L1 expression
- Clinical scenarios can exceptions for nivolumab/pembrolizumab (patients who have failed EGFR/ALK-specific treatment)

Preanalytical Optimization of Cytology and small biopsy Specimens

Scale of Sensitivities



Analytical Sensitivity

- How sensitively can a test detect a rare change?
- Low AS can be overcome with enrichment (circling of tumor)
- FN related to allelic dilution (low tumor burden - % of tumor cells

Clinical Sensitivity

- How many of the possible changes are detected?
- Inherent in test design
- FN related to genetic alterations falling outside the range of testing

Preanalytical Processing

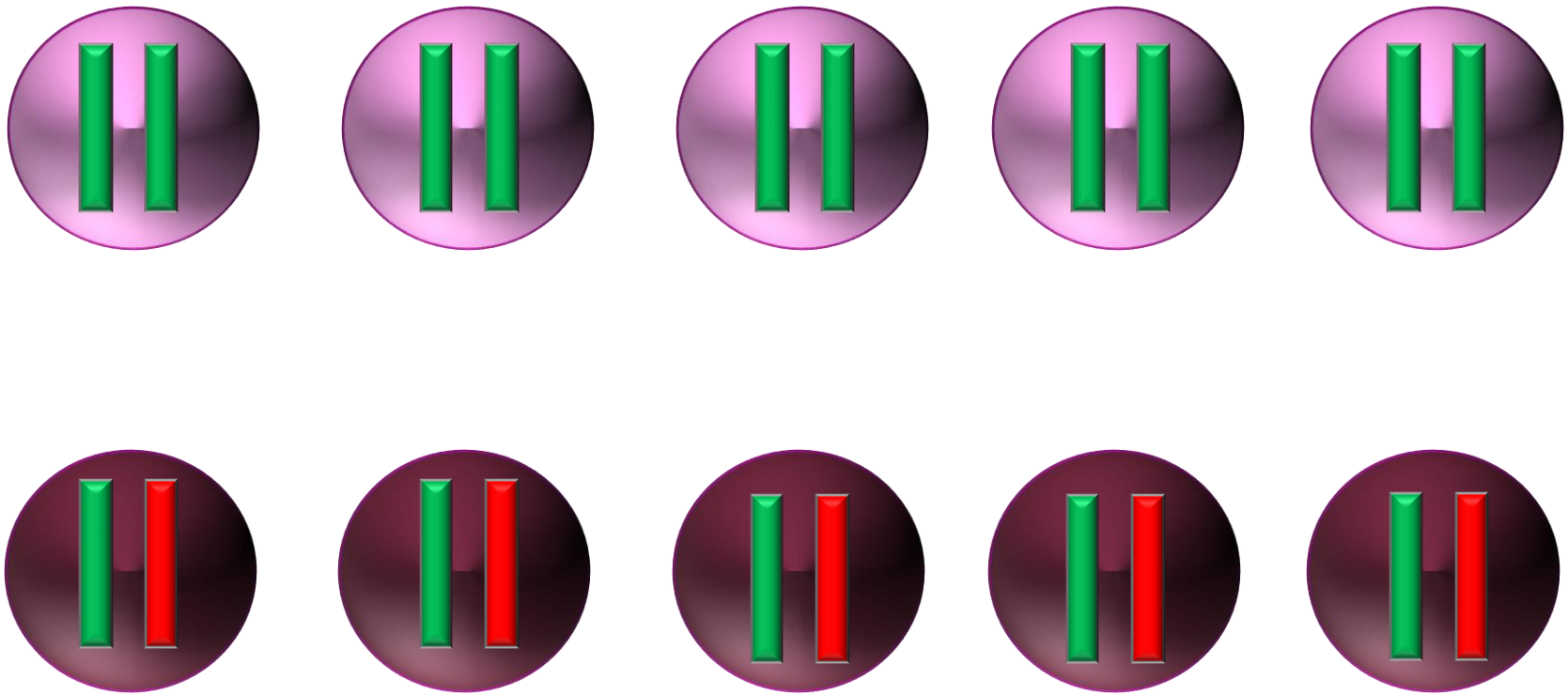
- Assessment for adequacy:
  - » Ratio of tumor to non-tumor nucleated cells in a specimen
  - » An extremely small specimen with high tumor cellularity may be superior to an abundant specimen with low tumor cellularity
- Evaluation of specimen quantity is an important first step
  - » Limiting material used for morphological diagnosis to necessary amount
- Thinking of ways of to better utilize the small cytology specimens

