RNA-based Next Generation Sequencing for the Detection of Actionable Fusions

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LABORATORIO DE DIANAS TERAPÉUTICAS

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- **Begin with the end in mind**
- **Seek first to understand & then to be understood**
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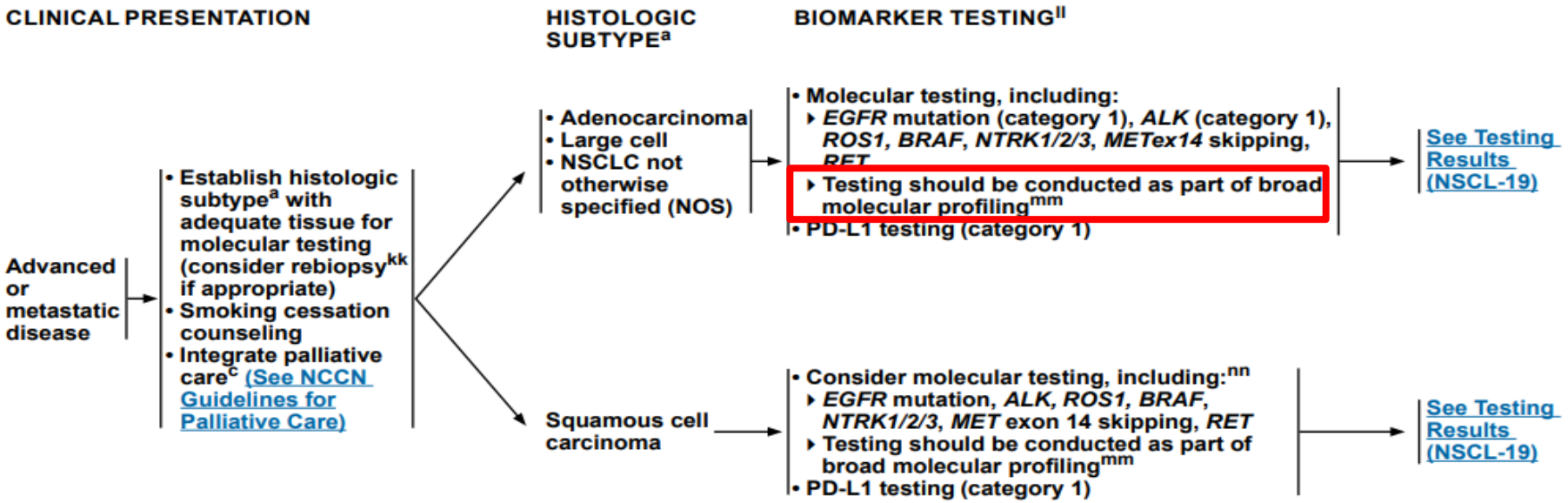
Begin With The End In Mind

NCCN Guidelines / Recommendations



[a] See Principles of Pathologic Review (NSCL-A).
[c] Temel JS, et al. N Engl J Med 2010;363:733-742.
[kk] If there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *ROS1*, *BRAF*, *NTRK1/2/3*, *MET*, *and RET*, repeat biopsy and/or plasma testing should be done. If these are not feasible, treatment is guided by available results and, if unknown, these patients are treated as though they do not have driver oncogenes.
[ll] See Principles of Molecular and Biomarker Analysis (NSCL-H).

[mm] The NCCN NSCLC Guidelines Panel strongly advises 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. See Emerging Biomarkers to Identify Patients for Therapies (NSCL-I).
[nn] Lam VK, et al. Clin Lung Cancer 2019;20:30-36.e3; Sands JM, et al. Lung Cancer 2020;140:35-41.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

