Rapid Molecular Biomarker Testing at Primary Diagnosis and Beyond: We Can Do a Lot With a Little

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The problem:

- Therapy planning for patients with a cancer diagnosis requires knowledge of biomarker status, especially in lung cancer.
- Patients with advanced cancer need testing of the most recent tissue.
- Biomarker-driven decisions are time-sensitive.
- Testing should be done rapidly.
- New recommendations suggest “up-front” testing of diagnostic tissue

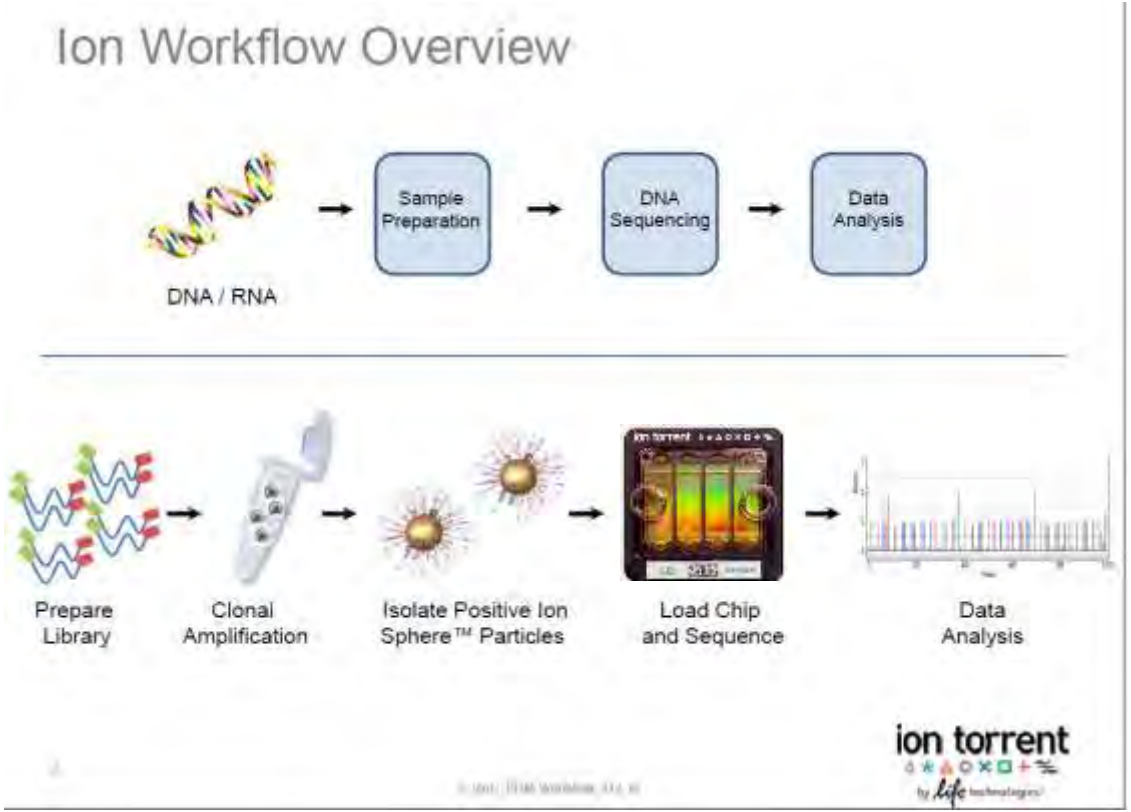
Practical Issues

- Tissue acquisition is often minimally-invasive
- Small biopsies requiring lots of testing
  - H&E levels
  - Immunohistochemistry stains
  - Molecular analysis for multiple analytes
- Tumor is not always predominant population

The solution

- Do multiple biopsies or passes
  - More invasive and expensive
- Order a liquid biopsy
  - Requires special processing
  - Highly sensitive assays are needed
  - Does not always reflect tissue findings
- Use technology that can handle small tumor tissue volumes

Next Generation Sequencing (NGS) by Semi-Conductor Technology



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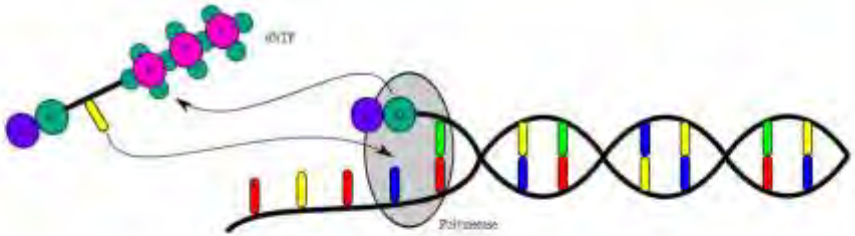
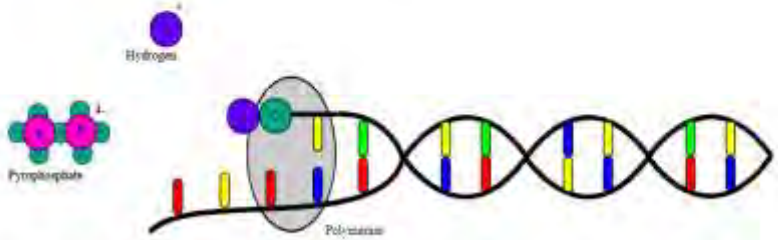
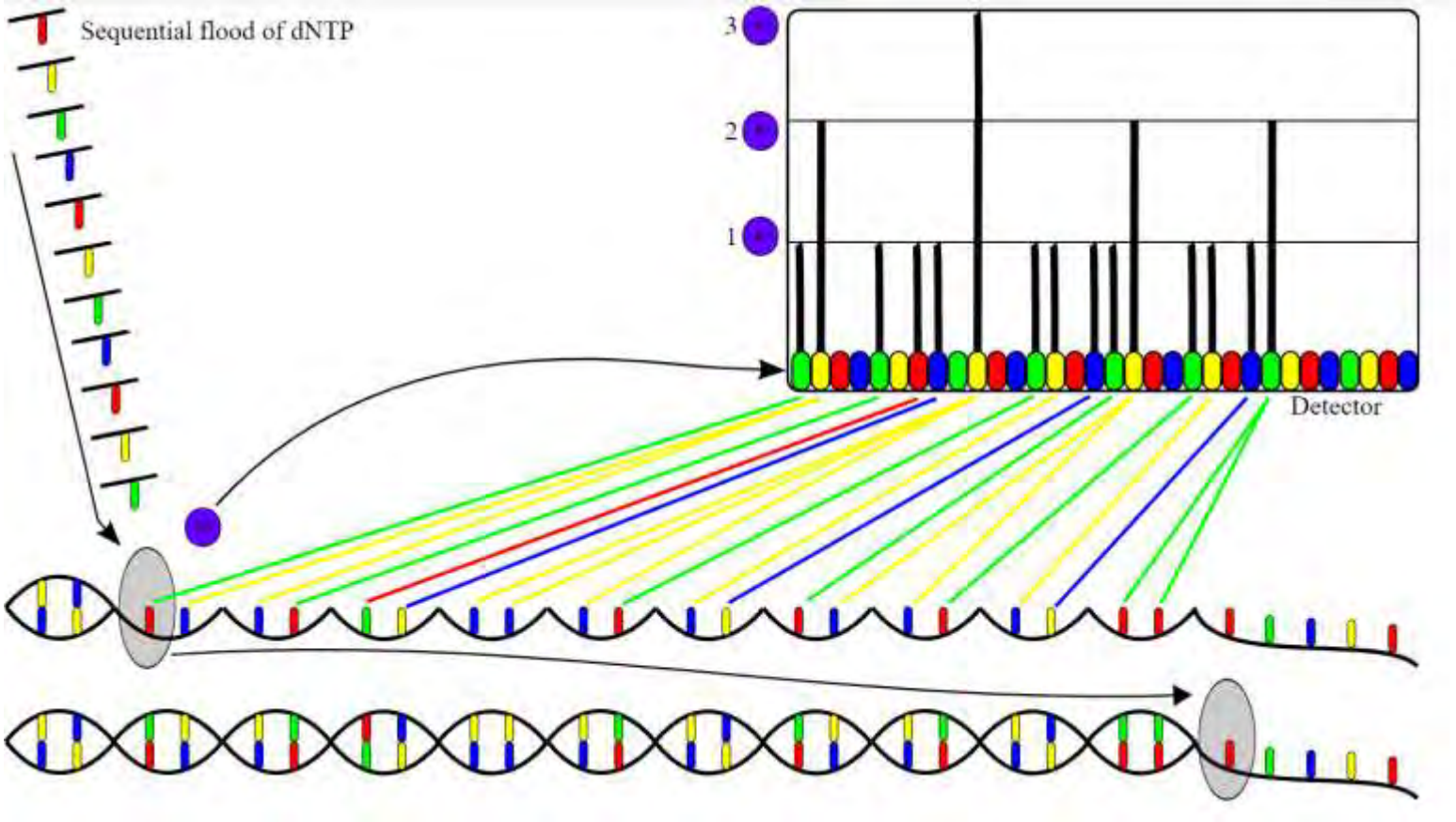
Semi-conductor sequencing