Analytical Sensitivities of Different Sequencing Platforms

Platform	Limit of Detection – Mutant Allelic Frequency	Comments	Percentage of Tumor Cells for Testing (Tumor Burden)*
Sanger Sequencing	15-20%	Not a quantitative method	30-40%
Melt Curve Analysis	≈10%	Not a quantitative method	≈20%
Pyrosequencing	≈5%	Conservatively at 10%	10-20%
NGS	1-2%	May detect less than that	5-10%

1. Tsiatis et al. J Mol Diagn 2010;12:425-32.
2. Lin et al. Am J Clin Pathol 2014;141:856-66.

*Assuming that tumor cells are heterozygous for the mutation



: Non-tumor cell

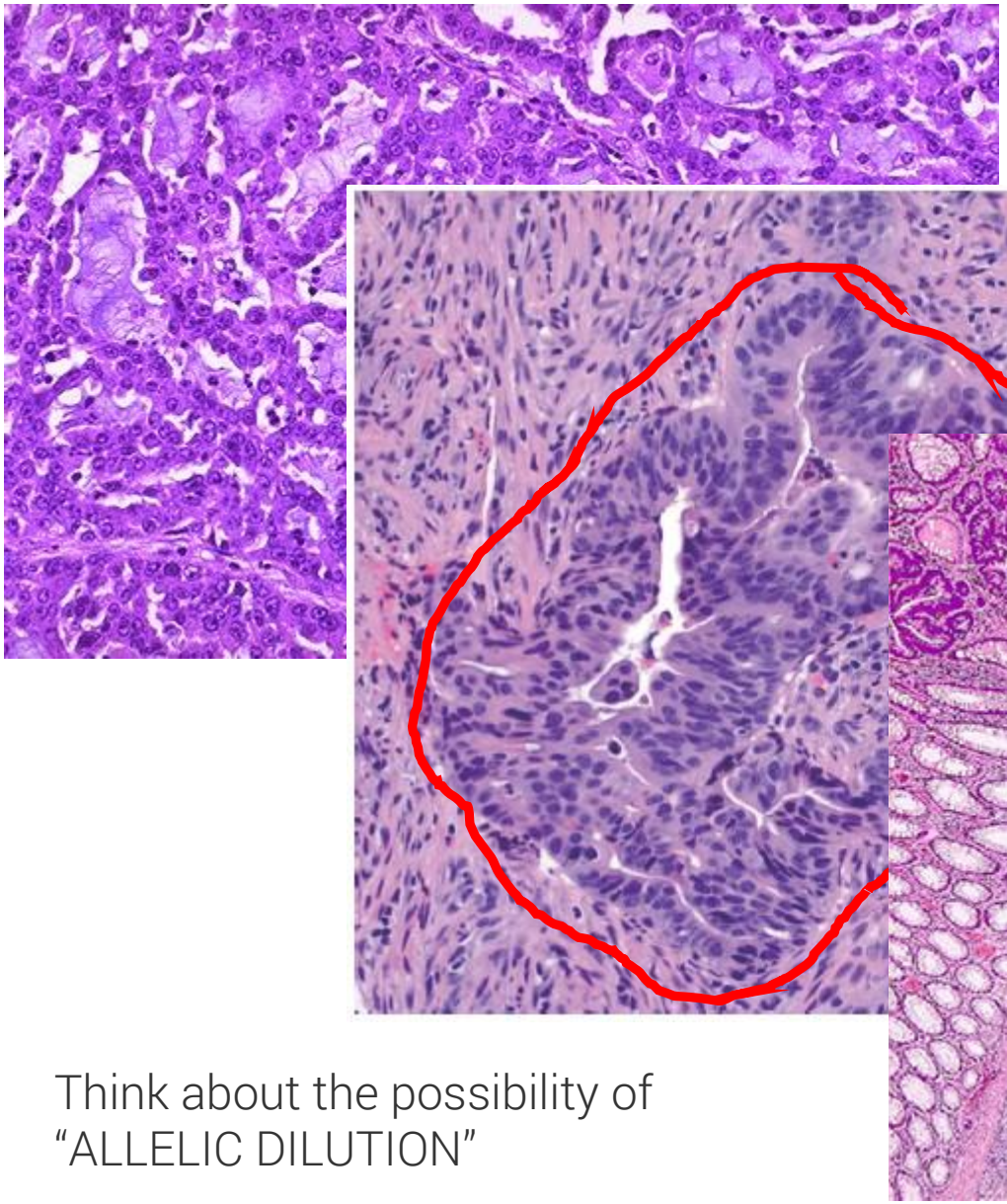
: Tumor cell

50%

: Non-mutated allele

: Mutated allele

25%



Think about the possibility of
“ALLELIC DILUTION”

How many cells do I need?