NCCN Clinical Practice Guidelines. Non-Small Cell Lung Cancer. Version 4.2021

Begin With The End In Mind

Only a minority of patients currently benefit

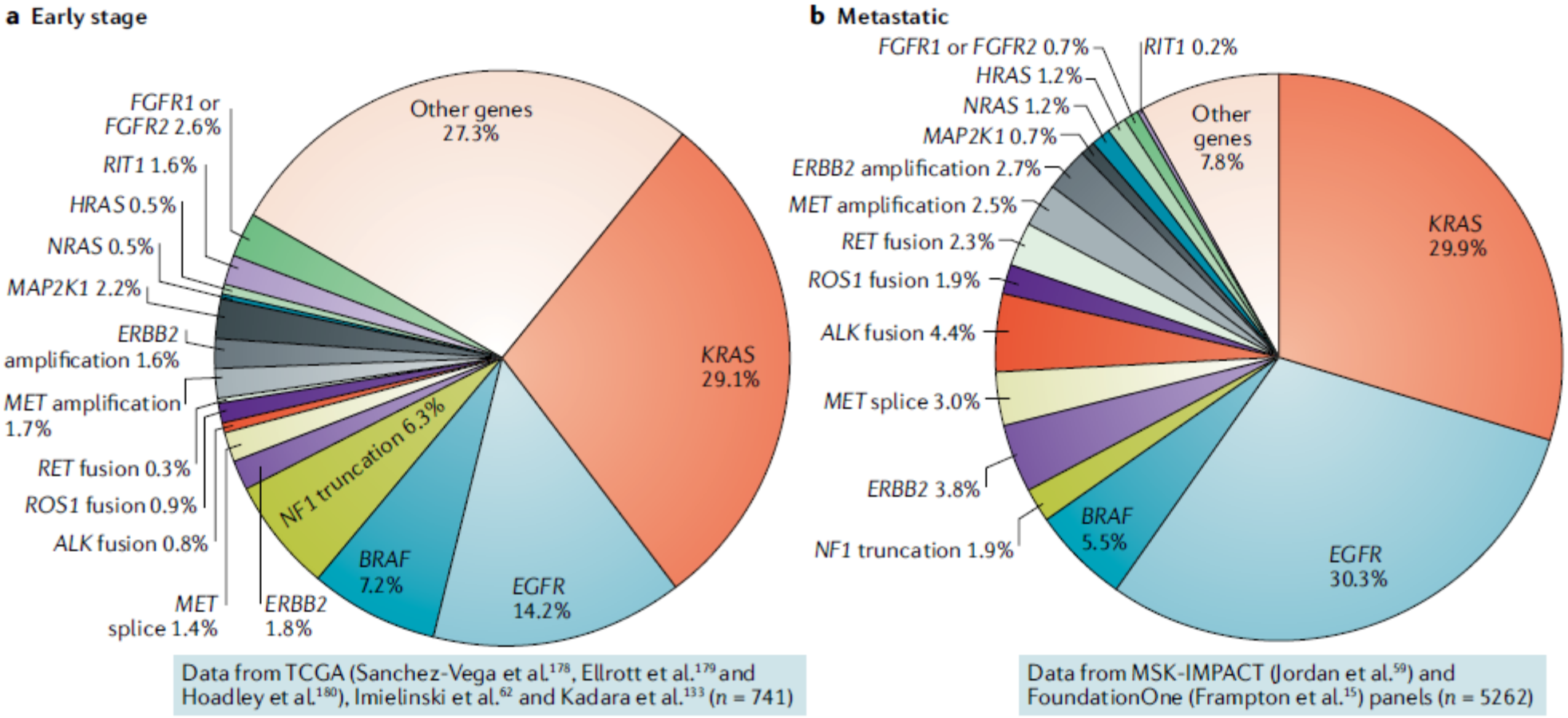
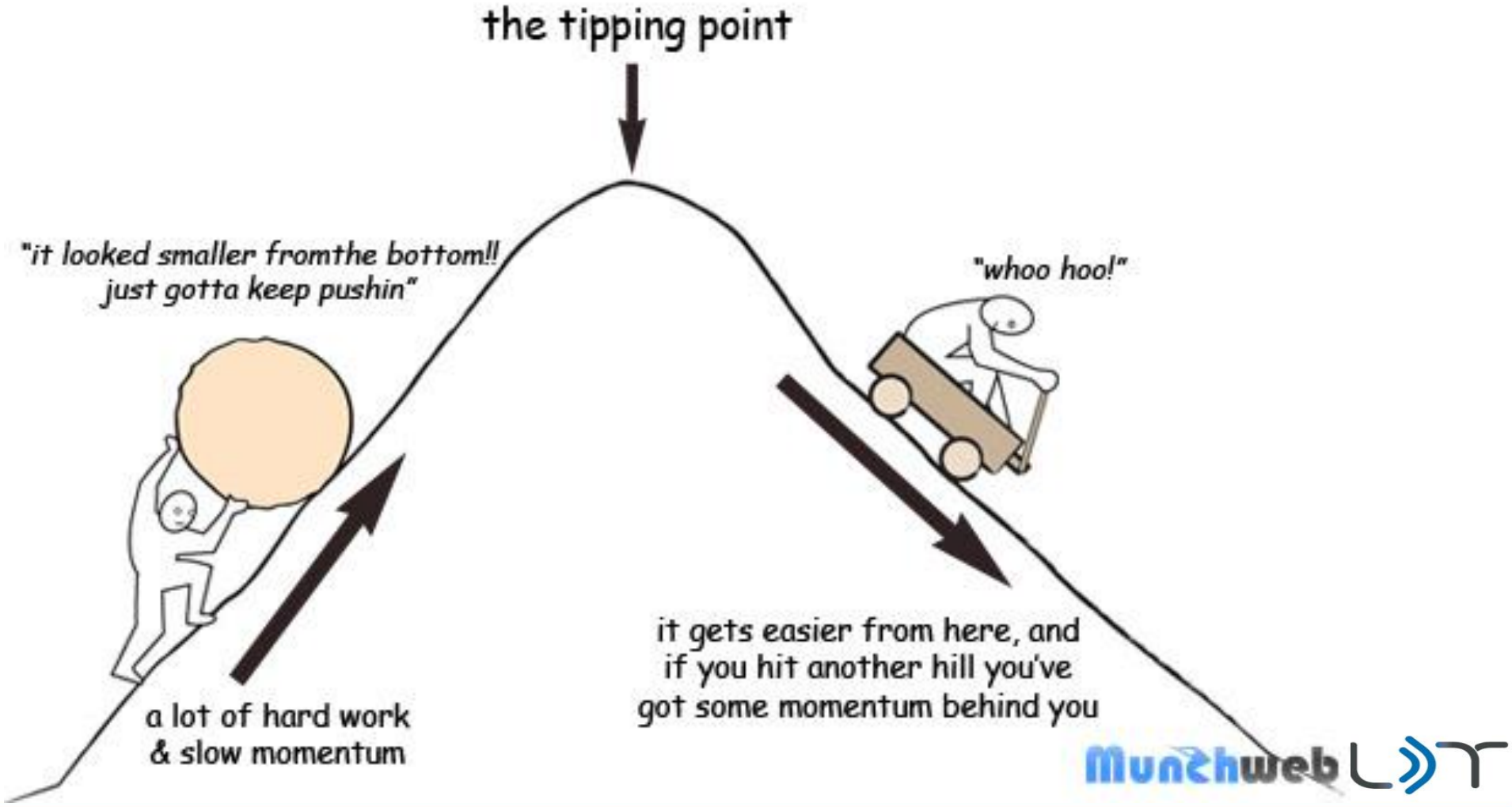


Fig. 1 | **Single oncogenic driver paradigm of lung adenocarcinoma molecular classification.**

Skoulidis F & Heymach JV. Nat Rev Cancer 2019

The Tipping Point

The need to search for low prevalence fusions



Begin With The End In Mind

Guidelines / Recommendations

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

REVIEW ARTICLE

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group

levels of recommendations for the use of NGS. Based on the current evidence, ESMO recommends routine use of NGS on tumour samples in advanced non-squamous non-small-cell lung cancer (NSCLC), prostate cancers, ovarian cancers and cholangiocarcinoma. In these tumours, large multigene panels could be used if they add acceptable extra cost compared with small panels. In colon cancers, NGS could be an alternative to PCR. In addition, based on

Mosele F *et al*. Ann Oncol 2020

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Seek First to Understand and Then to Be Understood

