NCCN 2021 Virtual Congress:
Biomarkers in Solid Tumors

Friday, November 12, 2021
10:20 AM – 11:05 AM EST

General Principles of Biomarker Testing:
When Should Broad Genomic Profiling Be Performed and What Do Different Tests Offer?

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Abramson Cancer Center at the University of Pennsylvania

National Comprehensive Cancer Network®

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Learning objectives

- Describe different types of genomic testing
- Identify the factors that should be considered when ordering genomic testing
- Describe the types of tests that can detect fusion genes
- Review the utility, benefits and limitations of tissue vs liquid biopsy testing

Penn Medicine
Abramson Cancer Center

Case study: 48 year old male with NSCLC

- Never smoker
- Presented with cough, fatigue, DOE
- Imaging: multiple RLL nodule; brain MRI: involvement of clivus
- Pathology: lung adenocarcinoma

- Testing was ordered for molecular studies on the initial specimen

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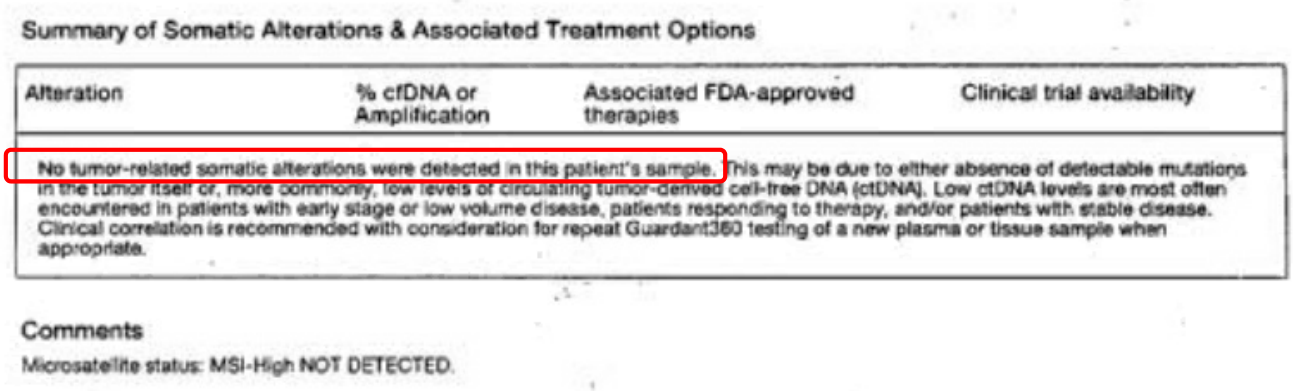
IHC for PDL1

PD-L1 POSITIVE, High Expression, (Tumor proportion score: 70%)

No additional molecular testing performed at this time

Case study: 48 year old male with NSCLC

Liquid biopsy panel results



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Comments

Microsatellite status: MSI-High NOT DETECTED.

Circulating tumor DNA (ctDNA) based testing was performed at diagnosis and at two subsequent intervals, with no tumor-related somatic alterations detected.

Case study: 48 year old male with NSCLC recurrence at 2 years

Tissue-based molecular testing was performed at recurrence

DNA panel testing for somatic mutations

Tissue Source: Pericardial Fluid
Block: A4
Estimated Tumor Percentage: 10-19%
Indication for Study: Adenocarcinoma present

Variant Report

VARIANTS OF UNCERTAIN SIGNIFICANCE (see interpretation and comments):

GENE PROTEIN CHANGE cDNA CHANGE

NTRK1 p.T195M c.584C>T

RET p.D102N c.304G>A

TET2 p.Y1679H c.5035T>C

Tissue based testing was performed on a pericardial fluid sample. Three variants of uncertain significance (VUS) were observed, but no tumor-related somatic alterations were detected

Of note: two of the VUS were in genes that are associated with therapies, but these variants did not have data to suggest they are tumor driver mutations

Case study: 48 year old male with NSCLC recurrence at 2 years

Positive result for an EML4/ALK rearrangement

RNA testing panel:

Tissue Source: Pericardial Fluid

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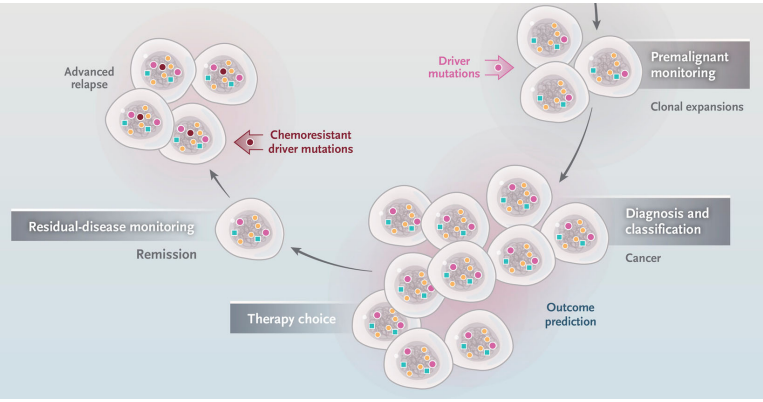
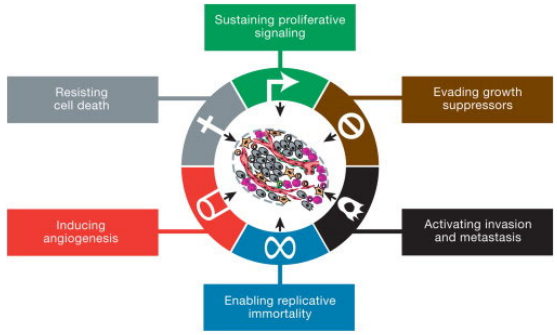
- Why did the initial testing results as quantity not sufficient?
- Why were three separate molecular tests ordered?
- Why wasn't the EML4/ALK rearrangement detected in all three tests?
- How to interpret these discrepant results?

Introduction and background

- Cancers all have a genetic component (in part), with an accumulation of mutations in genes that regulate cell division, survival, invasion or other hallmarks of cancer
- Detection of driver mutations can help with therapy choice
- Continuous monitoring can identify clones that may be chemo-resistant

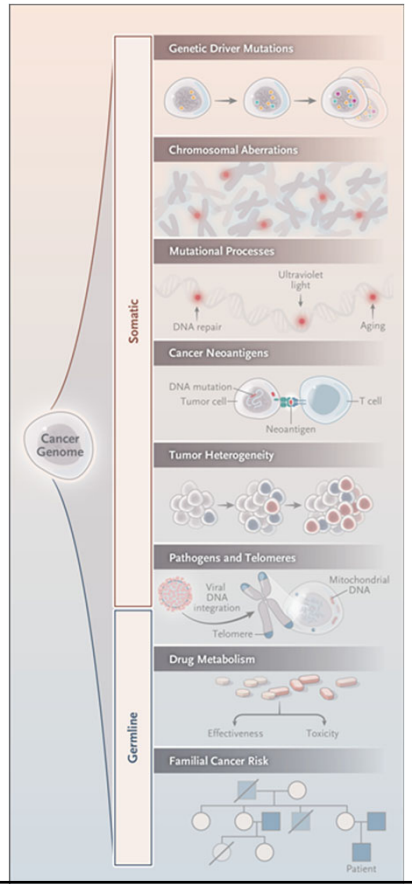


Nangalia and Campbell. N Engl J Med 2019; 381:2145-2156



Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011.144(5):646-74 PMID: 21376230.