Polymerase integrates a nucleotide.

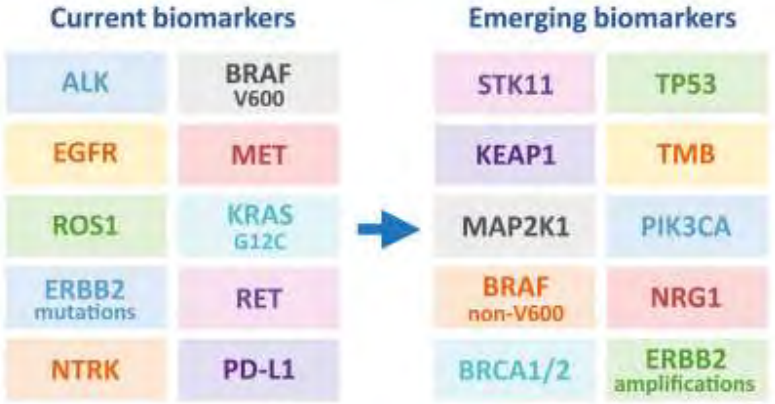


Hydrogen and pyrophosphate are released.



Biomarkers that should be assessed

Up-front testing on Non- Small Cell Lung Cancer (NSCLC) at diagnosis

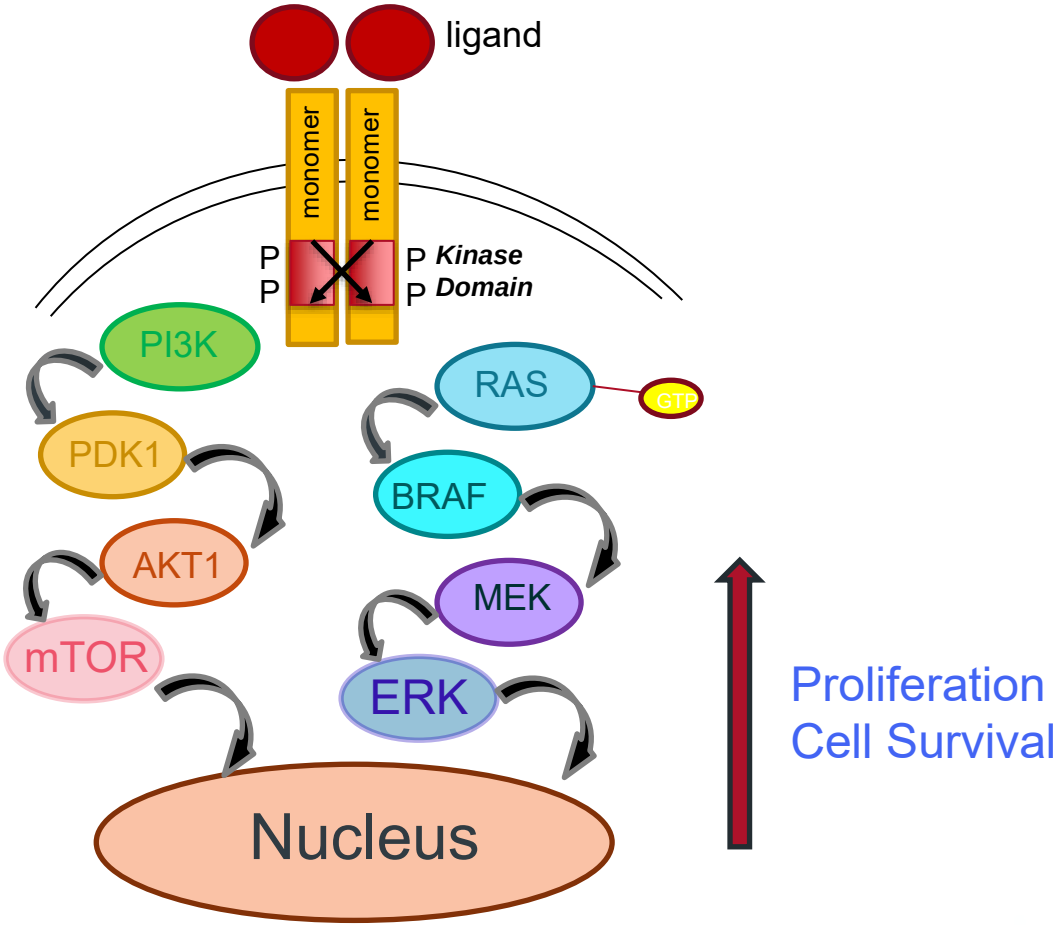


de Jager VD, et al. Future perspective for the application of predictive biomarker testing in advanced stage non-small cell lung cancer. Lancet Reg Health Eur. 2024 Mar 1;38:100839. e