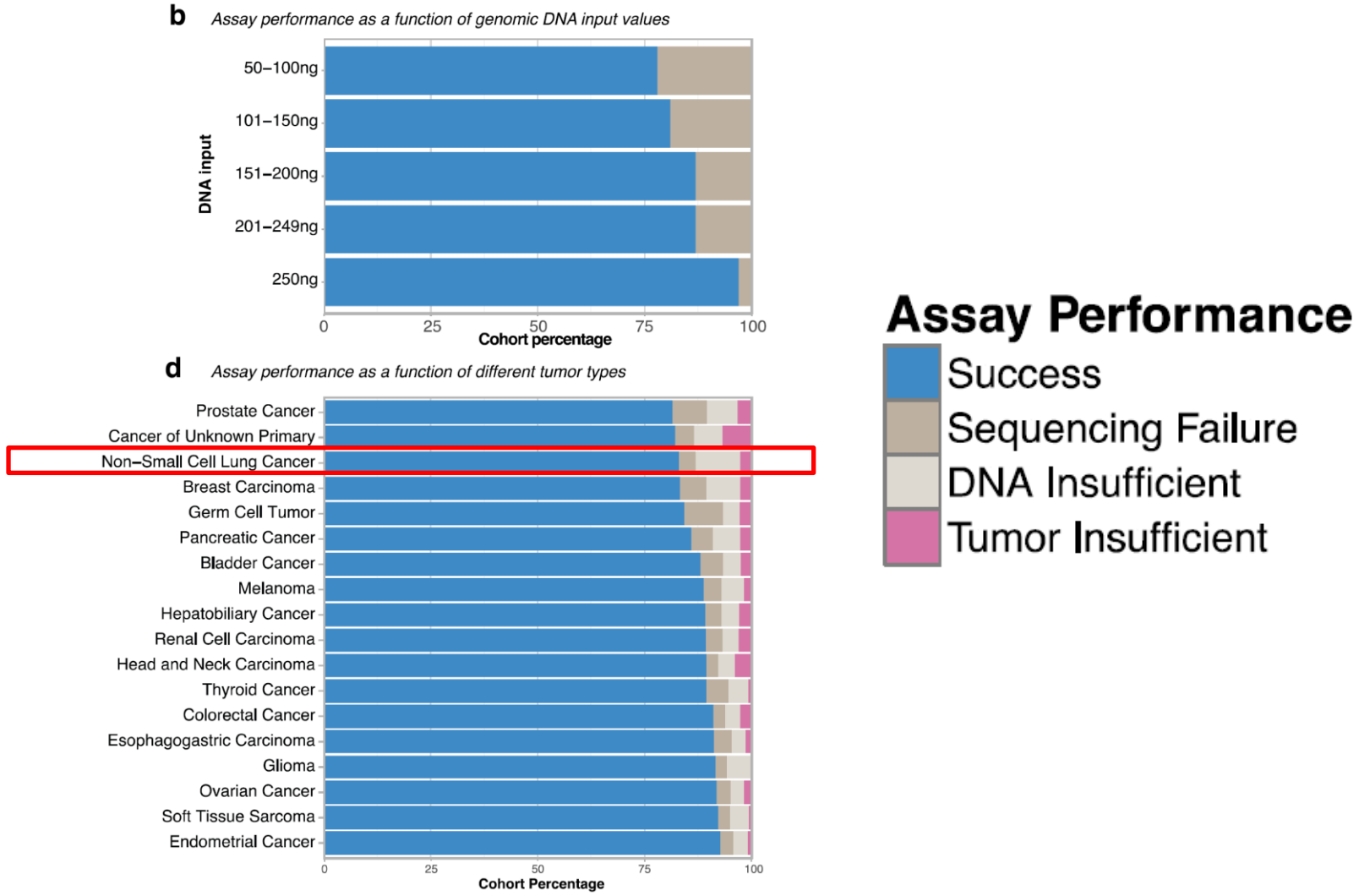
Next Generation Sequencing (NGS) pros & cons

NGS	Sequence thousands of genomic alterations
Advantages	Comprehensive profiling Usually high specificity and sensitivity
Challenges	Longer turnaround time High cost High input material for some panels Reduced sensitivity of DNA-only NGS Molecular redundancy Panel design (width)

Based on the presenter´s experience

Seek First to Understand and Then to Be Understood

Assay performance as a function of DNA/RNA input



Zehir A *et al*. Nat Med 2017

Seek First to Understand and Then to Be Understood

Better sensitivity of RNA-based NGS

PRINCIPLES OF MOLECULAR AND BIOMARKER ANALYSIS

Molecular Diagnostic Studies in Non-Small Cell Lung Cancer

- Numerous gene alterations have been identified that impact therapy selection. Testing of lung cancer specimens for these alterations is important for identification of potentially efficacious targeted therapies, as well as avoidance of therapies unlikely to provide clinical benefit.
- Some selection approaches for targeted therapy include predictive immunohistochemical analyses, which are distinct from immunohistochemical studies utilized to identify tumor type and lineage.
- Major elements of molecular testing that are critical for utilization and interpretation of molecular results include:
  - Use of a laboratory that is properly accredited, with a minimum of CLIA accreditation
  - Understanding the methodologies that are utilized and the major limitations of those methodologies
  - Understanding the spectrum of alterations tested (and those not tested) by a specific assay
  - Knowledge of whether a tumor sample is subjected to pathologic review and tumor enrichment (ie, microdissection, macrodissection) prior to testing
  - The types of samples accepted by the testing laboratory
- Specimen Acquisition and Management:
  - Although tumor testing has been primarily focused on use of FFPE tissues, increasingly, laboratories accept other specimen types, notably cytopathology preparations not processed by FFPE methods. Although testing on cell blocks is not included in the FDA approval for multiple companion diagnostic assays, testing on these specimen types is highly recommended when it is the only or best material.
  - A major limitation in obtaining molecular testing results for NSCLC occurs when minimally invasive techniques are used to obtain samples; the yield may be insufficient for molecular, biomarker, and histologic testing. Therefore, bronchoscopists and interventional radiologists should procure sufficient tissue to enable all appropriate testing.
  - When tissue is minimal, laboratories should deploy techniques to maximize tissue for molecular and ancillary testing, including dedicated histology protocols for small biopsies, including "up-front" slide sectioning for diagnostic and predictive testing.
- Testing Methodologies
  - Appropriate possible testing methodologies are indicated below for each analyte separately; however, several methodologies are generally considerations for use:
    - Next-generation sequencing (NGS) is used in clinical laboratories. Not all types of alterations are detected by individual NGS assays and it is important to be familiar with the types of alterations identifiable in individual assays or combination(s) of assays.
    - It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach, most typically performed by NGS. For patients who, in broad panel testing don't have identifiable driver oncogenes (especially in never smokers), consider RNA-based NGS if not already performed, to maximize detection of fusion events.
    - Real-time polymerase chain reaction (PCR) can be used in a highly targeted fashion (specific mutations targeted). When this technology is deployed, only those specific alterations that are targeted by the assay are assessed.
    - Sanger sequencing requires the greatest degree of tumor enrichment. Unmodified Sanger sequencing is not appropriate for detection of mutations in tumor samples with less than 25% to 30% tumor after enrichment and is not appropriate for assays in which identification of subclonal events (eg, resistance mutations) is important. If Sanger sequencing is utilized, tumor enrichment methodologies are nearly always recommended.
    - Other methodologies may be utilized, including multiplex approaches not listed above (ie, SNaPshot, MassARRAY).
    - Fluorescence in situ hybridization (FISH) analysis is utilized for many assays examining copy number, amplification, and structural alterations such as gene rearrangements.
    - IHC is specifically utilized for some specific analytes, and can be a useful surrogate or screening assay for others.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

Continued

NCCN Clinical Practice Guidelines. Non-Small Cell Lung Cancer. Version 4.2021

Seek First to Understand and Then to Be Understood

Molecular redundancy

Molecular Testing for Patients With Lung Cancer

Table 2. Comparison of CAP/IASLC/AMP Recommendations and ASCO Endorsed Recommendations

CAP/IASLC/AMP Recommendation	ASCO Endorsed Recommendation (with modifications or qualifying statements in ***bold italics***)
Expert Consensus Opinion: Laboratories should ensure that test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample.	Laboratories should ensure that test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample.