Types of genetic abnormalities detected in solid tumors



- **Driver mutations**: e.g. oncogenes and tumor suppressor genes
- **Chromosome abnormalities**: e.g. rearrangements, aneuploidy and amplification
- **Mutational processes:** e.g. DNA repair deficiencies and exposures
- **Tumor heterogeneity:** e.g. acquired mutations associated with clonal evolution and resistance mutations

Detection of these types of abnormalities is dependent on the test design and validation

Nangalia and Campbell. N Engl J Med 2019; 381:2145-2156

Please note: this article has a great video on mutations in cancer

Recurrent clinically relevant mutations in solid tumors

Pan-cancer biomarkers: fusions involving NTRK1, NTRK2, NTRK3, MSI						
Lung	**Colon**	**Breast**	**Brain**	**Melanoma**	**Bladder**	**Prostate**
ALK	AKT1	AKT1	ATRX	BRAF	FGFR3	AR
BRAF	BRAF	BRCA1	BRAF	CTNNB1	MSH6	BRCA1
DDR2	HRAS	BRCA2	CDKN2A	GNA11	PMS2	BRCA2
EGFR	KRAS	ERBB2	EGFR	GNAQ	TSC1	ERG
ERBB2	MET	ESR1	IDH1	KIT		PTEN
FGFR1	MLH1	FGFR1	IDH2	MAP2K1		
FGFR3	MSH2	FGFR2	PDGFRA	NF1		
KRAS	MSH6	PIK3CA	PTEN	NRAS		
MAP2K1	NRAS	TP53	TERT	PDGFRA		
MET	PIK3CA	*[HRD]*	KIT	PIK3CA		
NRAS	PMS2		NF1	PTEN		
PIK3CA	SMAD4			TP53		
RET	TP53			*[UV signature]*		
ROS1	*[MSI]*					
STK11						
TP53						

Genes in red are seen across multiple tumor types

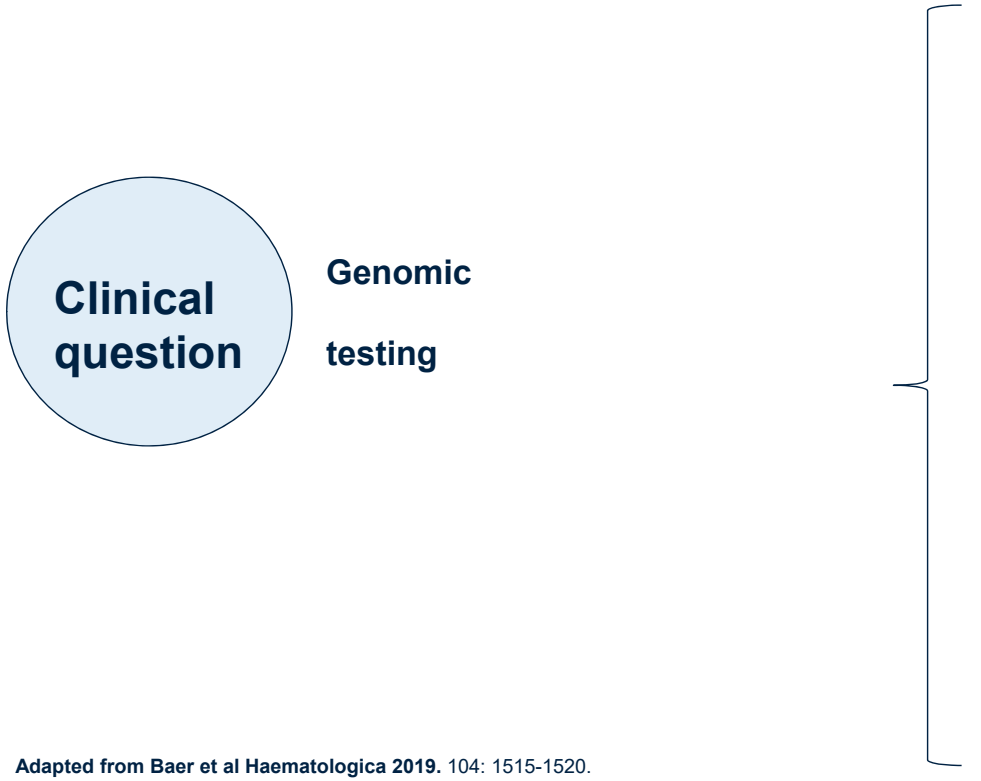
Specimen types for molecular testing of solid tumors

Specimen	Advantages	Considerations
Fine needle aspiration (FNA)/pleural fluid 	Less likely to be degraded; fast; may be enriched for tumor cells	Not always available; small specimen; validation dependent; paucicellular; degraded
FFPE: common for most tumors 	Common sample; well characterized; enrichment, enables broad application of NGS for all tumor samples	Formalin artifact: archival specimens, degraded RNA, cold ischemic time, small specimens
Blood (liquid biopsy*)	Non-invasive, broad testing	Specialized tubes

*more on liquid biopsy later in presentation

Turashvili et al 2012 PMID: 21963600
Wei et al 2016 PMID: 26682952

Summary: Genetic testing of solid tumors can help answer important clinical questions



Adapted from Baer et al Haematologica 2019. 104: 1515-1520.

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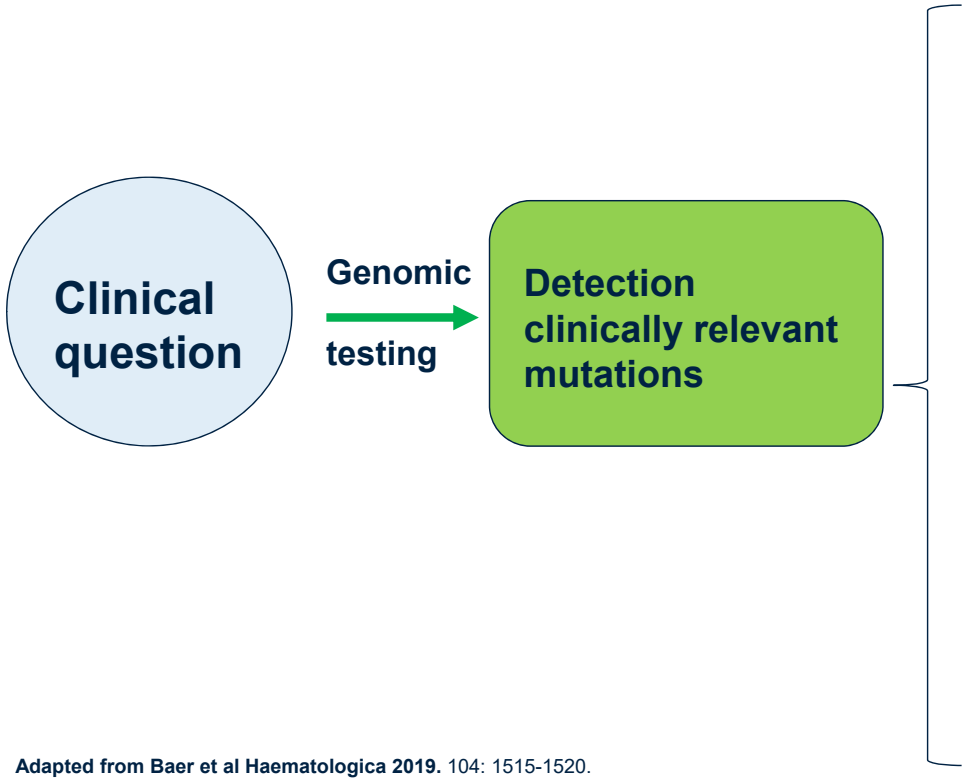