Solid Tumor Precision Panel (STPP) Assay Amplicon Based

DNA hotspots					CNVs		Inter-genetic fusions		Intra-genetic fusions
AKT1	CHEK2	FGFR3	KIT	NTRK3	ALK	FGFR1	ALK	NTRK1	AR
AKT2	CTNNB1	FGFR4	KRAS	PDGFRA	AR	FGFR2	BRAF	NTRK2	EGFR
AKT3	EGFR	FLT3	MAP2K1	PIK3CA	CD274	FGFR3	ESR1	NTRK3	MET
ALK	ERBB2	GNA11	MAP2K2	PTEN	CDKN2A	KRAS	FGFR1	NUTM1	
AR	ERBB3	GNAQ	MET	RAF1	EGFR	MET	FGFR2	RET	
ARAF	ERBB4	GNAS	MTOR	RET	ERBB2	PIK3CA	FGFR3	ROS1	
BRAF	ESR1	HRAS	NRAS	ROS1	ERBB3	PTEN	MET	RSPO2	
CDK4	FGFR1	IDH1	NTRK1	SMO			NRG1	RSPO3	
CDKN2A	FGFR2	IDH2	NTRK2	TP53					
DNA					DNA		RNA		RNA

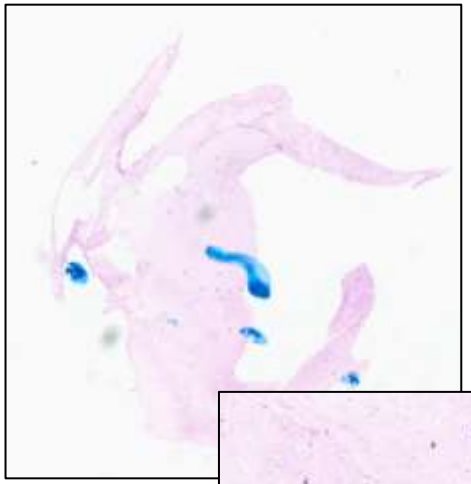
Receptor Tyrosine Kinase Pathways



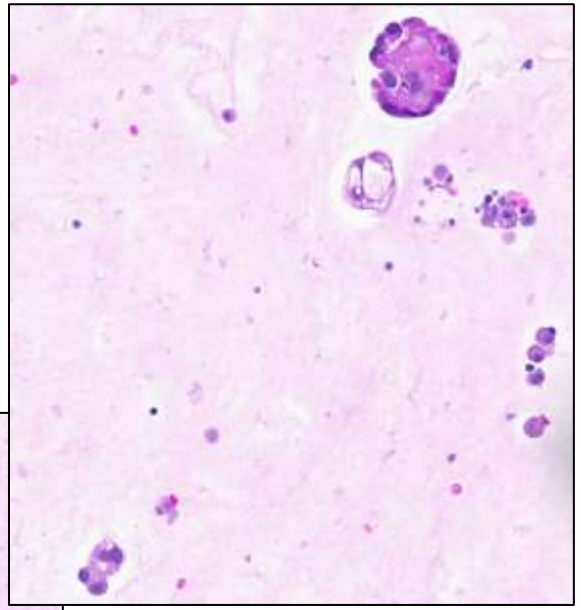
Non-Small Cell Lung Cancer (NSCLC) Cases

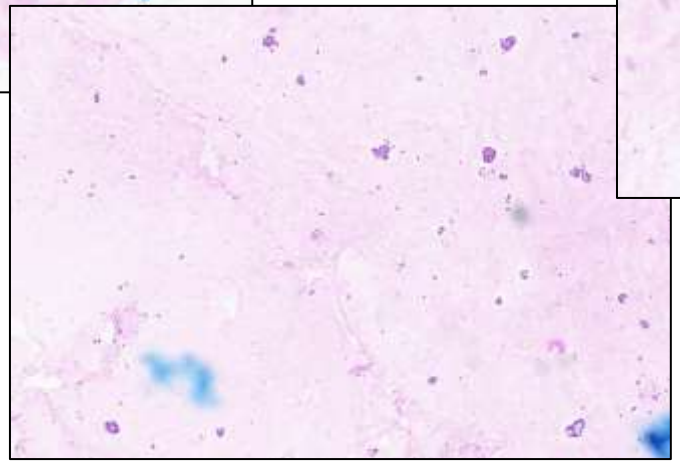
Case 1 Clinical History

- Female non-smoker in her seventies
- Presented to the Emergency Room with shortness of breath and weight loss
- Left-sided loculated pleural effusion
- Two left lung masses and mediastinal lymphadenopathy
- Thoracentesis performed



Pleural Fluid
Adenocarcinoma
TTF-1 +
Napsin +
TP53 +





DNA concentration:
1ng/µl
RNA concentration:
2ng/µl

Amplicon Based NGS panel (pleural fluid)

Gene	Alteration	Classification	VAF	Total Coverage
EGFR	c.2235_2249del;p.E746_A750del	Tier 1A - Pathogenic	33%	7692
TP53	c.841G>C;p.D281H	Tier 2C - Likely Pathogenic	27%	3754

Drugs Associated with Sensitivity for Patient's Tumor Type, Based on Genomic Analysis

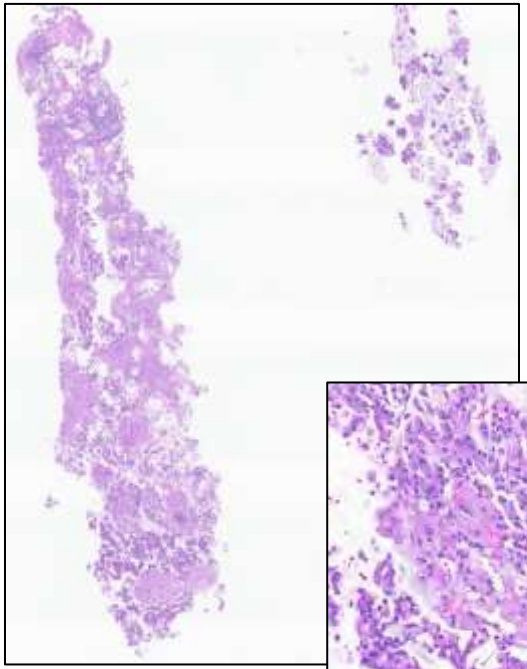
Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Afatinib	Primary sensitivity	EGFR Exon 19 Deletion (E746_A750del)	Non-Small Cell Lung Cancer	Single agent (FDA, NCCN), or may be considered in combination with cetuximab after progression on afatinib, erlotinib, gefitinib, or dacomitinib, and chemotherapy (NCCN).	Metastatic	FDA, NCCN
Dacomitinib	Primary sensitivity	EGFR Exon 19 Deletion (E746_A750del)	Non-Small Cell Lung Cancer		Metastatic	FDA, NCCN
Erlotinib	Primary sensitivity	EGFR Exon 19 Deletion (E746_A750del)	Non-Small Cell Lung Cancer	Single agent or in combination with ramucirumab (FDA, NCCN), or in combination with bevacizumab (NCCN, non-squamous only).	Metastatic	FDA, NCCN
Gefitinib	Primary sensitivity	EGFR Exon 19 Deletion (E746_A750del)	Non-Small Cell Lung Cancer		Metastatic	FDA, NCCN
Osimertinib	Primary sensitivity	EGFR Exon 19 Deletion (E746_A750del)	Non-Small Cell Lung Cancer	Preferred first-line therapy, per NCCN. Also approved as adjuvant therapy	Metastatic	FDA, NCCN