Kalemkerian G *et al*. J Clin Oncol 2018

Seek First to Understand and Then to Be Understood

NGS panel design is important

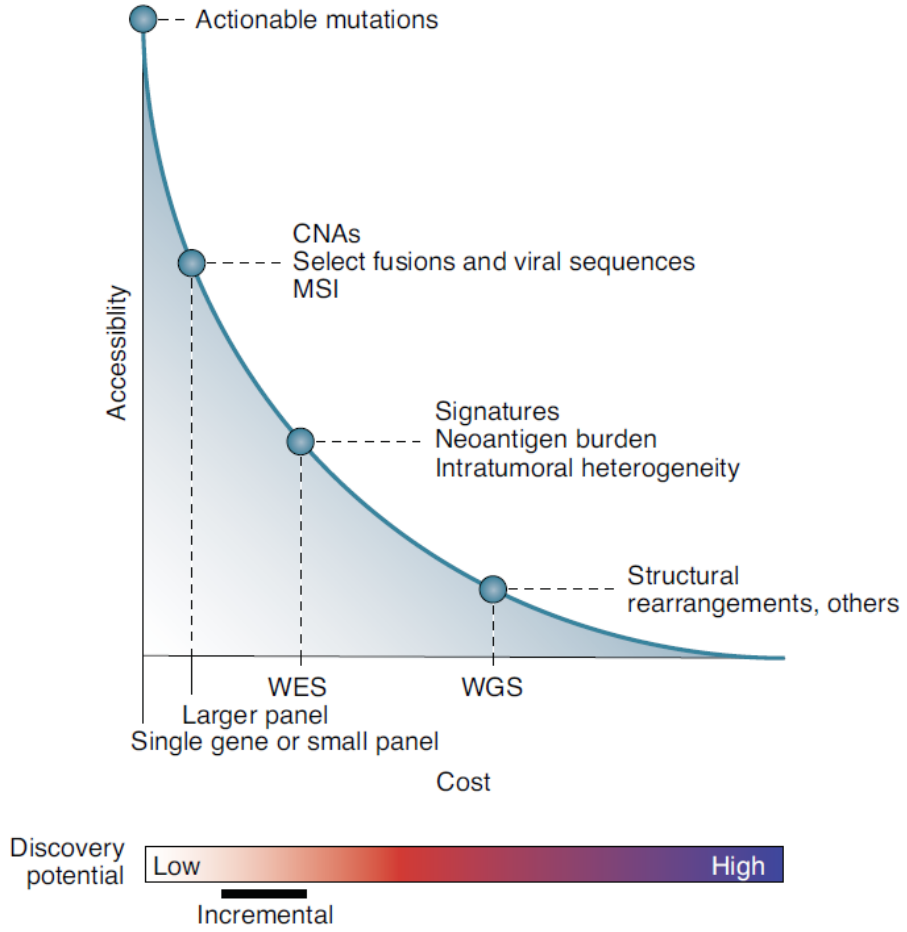


Fig. 1 | Balancing accessibility, utility, and discovery in clinical genomics.