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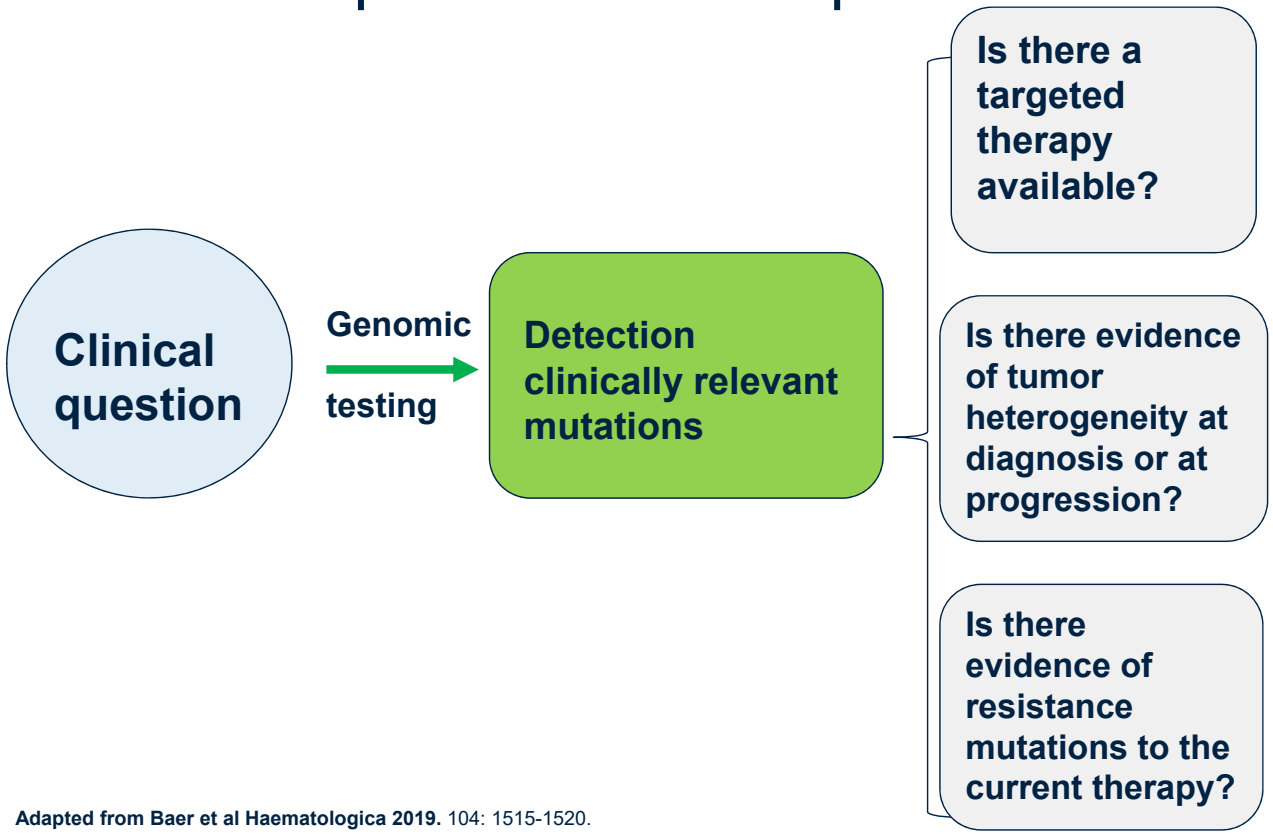

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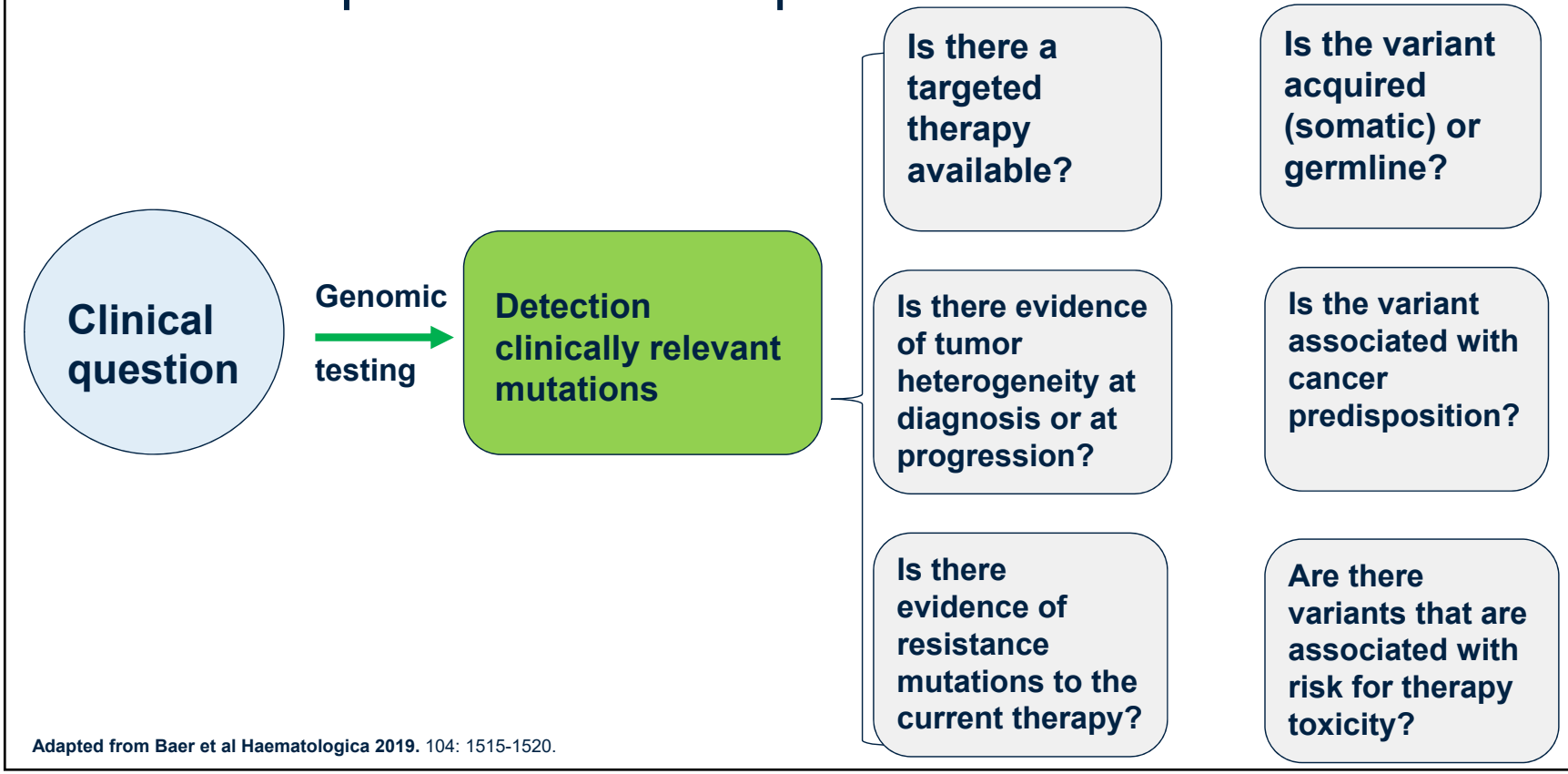

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Choosing the appropriate genetic testing for patients with solid tumors

Clinically relevant genetic information: big or small

- Mutations can be detected by many methods
- In oncology, single gene or panels are more common than whole exome or whole genome sequencing due to sequencing costs
- Targeted mutation analysis
  - Advantages = (usually) lower cost and more rapid TAT (1-7 days)
  - Disadvantage: limited scope; may miss other clinically useful alterations
- Broad genomic profiling: MPS/NGS
  - Advantage: Consolidation of sequential testing
  - Disadvantages: Coverage is variable, slower (1-3 weeks)

Pasmans et al Expert Rev Pharmacoecon Outcomes Res. 2021.21:413-414. PMID: 33852815.