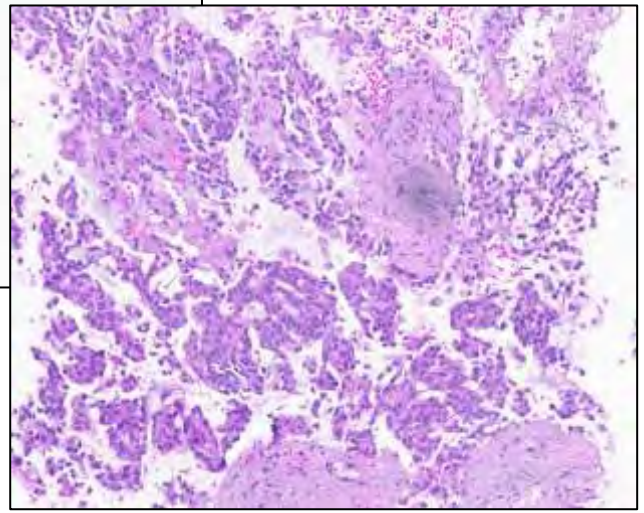
- No surgical intervention
- Continued reduction or stabilization of lesions and effusion
- Effect of TP53 variant[1] ?

[1]The Role of *TP53* Mutations in *EGFR*-Mutated Non-Small-Cell Lung Cancer: Clinical Significance and Implications for Therapy. Cancers (Basel). 2022 Feb 23;14(5):1143.

Case 2 Clinical History

- 70 year old male
- Former remote smoker 1967-1980
- Presented with cirrhosis (NASH?) and hepatocellular carcinoma
- Incidental right upper lobe lesion identified





Right middle lobe lung biopsy

Adenocarcinoma, focal papillary features

TTF-1 +

Napsin +

DNA concentration: 0.9 ng/µl

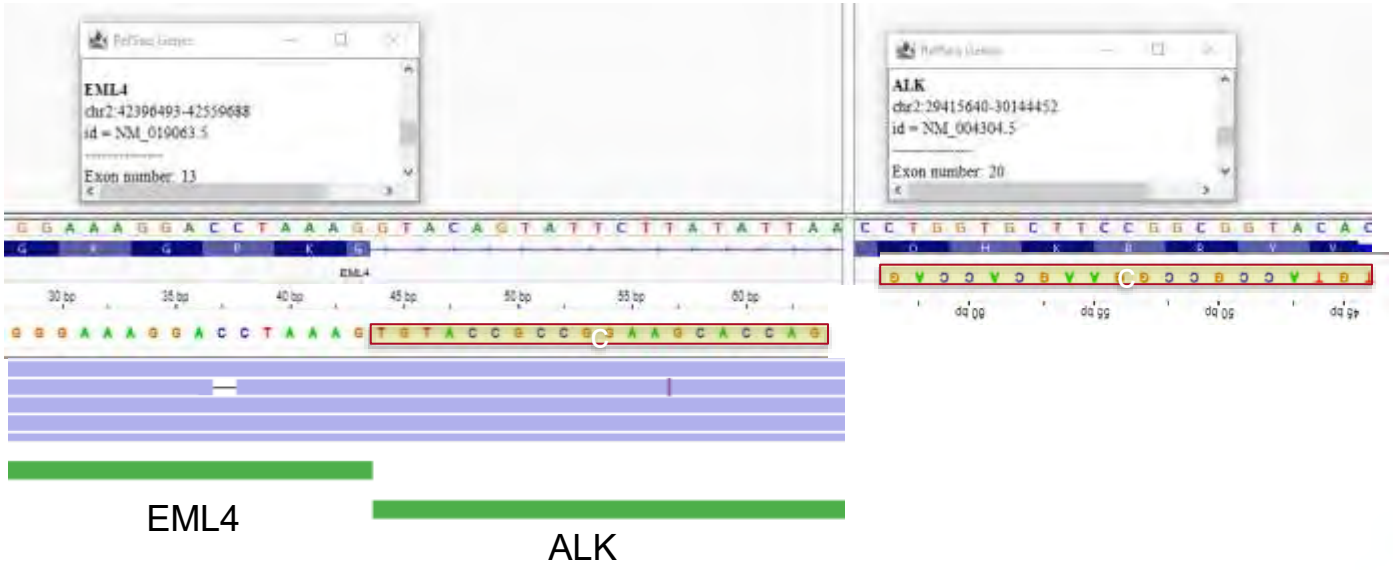
RNA concentration: 2.7 ng/µl

Amplicon Based NGS panel (on biopsy)

SUMMARY

Clinically Significant Alterations (Tier 1 or Tier 2 and/or Pathogenic or Likely Pathogenic):

EML4::ALK Fusion (Tier 1A)



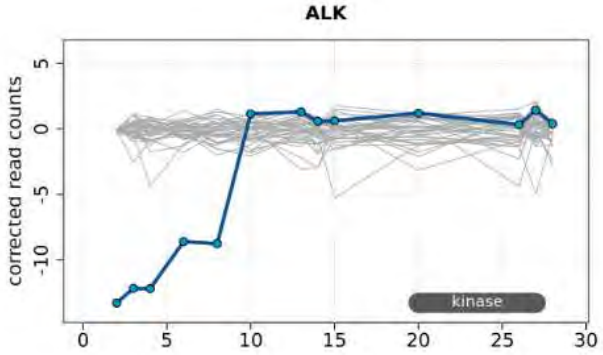
Follow up treatment

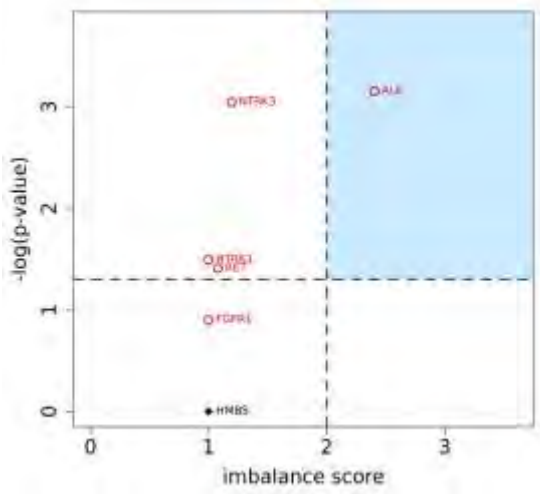
- Referred to an outside oncologist
- Other co-morbidities being addressed
- Lost to follow up