Donoghue MTA *et al*. Nat Cancer 2020

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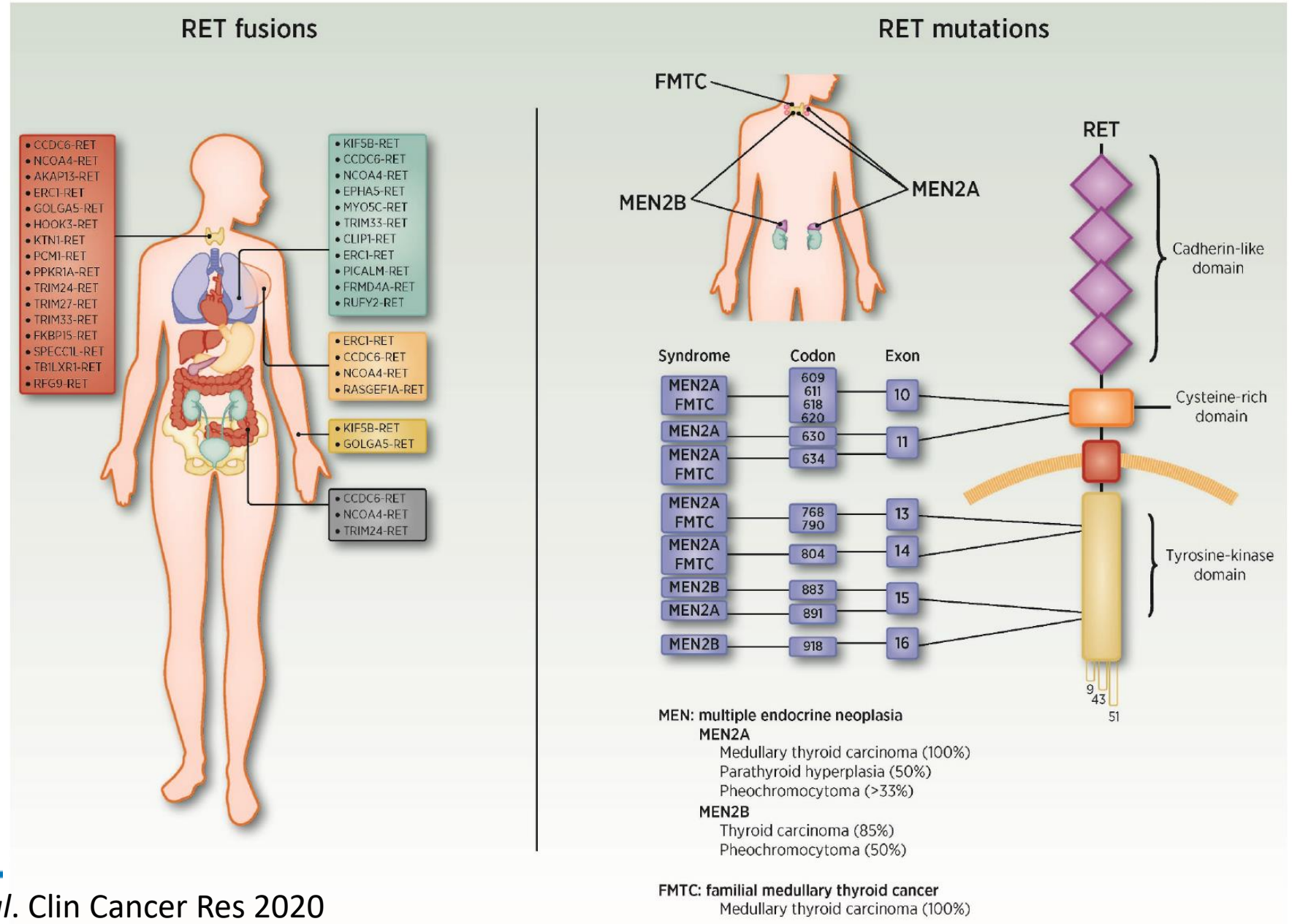
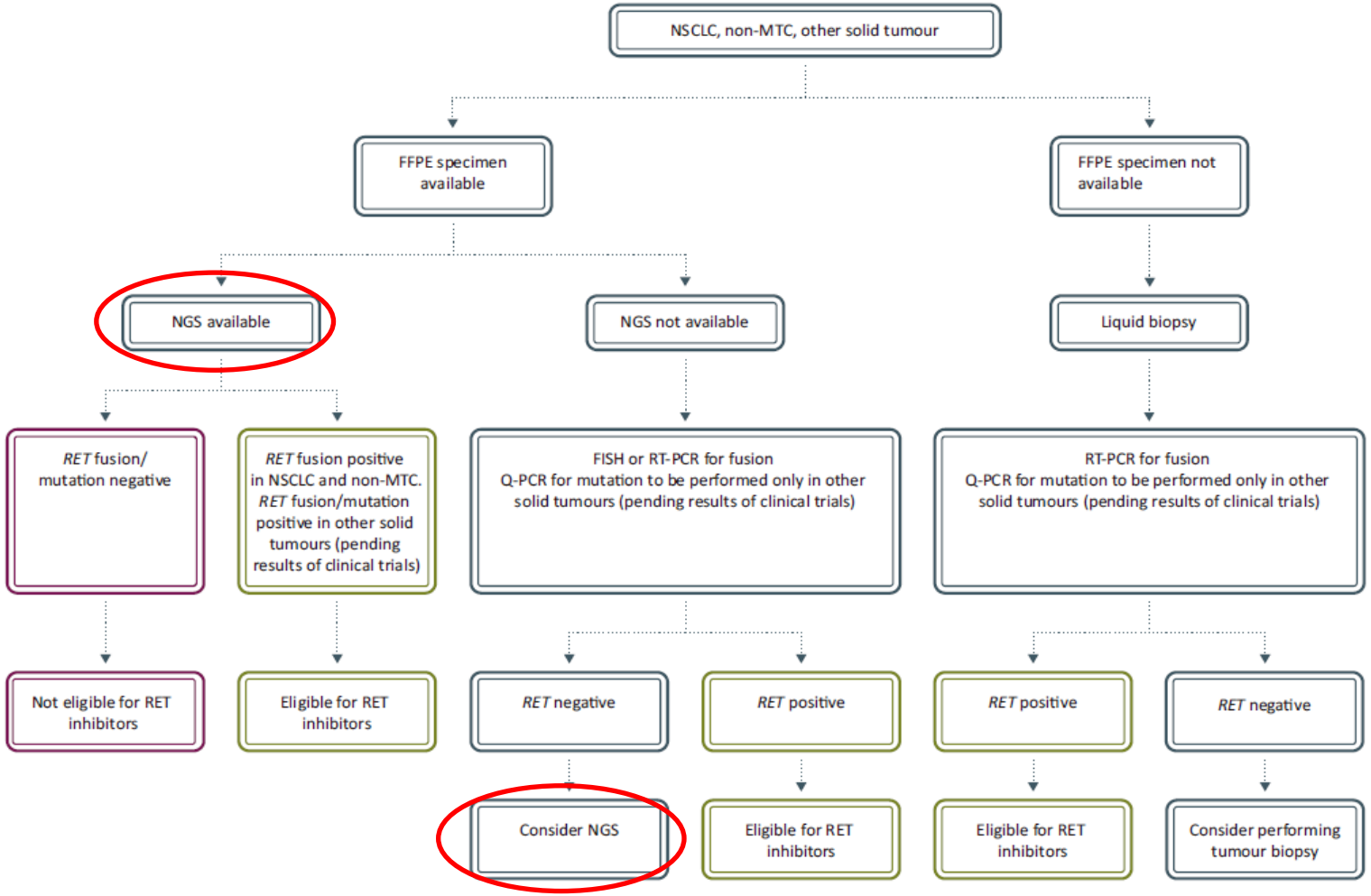
RET Fusions and Mutations