Advantages for using targeted mutational analysis

- Predictive, disease-specific, evidence-based tests
- Gene choice is “a la carte”: only interrogate a gene or a specific mutation that is of clinical interest for that disease
- Techniques include polymerase chain reaction (PCR), Sanger sequencing, and fluorescence in situ hybridization (FISH)
- Faster turnaround time (TAT)
  - Generally from hours to weeks from lab receipt to reporting
- Often accommodates low input, poor quality samples

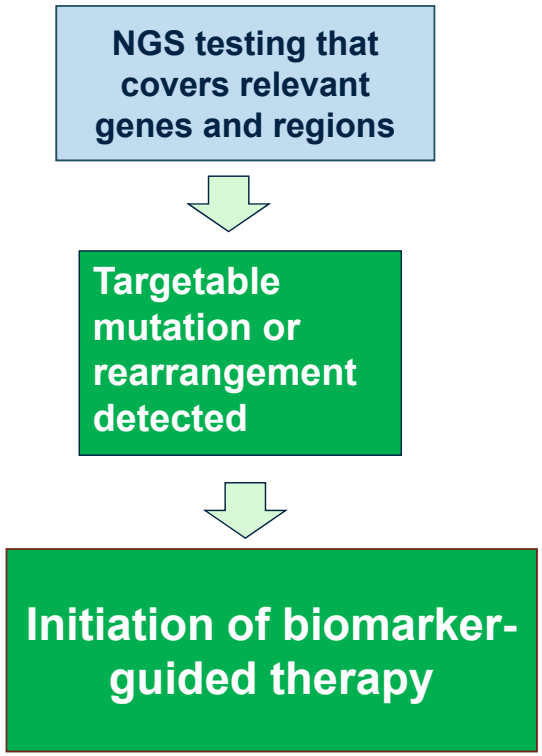
Hutchinson et al Clin Cancer Res. 2013. 19 :6696-702. PMID: 24345920

Considerations for ordering broad genomic profiling

- Testing of many genes and mutation types
- Commercial testing available, with FDA-approved options
- Multi-gene sequencing avoids sequential testing, providing analysis of a large number of genes in tandem
- "Comprehensiveness" is variable
  - From testing a select group of known, targetable cancer-related genes to an unbiased, comprehensive DNA- and RNA-based analyses (WGS+WTS)
- Tumor only (most labs) or tumor-normal pairs (matched T/N)
    - Matched T/N can definitively determine a mutation as somatic or germline variants (i.e. predisposition mutations that confer increased cancer risk)

Cobain EF, et al. Assessment of Clinical Benefit of Integrative Genomic Profiling in Advanced Solid Tumors. *JAMA Oncol*. 2021;7:525–533

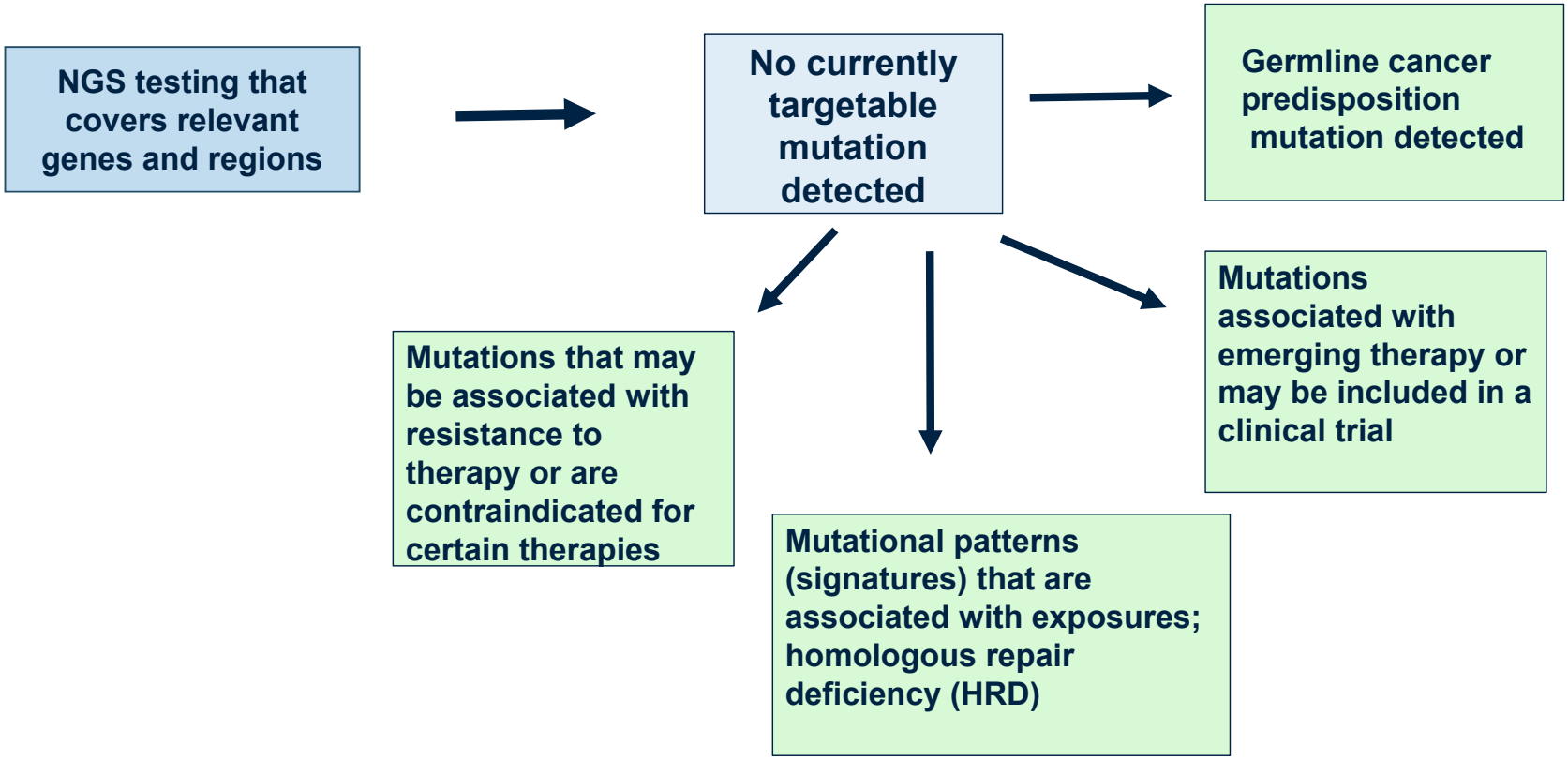
Genomic testing algorithms for a patient with advanced cancer



Important considerations for ordering broad genomic profiling:

- Are there potentially targetable mutations in this tumor type?
- What type of abnormalities are captured?
- What is the minimum input of nucleic acid?
- What is the minimum tumor percentage for the assay?
- *Should you order sequencing from both DNA and RNA?*

Genomic testing algorithms for a patient with advanced cancer



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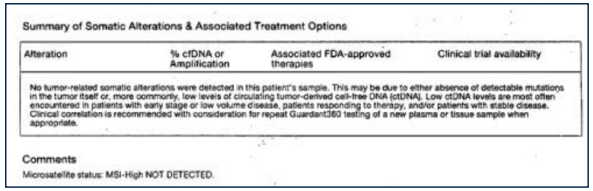

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TUMOR MUTATIONAL BURDEN (TMB; see interpretation and comments):

1.7 mutations per megabase (u/MB)

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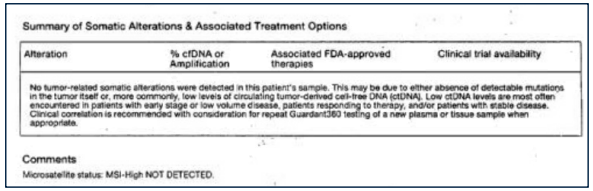

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Testing for fusion genes as drivers of this cancer