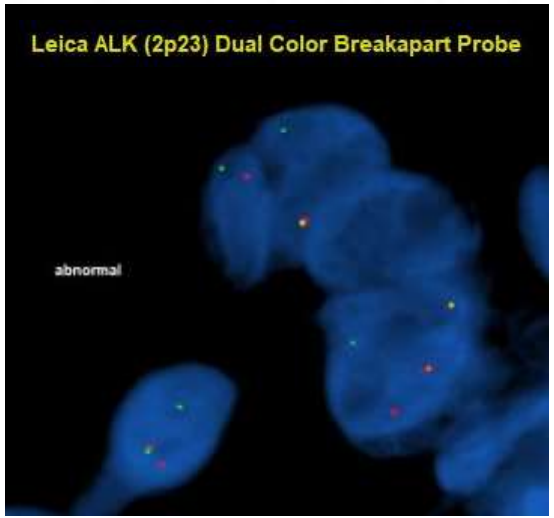
Drugs Associated with Sensitivity for Patient's Tumor Type, Based on Genomic Analysis

Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Alectinib	Primary sensitivity	ALK Fusion	Non-Small Cell Lung Cancer	Approved by FDA for patients with ALK-positive, metastatic NSCLC.	Metastatic	FDA, NCCN
Brigatinib	Primary sensitivity	ALK Fusion	Non-Small Cell Lung Cancer	Approved by FDA for patients with ALK-positive, metastatic NSCLC.	Metastatic	FDA, NCCN
Ceritinib	Primary sensitivity	ALK Fusion	Non-Small Cell Lung Cancer	Approved by FDA for patients with ALK-positive metastatic NSCLC.	Metastatic	FDA, NCCN
Crizotinib	Primary sensitivity	ALK Fusion	Non-Small Cell Lung Cancer	Approved by FDA for patients with ALK-positive or ROS1-positive metastatic NSCLC.	Metastatic	FDA, NCCN
Lorlatinib	Primary sensitivity	ALK Fusion	Non-Small Cell Lung Cancer	Approved by FDA for patients with ALK-positive, metastatic NSCLC.	Metastatic	FDA, NCCN

Expression Imbalance (different patient)







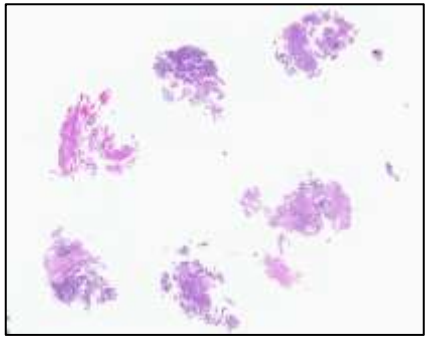
Positive for rearrangement of the *ALK* (2p23) locus (92%)

Liver Biopsy – Poorly Differentiated Carcinoma of Unknown Origin

ALK Amplification
CN=5.0 by NGS

Case 3 Clinical History

- Female in her mid-50s – Never Smoker
- Some left axillary discomfort, otherwise asymptomatic
- Screening mammogram and MRI revealed incidental Right Hilar Lymphadenopathy
- Right lower lobe lung mass biopsied



Lymph Node Station 7 Biopsy

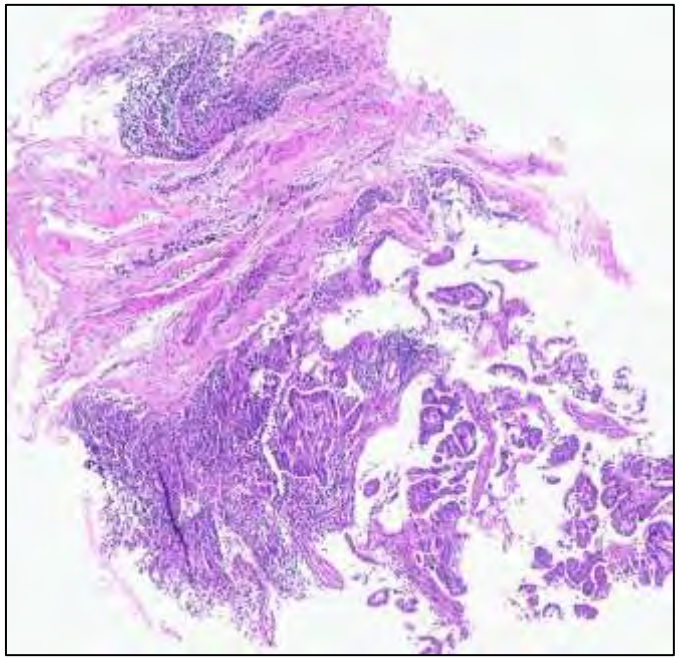
Adenocarcinoma

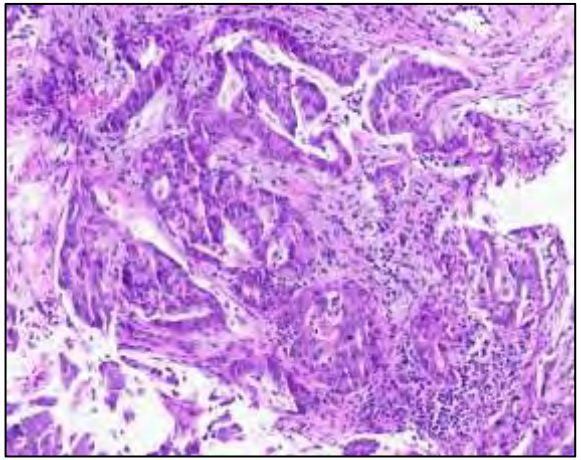
Papillary and micropapillary type

TTF1 +

Napsin A +

PDL1 – TPS score 20% (partial positive)





DNA concentration:
1.0 ng/µl
RNA concentration:
2.87 ng/µl

Amplicon Based NGS panel (on tissue)

Clinically Significant Alterations

CCDC6::RET Fusion (Tier 1A)

TP53 p.R267P (Tier 2C)

Current Treatment

- Started on neoadjuvant chemotherapy without immunotherapy due to *RET* fusion**
- Undergoing resection after neoadjuvant therapy

In advanced or metastatic disease

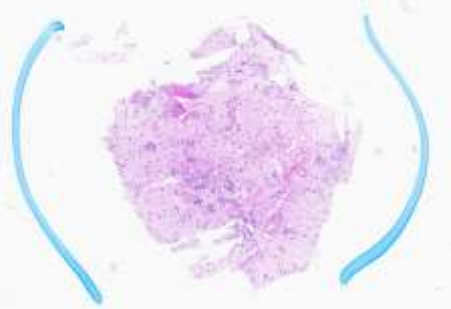
Drugs Associated with Sensitivity for Patient's Tumor Type, Based on Genomic Analysis