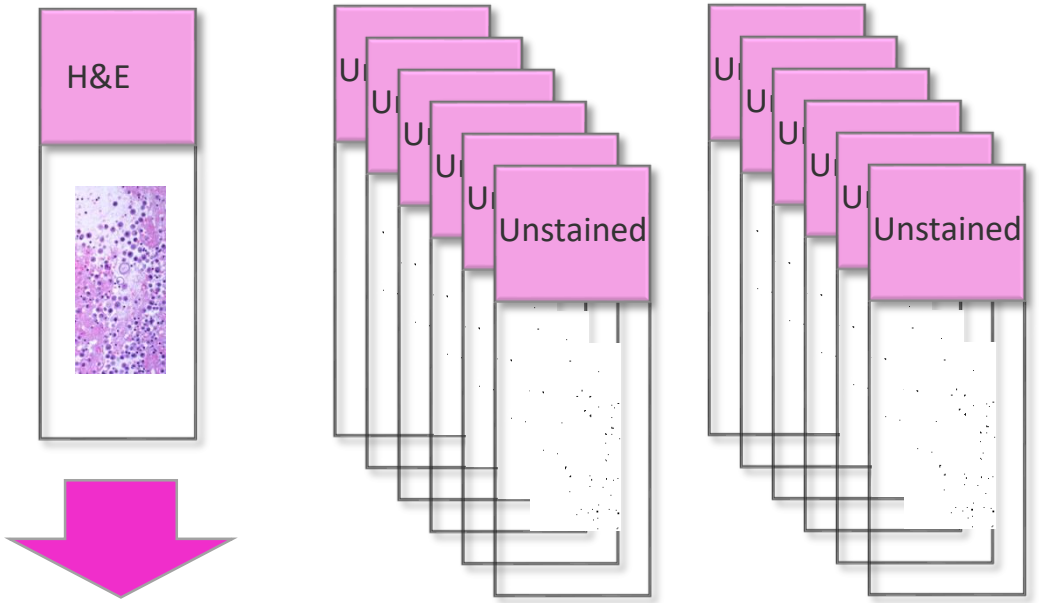
- How much DNA does one cell contain?
  - » 6-7 pg of DNA

- How many cells are needed for 1 ng of DNA?
  - » 1000/6 = 166.66
  - » 1000/7 = 142.85
  - » 140-170 cells

Cell requirements for common tests

Test	DNA Input	Number of Cells	Comments
Single gene assays (pyro-quant, PCR)	1 ng	160	
Sanger sequencing	10 ng	1600	
NGS	10-50 ng or more	1600-8000	10 ng min, shoot for 50 or more
SNParray	50-80 ng	8000-13000	
PD-L1 IHC	N/A	100	On 1 slide
ALK/ROS1/RET FISH	N/A	100	
MET FISH	N/A	40	
ALK/ROS1 IHC	N/A	50-10	

Initial Processing of Specimens



Adequacy assessment:

- % of tumor cells based on platform, for sequencing, PCR, etc.
- ≥100 viable tumor cells for PD-L1, ALK/ROS1/RET FISH
- ≥40 viable tumor cells for MET FISH
- ≥50-100 viable tumor cells for ALK/ROS1 IHC

Slide requirements for common tests

Test	DNA Input	Number of Slides	Comments
NGS	10-50 ng	10-20 	
EGFR	1-10 ng	1-2 	
BRAF	1-5 ng	1 	
ALK/ROS1 (FISH or IHC)	N/A	2 	More if equivocal/positive ROS1
PD-L1 IHC	N/A	2 	3 if sent outside
KRAS	1-5 ng	1 	
RET/MET FISH	N/A	2 	
MET exon 14 mutation, ERBB2 mutation	N/A	Varies 	

Work with your molecular lab

- Consider including pertinent IHC slides along with slides/blocks sent for testing
- Consider including tumor burden estimate in the report comment
  - » e.g. “The tumor cells represent approximately 30% of the entire cell population.”
- Consider including molecular adequacy information in the report comment
  - » e.g. “The cell block H&E matches the smears in cellularity and may be used for ancillary testing.”
  - » Or “The cell block material is scant; smears from passes 1 and 2 are the most cellular and may be used for ancillary testing.”

Summary – Test Ordering

- Consider the recommended testing based on pathology diagnosis and clinical presentation
- Panel testing is recommended whenever possible
- Recommended testing will cover the majority of actionable information for treatment
- For small biopsy/cytology specimens, it becomes very important to understand what testing you can do and what the chances of getting actionable information are

Summary – Molecular Laboratory

- Labs are encouraged to validate testing for cytology/low input specimens
- Existing platforms can be adapted for low input specimens
- Novel techniques may be suitable for low input specimens
- Strategies can be developed to optimize the collection/adequacy assessment/usage of cytology specimens for molecular testing

Thank you!

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