Detection of fusion genes in solid tumors

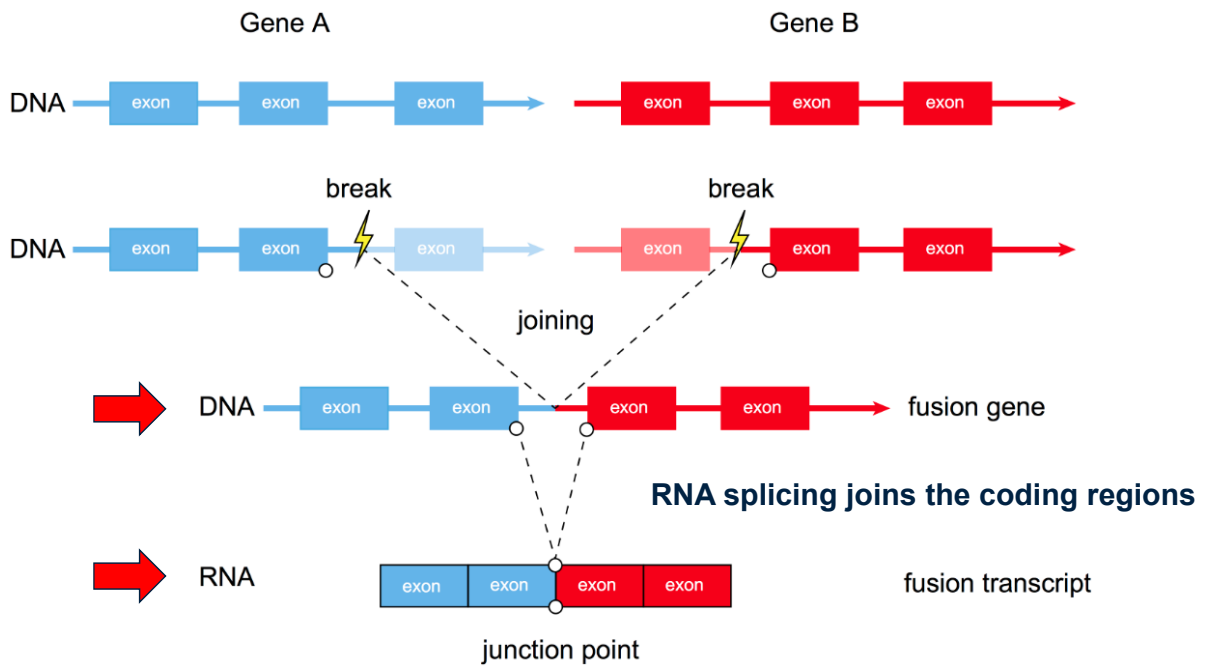
Oncogenic gene fusions are common in patients with solid tumors and occur across a wide spectrum of tumor types

- Gene fusions arise as a result of genomic rearrangements, and can drive both the development and progression of cancer
- Frequently involve tyrosine kinases resulting in constitutive activation, increasing downstream signaling and tumor growth
- Associated with “oncogene addiction” making them good targets for therapy
- Effective inhibitors are available for many of these fusions
- Can be detected by FISH, RT-PCR, or NGS
- Can use DNA or RNA as a substrate

Schram, *et al. Nat Rev Clin Oncol* 14, 735–748 (2017)

Yakushina VD, Lerner LV, Lavrov AV. Gene Fusions in Thyroid Cancer. Thyroid. 2018 Feb;28(2):158-167. PMID: 29281951.

Fusion genes can lead to activation of a regulated oncogene through multiple mechanisms



https://tumorfusions.org/

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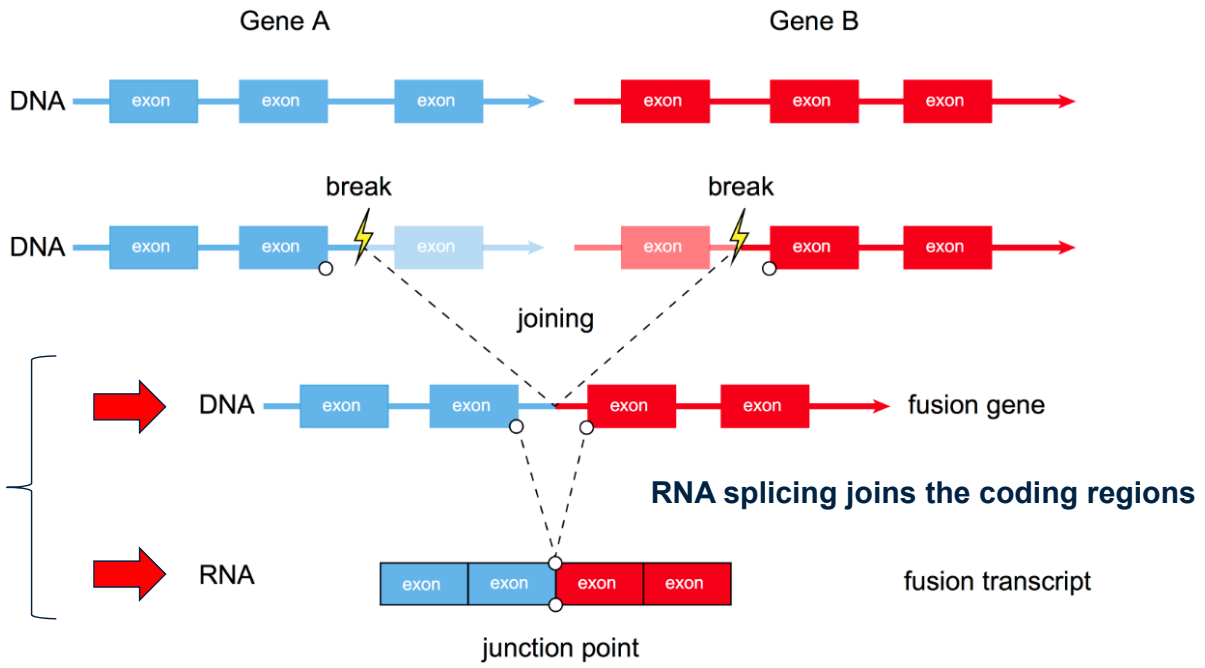
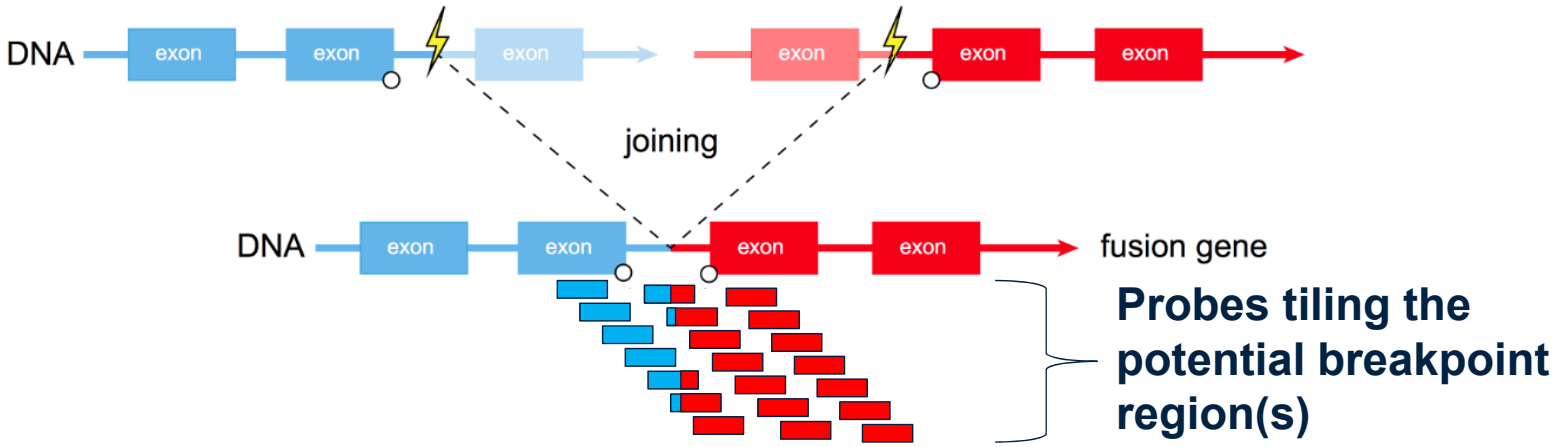


Which is the best nucleic acid for fusion detection? …it depends

https://tumorfusions.org/

Fusion gene detection in DNA requires coverage of the breakpoint



Breakpoints can occur anywhere within the introns (and occasionally in the exons). Reads that map to both genes are consistent with a fusion.