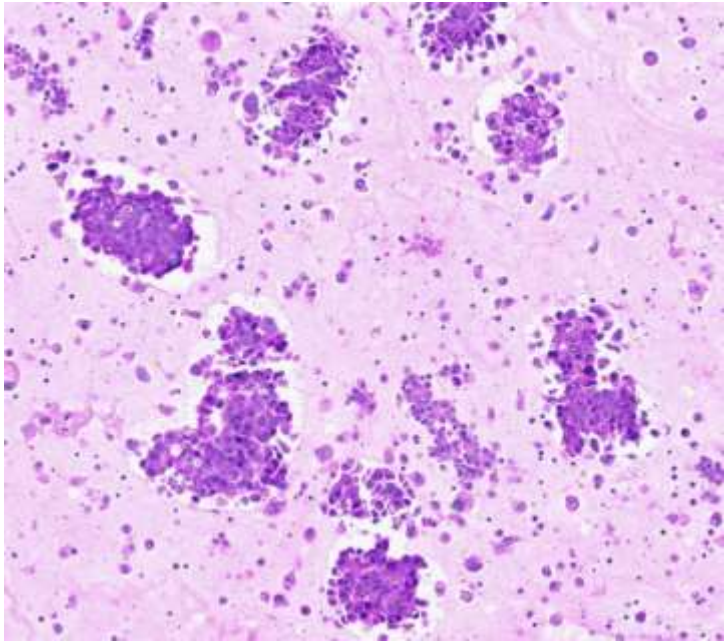
Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Cabozantinib	Primary sensitivity	RET Fusion	Non-Small Cell Lung Cancer	Recommended by NCCN under Category 2A.	Metastatic	NCCN
Pralsetinib	Primary sensitivity	RET Fusion	Non-Small Cell Lung Cancer	Indicated for adult patients with metastatic RET fusion-positive NSCLC.	Metastatic	FDA, NCCN
Selpercatinib	Primary sensitivity	RET Fusion	Non-Small Cell Lung Cancer	Approved for adult patients with metastatic RET fusion-positive NSCLC.	Metastatic	FDA, NCCN
Selpercatinib	Primary sensitivity	RET Fusion	Non-Small Cell Lung Cancer	Indicated for locally advanced or metastatic solid tumors with a RET fusion, who had progression on prior systemic treatment or have no satisfactory alternative options.	Metastatic	FDA

**BMC Cancer. 2024 Feb 5;24(1):178.
**JCO Precis Oncol. 2019;3:PO.18.00386. doi: 10.1200/PO.18.00386.
** https://ascopubs.org/doi/10.1200/JCO.2018.36.15_suppl.9034

Case 4 Clinical History

- 86 year-old male with a history of bladder cancer (TURBT and BCG)
- Multiple comorbidities
- Former, remote smoker (Quit Date-1974)
- Presented to Emergency Room after dyspnea, weakness and syncopal episode
- Large loculated pleural effusion
- Malignant pleural effusion with thoracentesis
- Mediastinal lymphadenopathy
- Bone and liver lesions

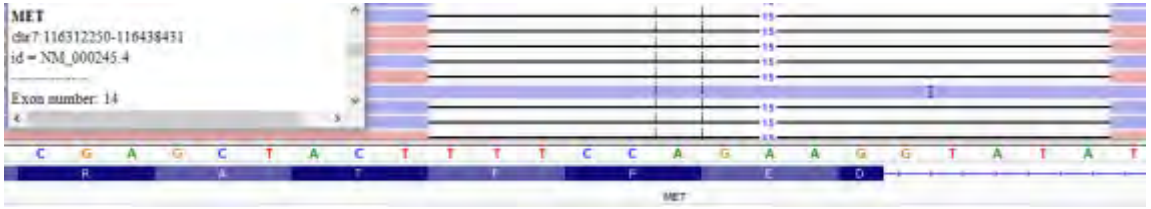




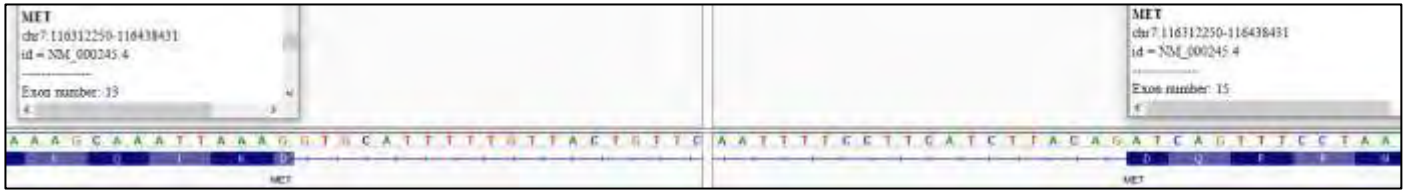
Pleural Fluid
Adenocarcinoma
TTF1 +
Napsin A +
PDL1 – TPS score 90%

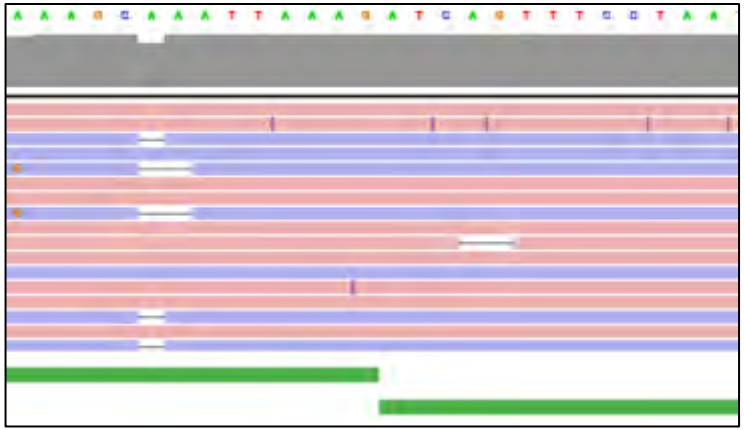
DNA concentration:
16.8 ng/µl
RNA concentration:
21.5 ng/µl

Clinically Significant Alterations	VAF
MET c.3077_3082+9del (Tier 1A)	46%
MET Exon 14 Skipping (Tier 1A)	