Belli C *et al*. Clin Cancer Res 2020

RET Fusions

Algorithm: ESMO

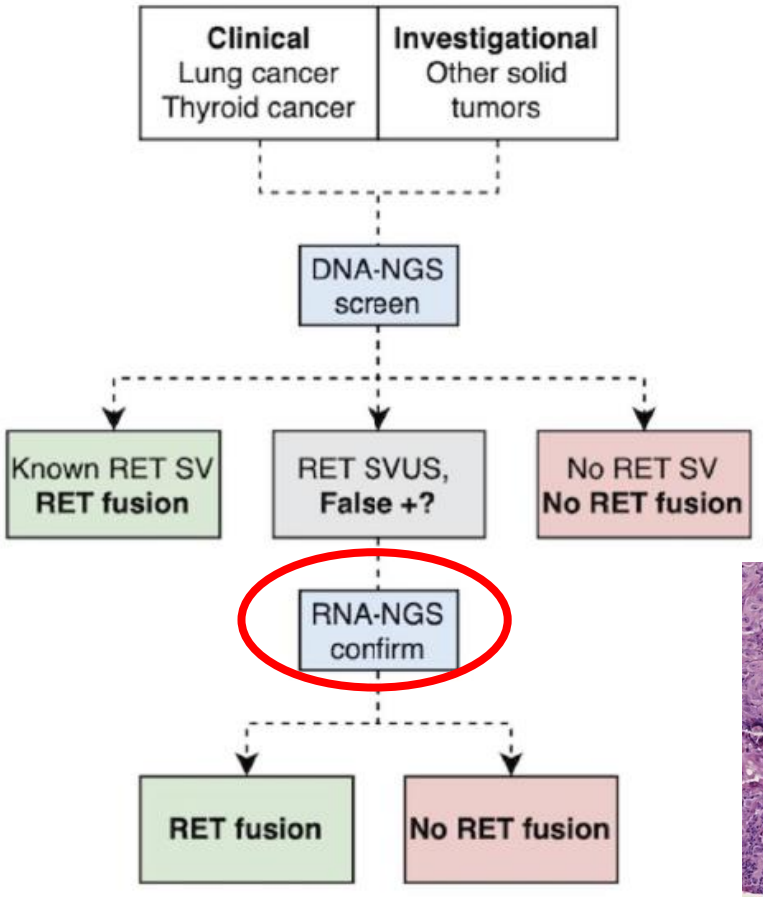


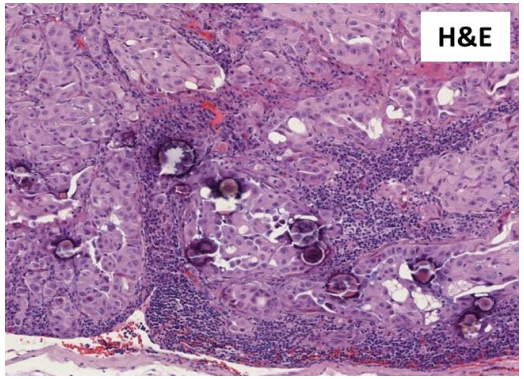
Belli C *et al*. Ann Oncol 2021

RET Fusions

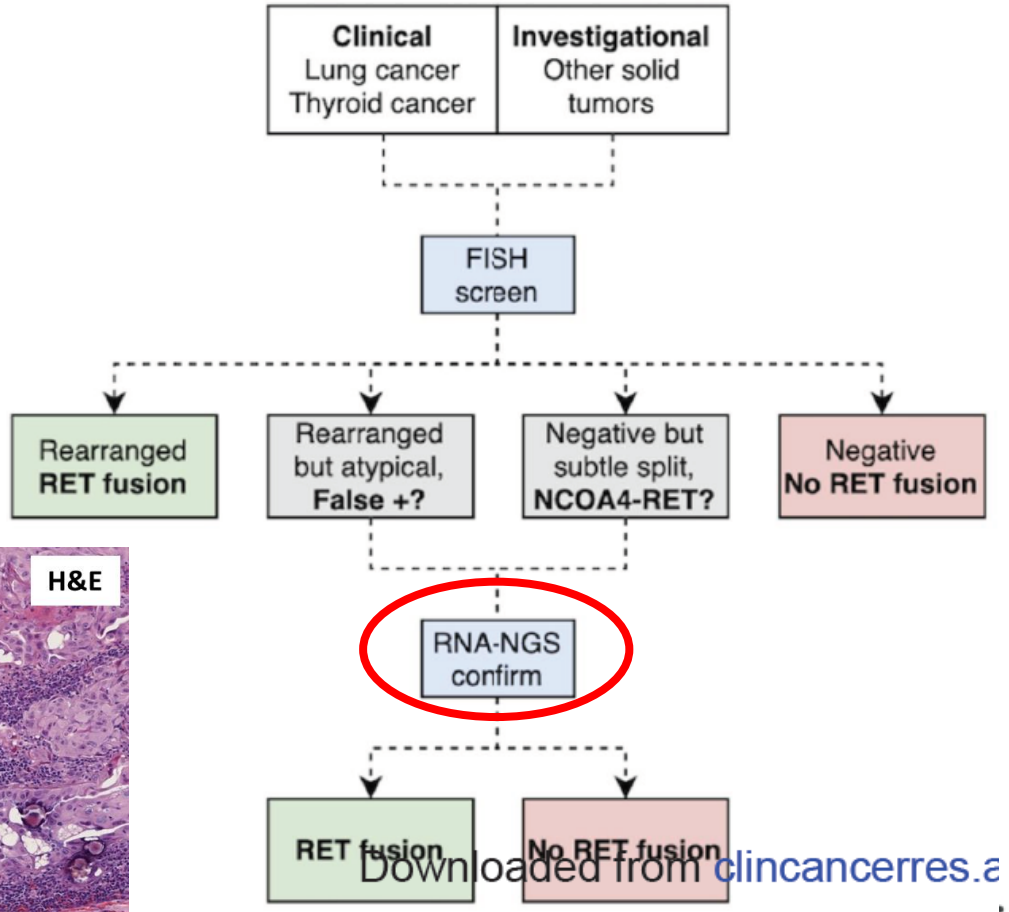
Algorithm: MSKCC

a





b



Yang S-R *et al*. Clin Cancer Res 2020

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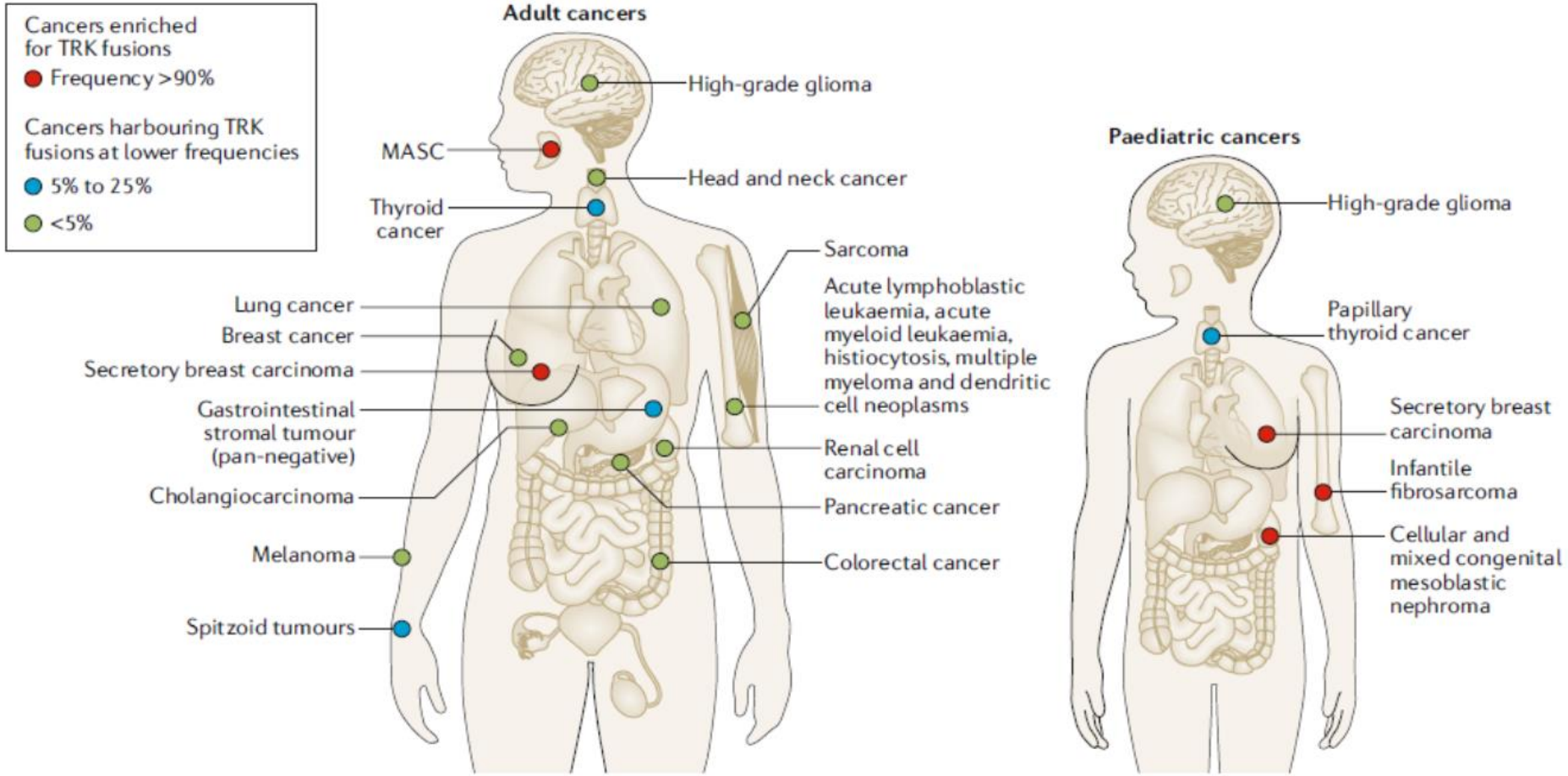
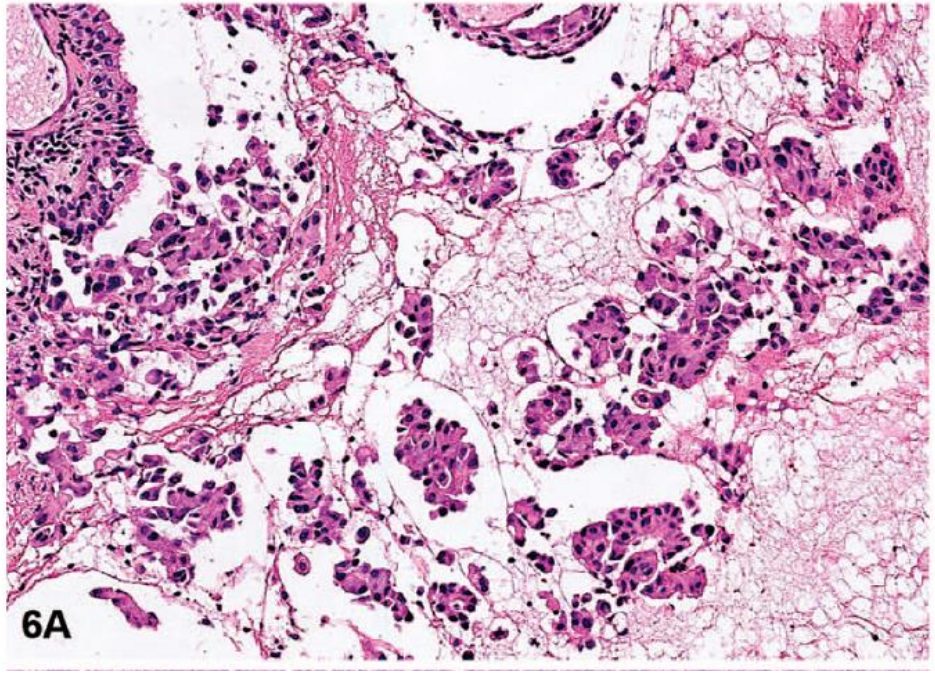
NTRK Fusions