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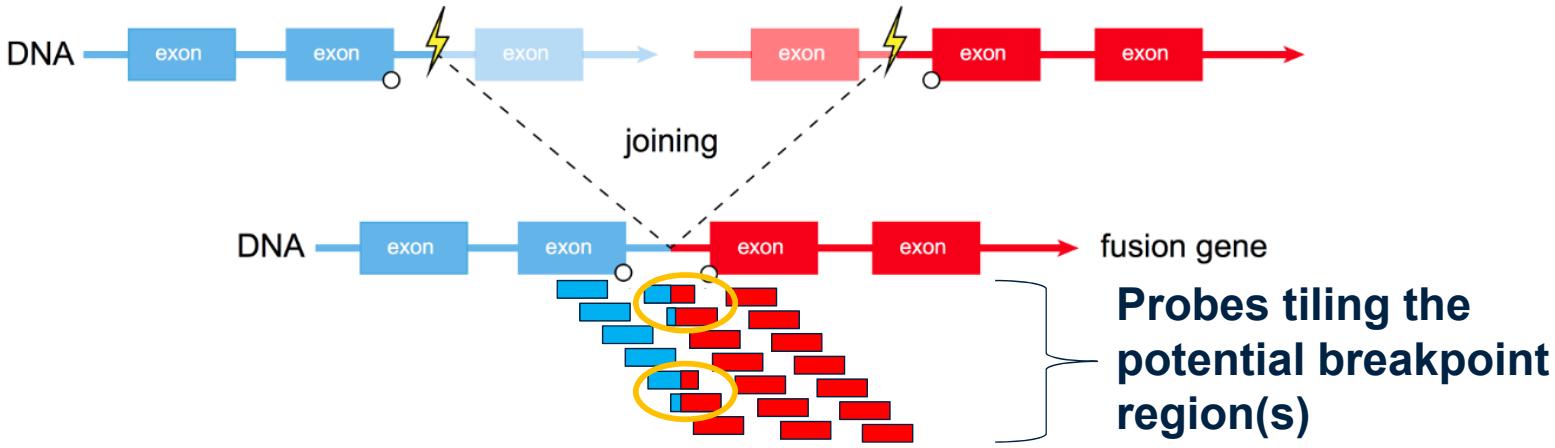

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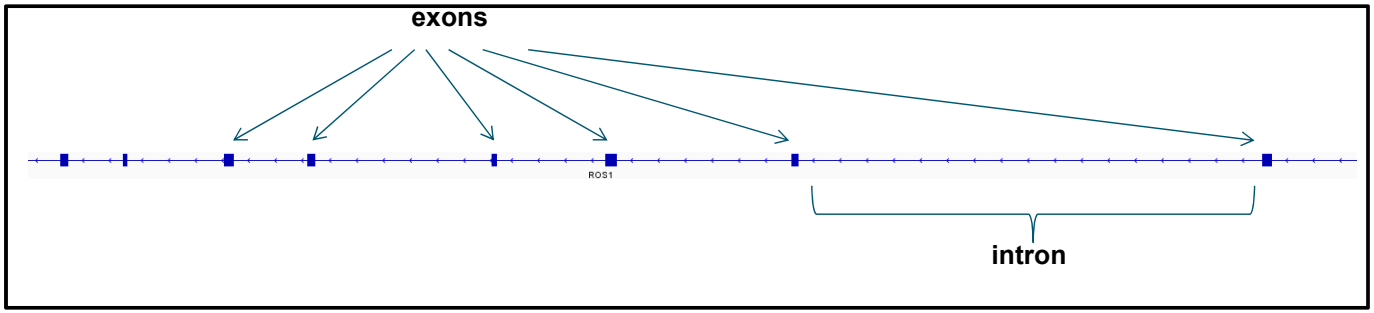
There are multiple challenges for detecting fusions using DNA based testing



Example: NTRK3
Common intron involved is very large. Most DNA testing identifies ETV6/NTRK3 Uses intronic sequence from ETV6

- Since the vast majority of genomic rearrangements occur in introns, need to sequence introns
  - Introns tend to be much larger than exons
  - Introns tend to contain repetitive sequences
  - Hard to map and thus difficult to sequence

Slide courtesy of K. Davies and D. Aisner

Detection of fusions using RNA as a template

Amplification of known partners (e.g. traditional RT-PCR)

- **Detection of a specific fusion gene (e.g. Is ALK joined with EML4)?**

Wu et al. J Hematol Oncol. 2016.9:19.PMID: 26951079

Detection of fusions using RNA as a template

Amplification of known partners (e.g. traditional RT-PCR)

- **Detection of a specific fusion gene (e.g. Is ALK joined with EML4)?**

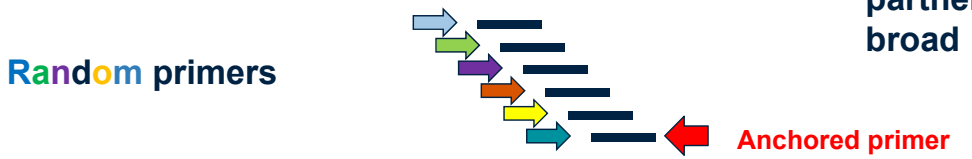
For rearrangements that are common/recurrent, detection of juxtaposition of known partners is fast and yields a discrete result (e.g. BCR/ABL1)

Wu et al. J Hematol Oncol. 2016.9:19.PMID: 26951079

Detection of fusions using RNA as a template

Fusion of critical genes with unknown partners is tricky but important

Using random primers for the detection of a partner to a recurrently rearranged gene is broad



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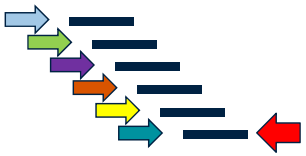
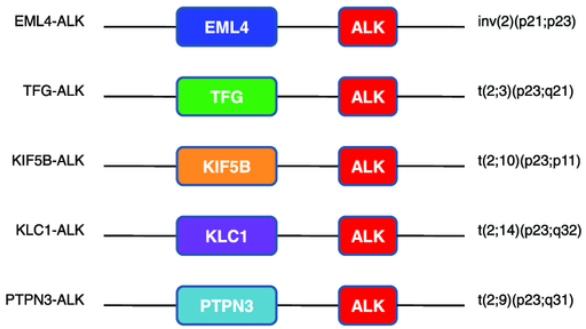


Anchored primer

Using random primers for the detection of a partner to a recurrently rearranged gene is broad



Detection of a critical gene with an unknown partner (e.g. Is there an ALK rearrangement in the tumor?)