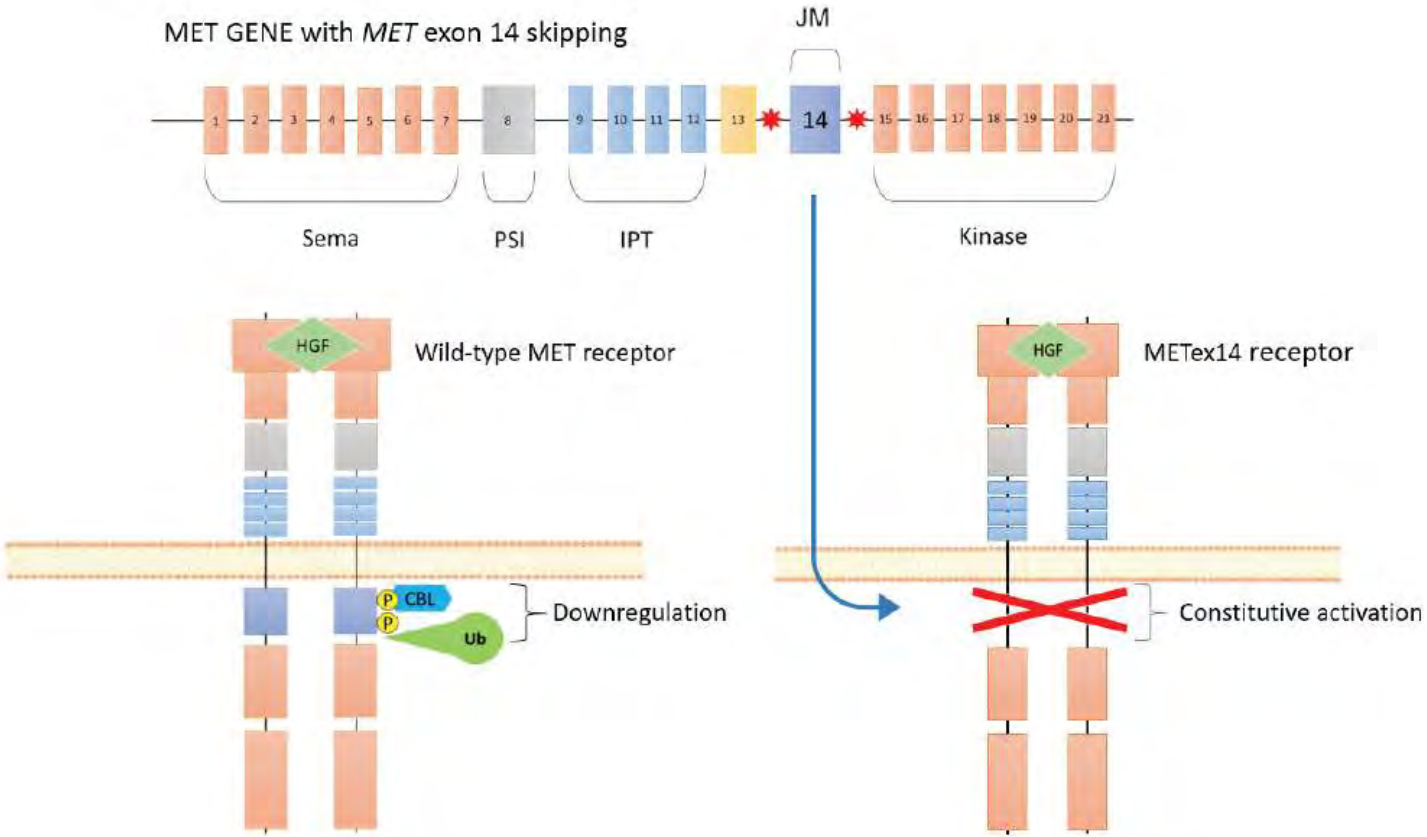


DNA





RNA



MET Exon 14 Skipping Variant

Drugs Associated with Sensitivity for Patient's Tumor Type, Based on Genomic Analysis

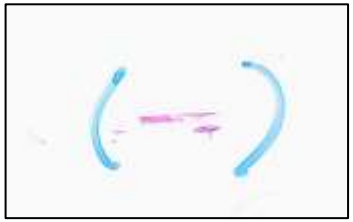
Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Capmatinib	Primary sensitivity	MET Exon 14 Skipping	Non-Small Cell Lung Cancer	Indicated for adult patients with metastatic non-small cell lung cancer with a mutation that leads to MET exon 14 skipping.	Metastatic	FDA, NCCN
Crizotinib	Primary sensitivity	MET Exon 14 Skipping	Non-Small Cell Lung Cancer	Recommended by NCCN under Category 2A for patients with high-level MET amplification or MET exon 14 skipping mutations.	Metastatic	NCCN
Tepotinib	Primary sensitivity	MET Exon 14 Skipping	Non-Small Cell Lung Cancer	Indicated for metastatic NSCLC harboring MET exon 14 skipping alterations.	Metastatic	FDA, NCCN

- Started on Capmatinib 400 mg BID (oral)
- Reduction of most lesions within two months
- Tolerating treatment well

Other Cases (non-NSCLC)

Case 5

- Male diagnosed with nodular melanoma of left neck in mid 60s
- Negative staging and sentinel node
- 5 years later presented with left shoulder and groin pain
- Widespread metastatic disease
- Left lung mass biopsied with metastatic melanoma
- Started Immune Checkpoint Inhibitors but developed hepatitis – treated with steroids

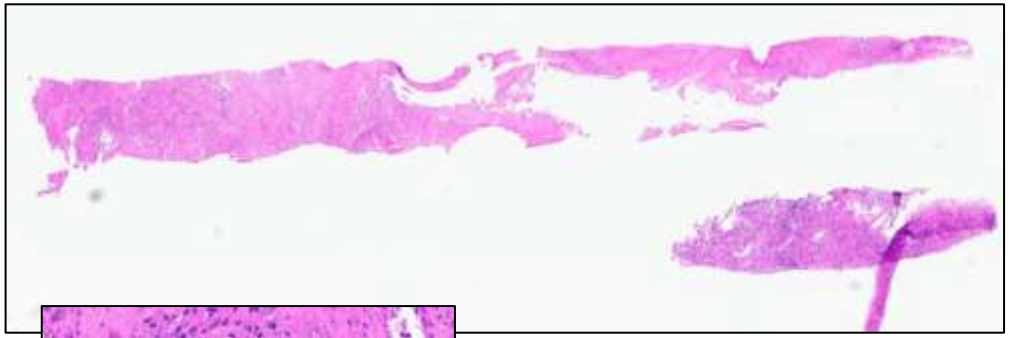


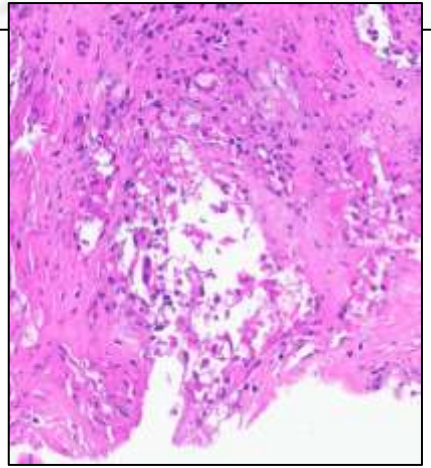
Left Lung Biopsy

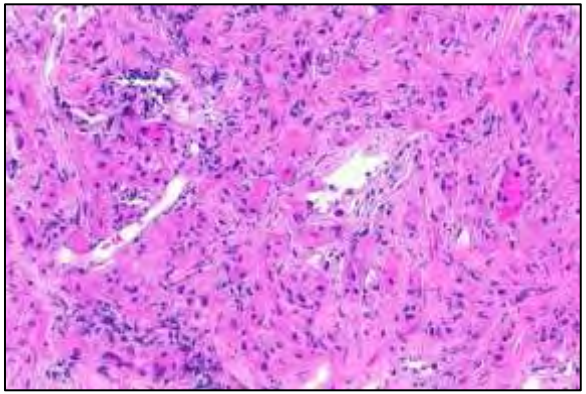
Poorly differentiated neoplasm, c/w melanoma