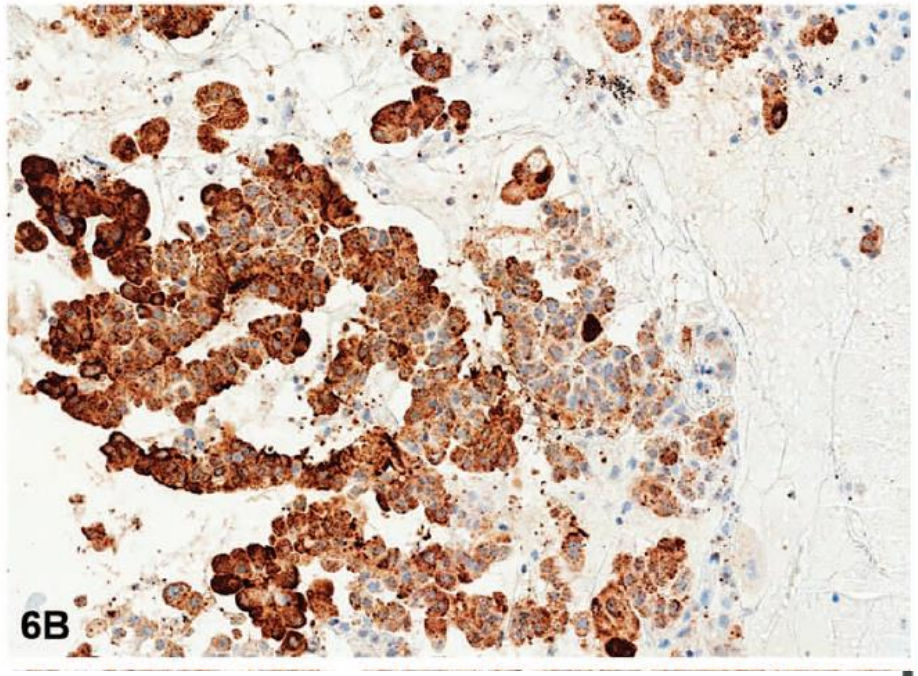


Cocco E *et al.* Nat Rev Clin Oncol 2018

NTRK Fusions

<1% of NSCLCs	Adenocarcinoma (usually[a])	Without driver gene alterations[a]	Cytoplasmic (alone or combined with membrane staining if *TPM3-NTRK1*)	*TPM3-NTRK1*, *IRF2BP2-NTRK1*, *SQSTM1-NTRK1*, *MPRIP-NTRK1*	Persevere in driver-negative lung adenocarcinomas: Trust intense and diffuse pan-TRK IHC staining in lung adenocarcinomas Make sure the NGS panel is broad enough and RNA based[a]