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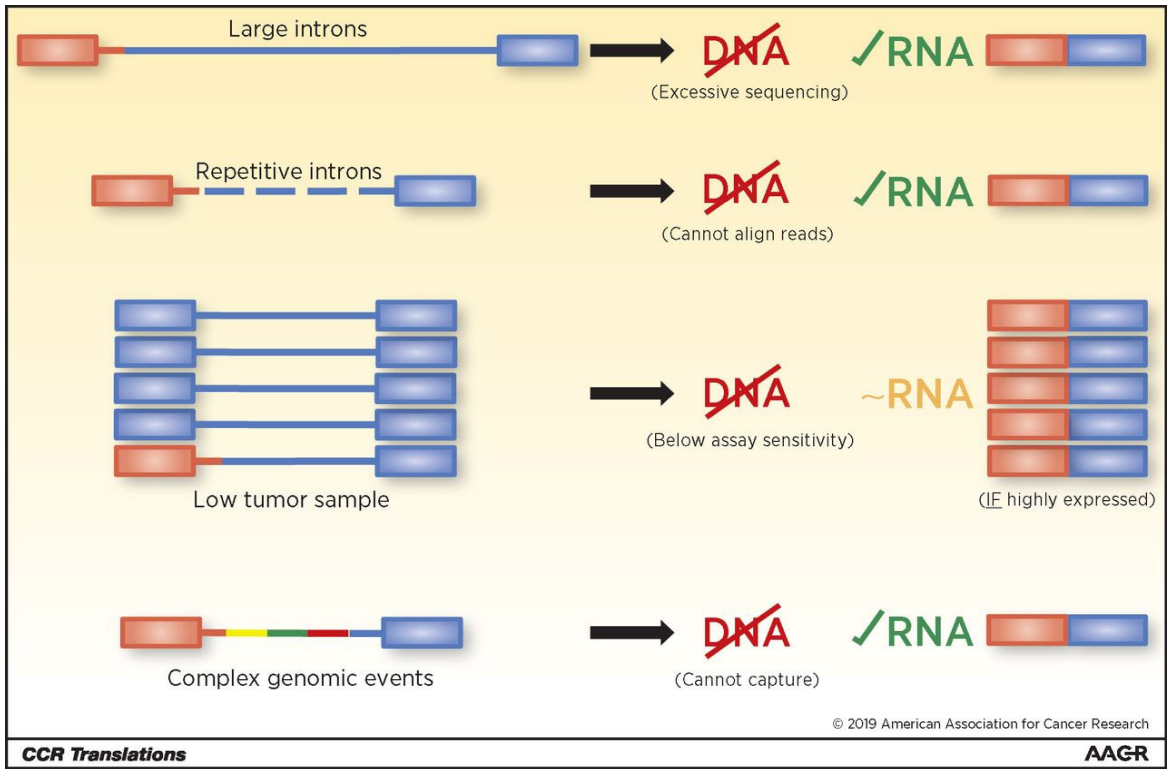
False-negative gene fusion results in DNA-based NGS analysis secondary to genomic complexity



Reflex to an RNA based test should be considered in the setting of a negative result for fusion genes from a DNA based test and no other driver genes detected

Kurtis D. Davies, and Dara L. Aisner Clin Cancer Res 2019;25:4586-4588

Case study: 48 year old male with NSCLC recurrence at 2 years

Positive result for an EML4/ALK rearrangement

RNA testing panel:

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The DNA tumor panel from tissue wasn't designed to detect fusions; the ctDNA assay was designed to detect EML4/ALK but didn't detect the fusion

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Transcript IDs: NM_019063.3/NM_004304.4

Using RNA from the tumor tissue as a substrate allowed detection of the EML4/ALK driver mutation in this patient

The DNA tumor panel from tissue wasn't designed to detect fusions; the ctDNA assay was designed to detect EML4/ALK but didn't detect the fusion

Liquid biopsy and tissue testing

What to do if there is insufficient (poor quality or low quantity) tumor tissue

- Potential for delay of treatment
- Use another block or sample (may need recuts)
- Rebiopsy

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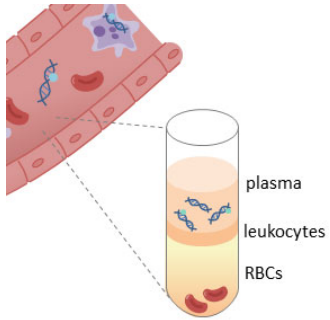
College of American Pathologists. The 'liquid' biopsy. https://www.cap.org/member-resources/articles/the-liquid-biopsy

What to do if there is insufficient (poor quality or low quantity) tumor tissue



- Potential for delay of treatment
- Use another block or sample (may need recuts)
- Rebiopsy
- Liquid biopsy
  - Typically uses peripheral blood (plasma) for detection of biomarkers associated with tumors
  - Following the appropriate collection for blood for testing is crucial to prevent degradation
  - Amount of circulating tumor DNA (ctDNA) varies based on tumor type, location, stage and other factors

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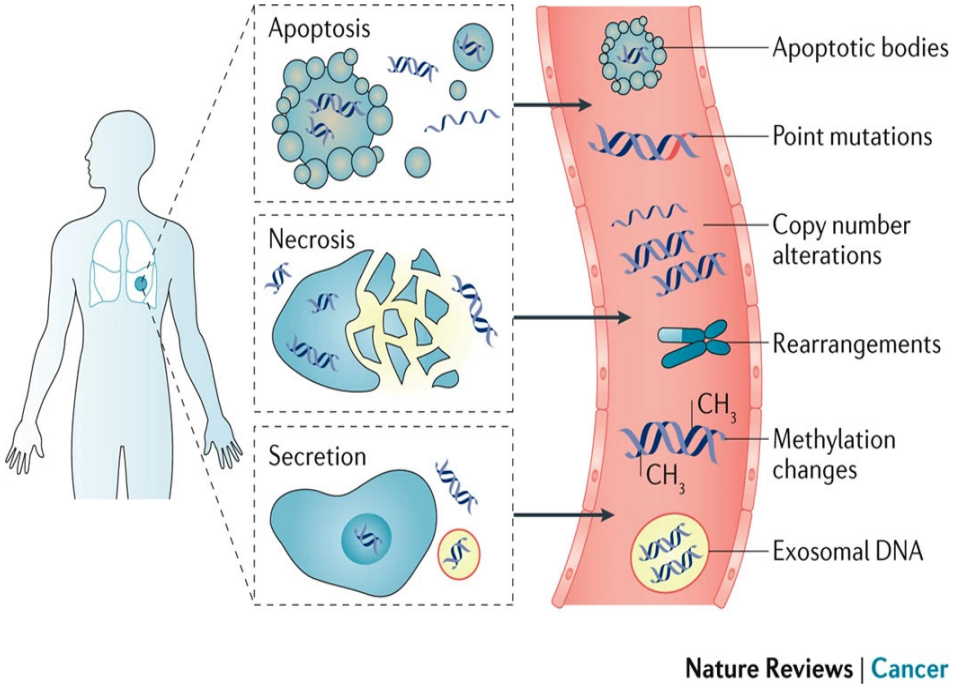
Liquid biopsy testing can detection actionable mutations by testing the patient's blood



- Minimally invasive test to detect mutations and gene rearrangements to help inform treatment strategies, and monitor cancer patients' disease
- Cell death is the most common way ctDNA is released from tumors
- Liquid biopsy testing detects fragments of DNA and include tumor DNA and DNA from normal cell turnover
- Useful when samples from the primary tumor are insufficient or inaccessible

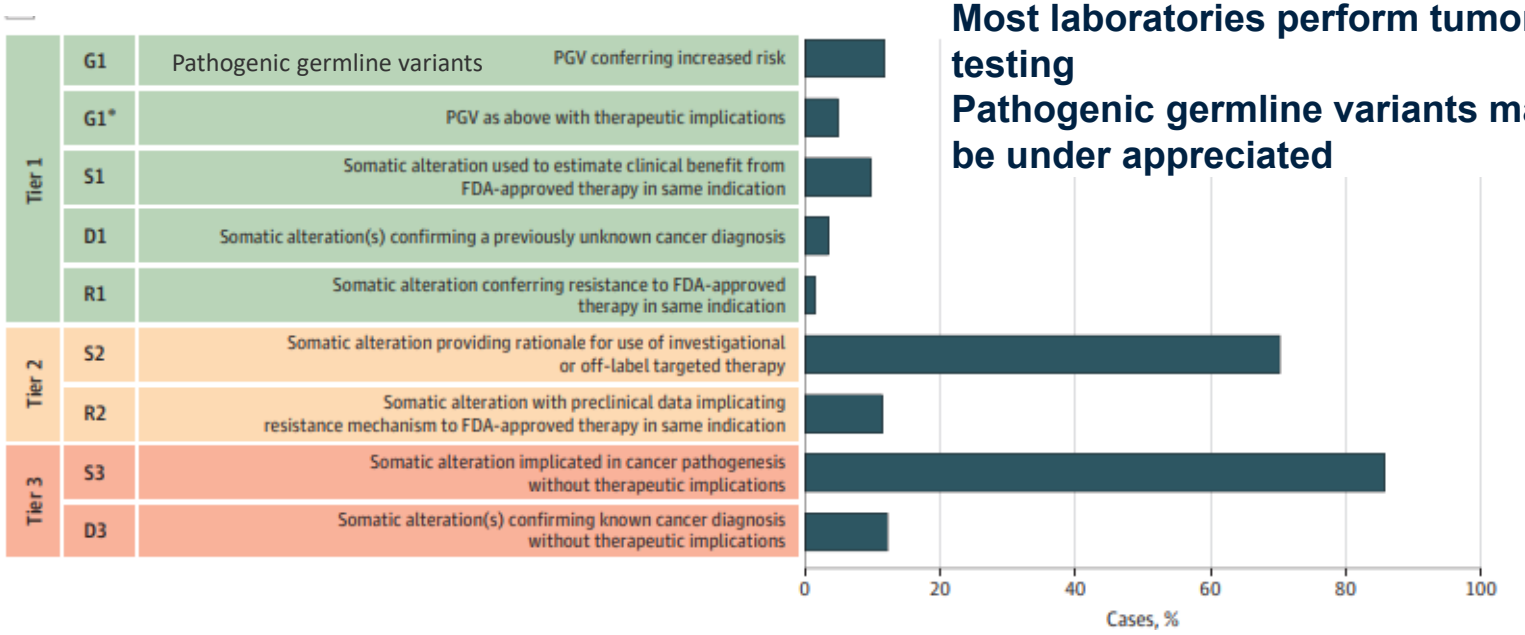
Wan, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 17, 223–238 (2017).

Considerations for tissue testing vs liquid testing

- Tissue biopsies and liquid biopsies can be considered complementary testing
- Detection of mutations/fusions by liquid biopsy is considered actionable
- Detection of mutations does not necessarily mean the mutations are from the known tumor
  - Metastatic sites or occult malignancies
- **If analysis of a liquid biopsy does not detect an actionable mutation, confirmation using tissue testing should be strongly considered per ASCO and AMP guidelines**

Wan, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 17, 223–238 (2017).

Summary: Clinically relevant molecular alterations in metastatic cancers includes germline and somatic alterations



Most laboratories perform tumor-only testing
Pathogenic germline variants may be under appreciated

Cobain EF, et al. Assessment of Clinical Benefit of Integrative Genomic Profiling in Advanced Solid Tumors. *JAMA Oncol.* 2021;7:525–533.

Summary: Detection of actionable fusion genes by DNA vs RNA based testing

	RNA	DNA
Advantages	Detects the result of the gene to gene fusion and exon deletion events; more copies of RNA in cell; introns removed in mRNA	Detects exactly where the DNA molecules are joined; DNA is stable; often already being assessed for other mutations
Disadvantages	RNA is vulnerable to degradation (risk of false negative); additional test=more tissue; may miss expression associated fusions (e.g. IGH/MYC)	Intron where breakage occurred may not be covered (false negative); more likely to be missed if low tumor percentage; introns can be low complexity so difficult to sequence; complex rearrangements may miss call rearrangements

Summary: Broad molecular profiling: tissue or liquid biopsy testing

Tissue biopsy	Liquid biopsy
• Involves sampling of the primary or metastatic tumor • Pathologists assess tumor percentage and histologic features • FFPE, FNA, fresh tissue • DNA quantity and quality are variable • RNA quantity and quality are variable • Very small samples, those with insufficient tumor percentage, necrotic samples may be inadequate • Old tissue samples may not represent the current tumor • Only the submitted region is sequenced	• Minimally invasive (e.g. blood draw, urine) • Consider upfront if the tumor is inaccessible or the patient is not medically fit for invasive tumor sampling • Can be performed (fairly) rapidly • Plasma is preferred over serum for ctDNA extraction • Consider at the time of initial diagnosis in all patients who need tumor molecular profiling; important if considering following patient by ctDNA • Detection of an actionable mutation is sufficient evidence to initiate targeted treatment • If no driver mutation is detected, it should be considered inconclusive and followed up with a secondary test (tissue based)

Lindeman NI, et al. *J Mol Diagnost*. 2018;20(2):129-159.Rolfo C, et al. *J Thorac Oncol*. 2018;13(9):1248-1268.

Thank you for your attention!

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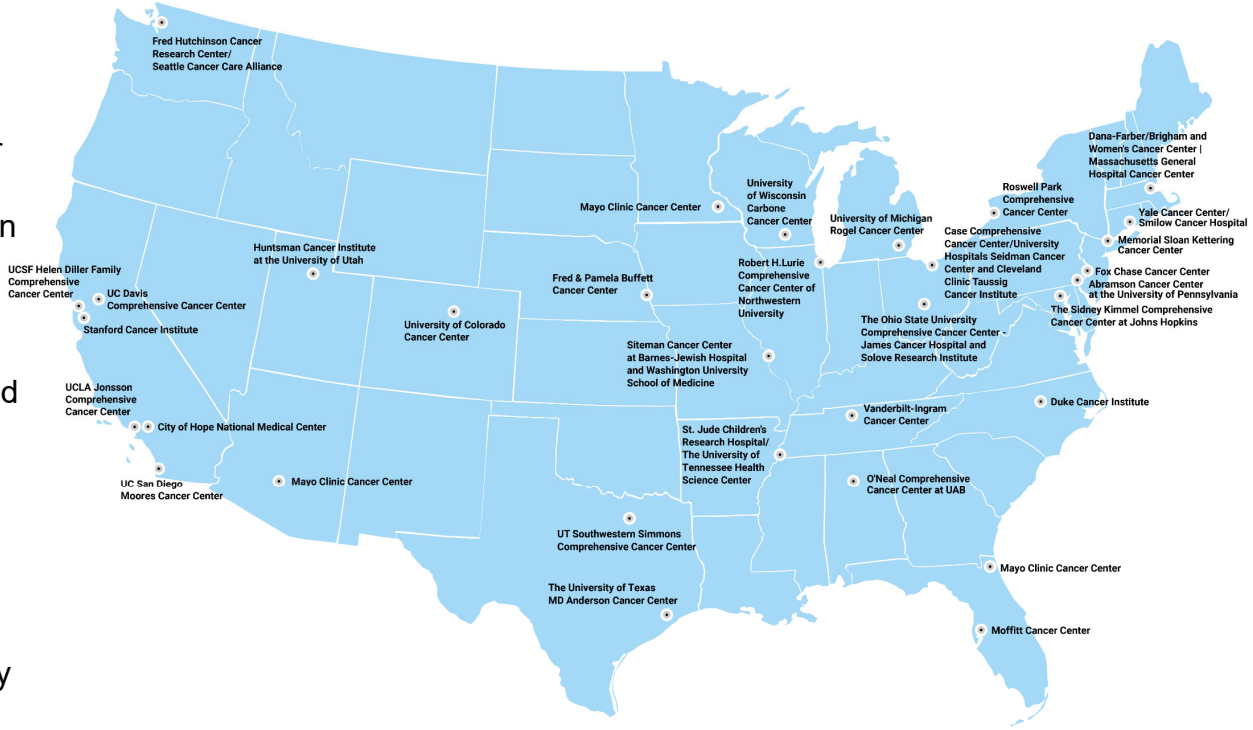

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