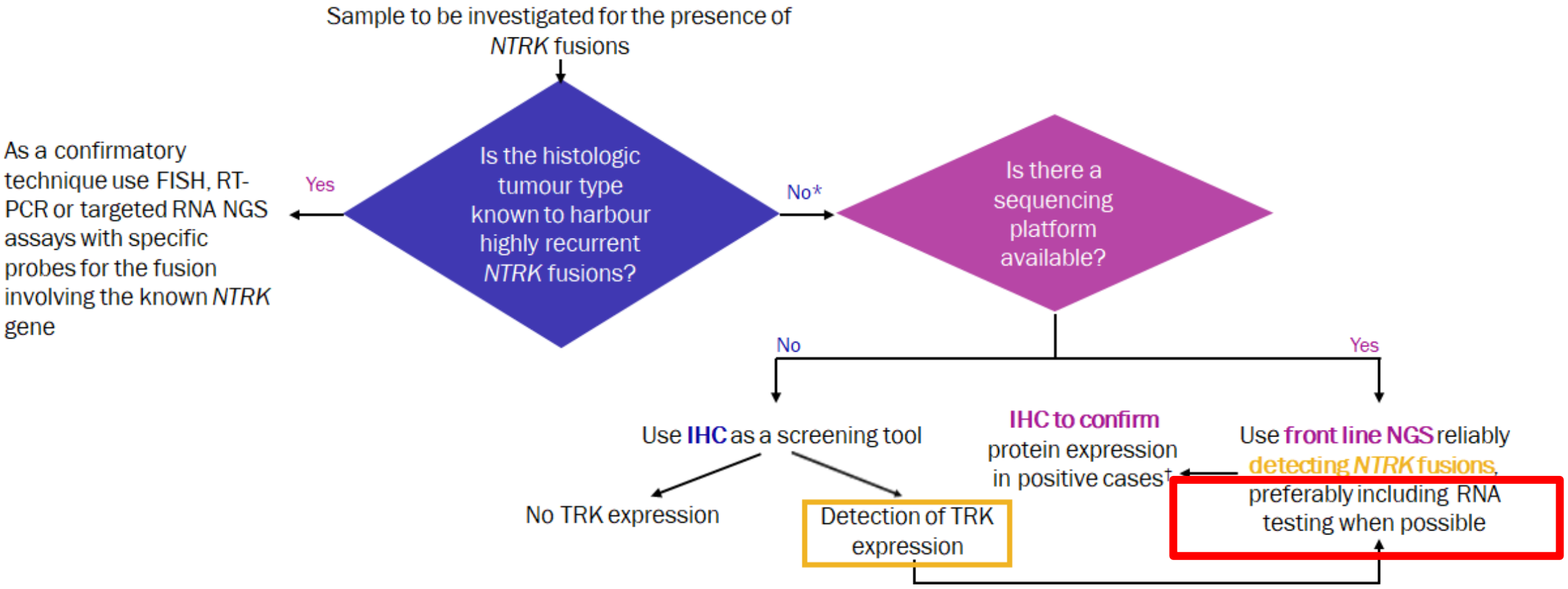




Conde E *et al.* Arch Pathol Lab Med 2020

NTRK Fusions

Algorithm: ESMO



Marchio C *et al.* Ann Oncol 2019

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Conclusions

TEAMWORK — PART 1
Debra Malina, Ph.D., *Editor*

Divided We Fall

attendings switched, bringing in a new set of opinions. Everyone was talking about how best to care for the patient, but at no point were we all talking to each other.

mendously variable. Second, quantity of communication predicted quality: teams that exchanged more information performed better than those that exchanged less. Third, errors more often

Rosenbaum L. New Engl J Med 2019

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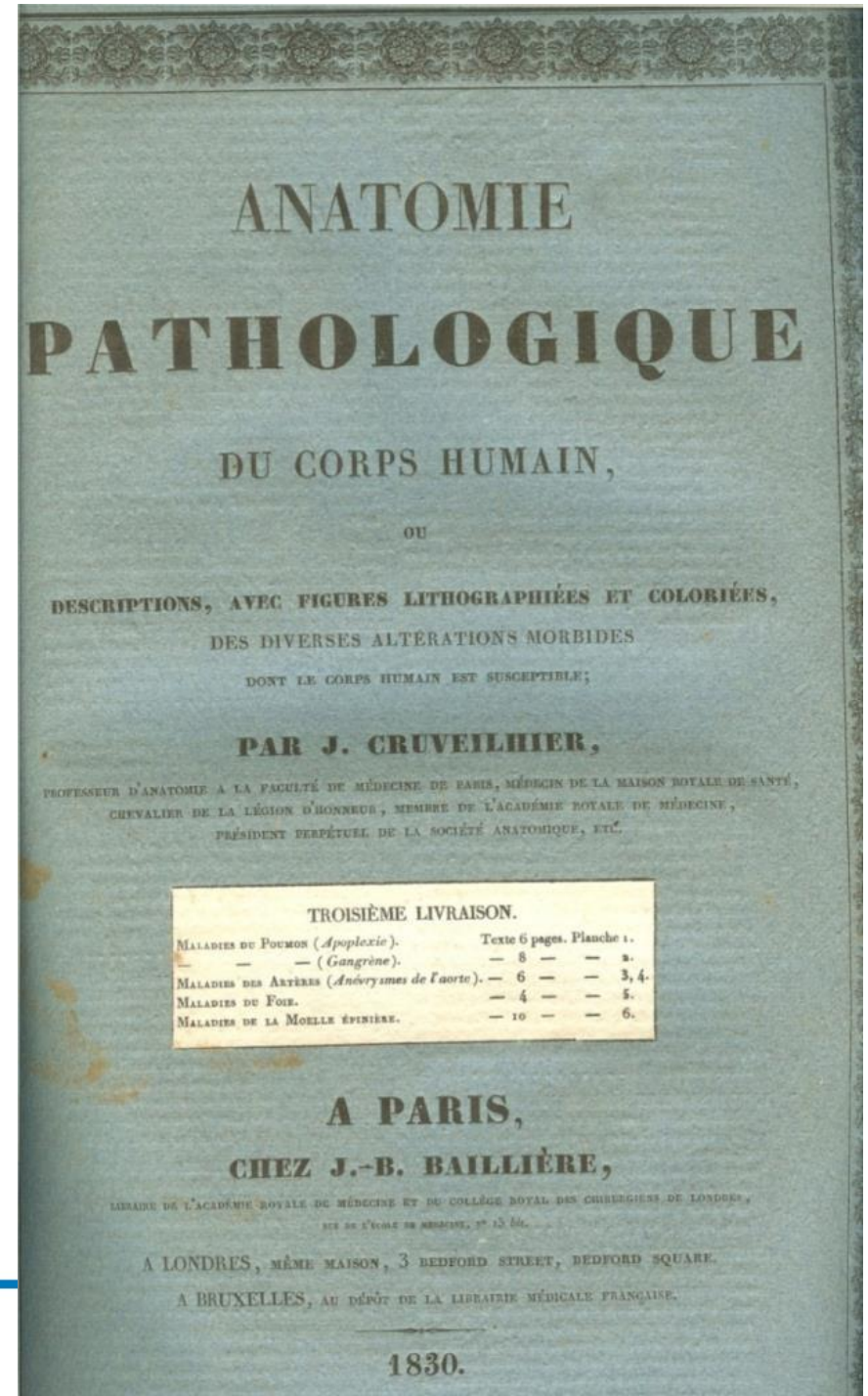

ANATOMIE
PATHOLOGIQUE
DU CORPS HUMAIN,

OU

DESCRIPTIONS, AVEC FIGURES LITHOGRAPHIÉES ET COLORIÉES,

DES DIVERSES ALTÉRATIONS MORBIDES

DONT LE CORPS HUMAIN EST SUSCEPTIBLE;

PAR J. CRUVEILHIER,

PROFESSEUR D'ANATOMIE A LA FACULTÉ DE MÉDECINE DE PARIS, MÉDECIN DE LA MAISON ROYALE DE SANTÉ,
CHEVALIER DE LA LÉGION D'HONNEUR, MEMBRE DE L'ACADÉMIE ROYALE DE MÉDECINE,
PRÉSIDENT PERPÉTUEL DE LA SOCIÉTÉ ANATOMIQUE, ETC.

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