SOX10 +

S100 +



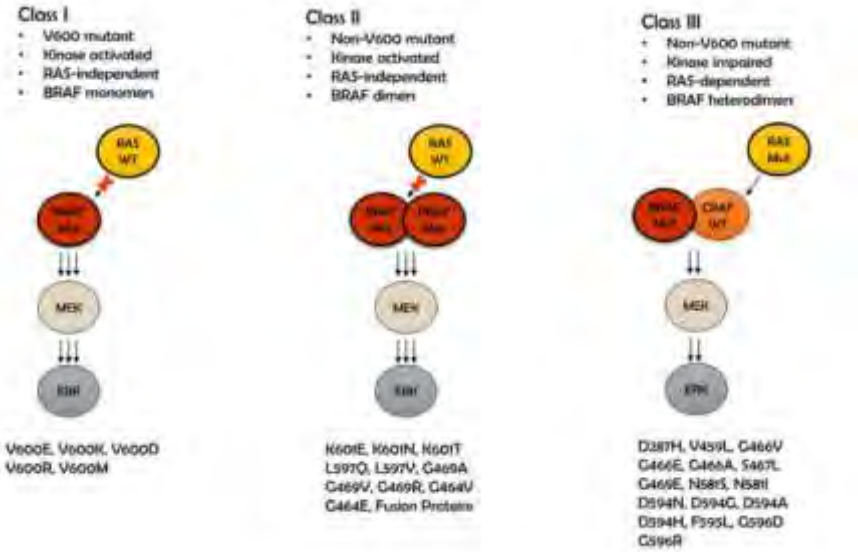




BRAF non-V600 variants

Clinically Significant Alterations

BRAF p.G469E (VAF: 18.1%) (Exon 11)

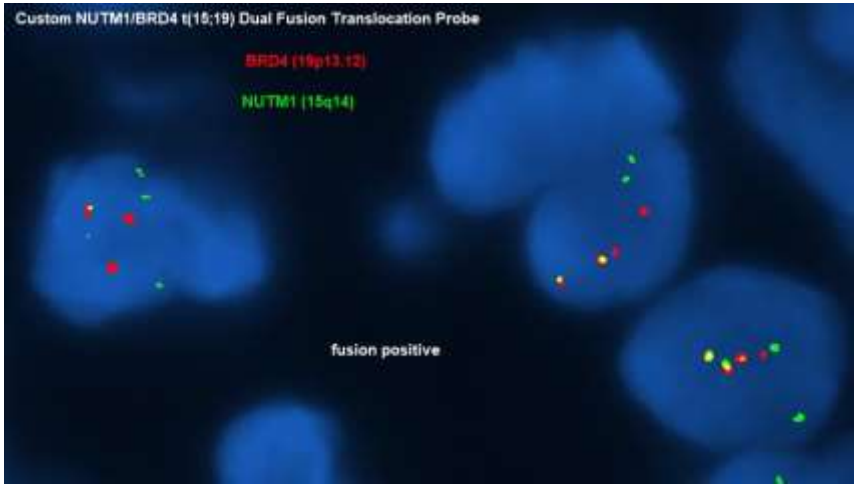


BRAF and MEK inhibition currently not recommended for non-V600E variants in exons 11 or 15 per NCCN 2.2024

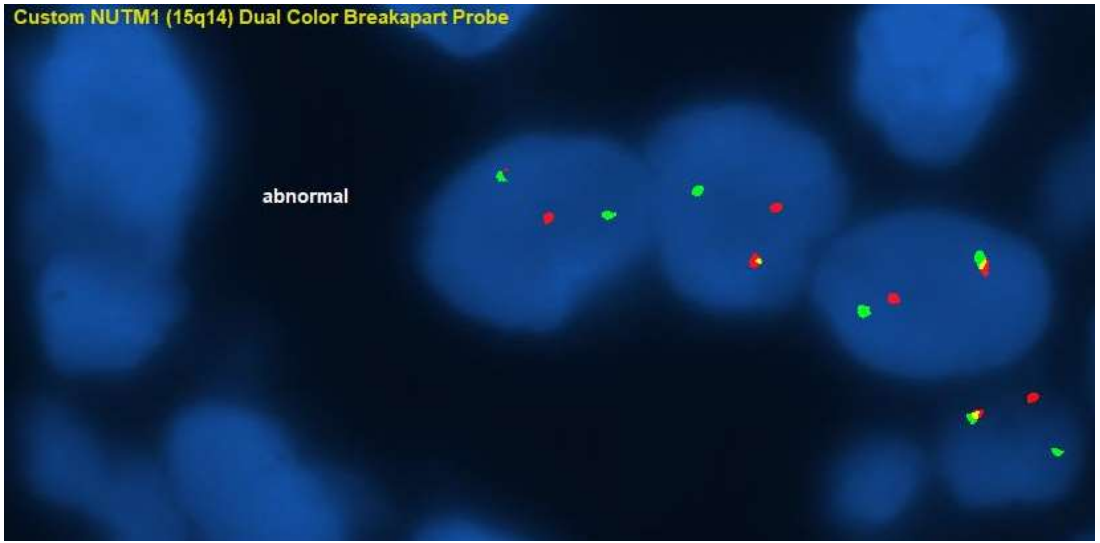
Śmiech M, Leszczyński P, Kono H, Wardell C, Taniguchi H. Emerging *BRAF* Mutations in Cancer Progression and Their Possible Effects on Transcriptional Networks. Genes (Basel). 2020 Nov 12;11(11):1342. doi: 10.3390/genes11111342. PMID: 33198372; PMCID: PMC7697059. Licensed by Creative Commons version 4.0.

Case 6

- 71 year-old female, former smoker 30+ years
- Questionable history of cutaneous melanoma
- Incidental 5 cm right lung mass identified on a coronary calcium score CT test
- FNA and biopsy were performed at outside institution



- Outside cytology FNA
- Right Upper Lobe Lung Mass
- Poorly Differentiated Carcinoma
- Multiple Negative Stains
- NUT IHC Positive



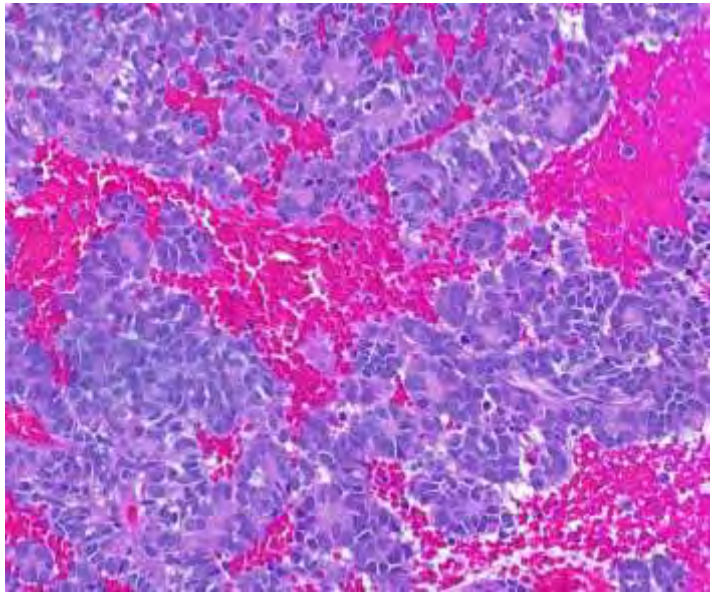
Continued Course

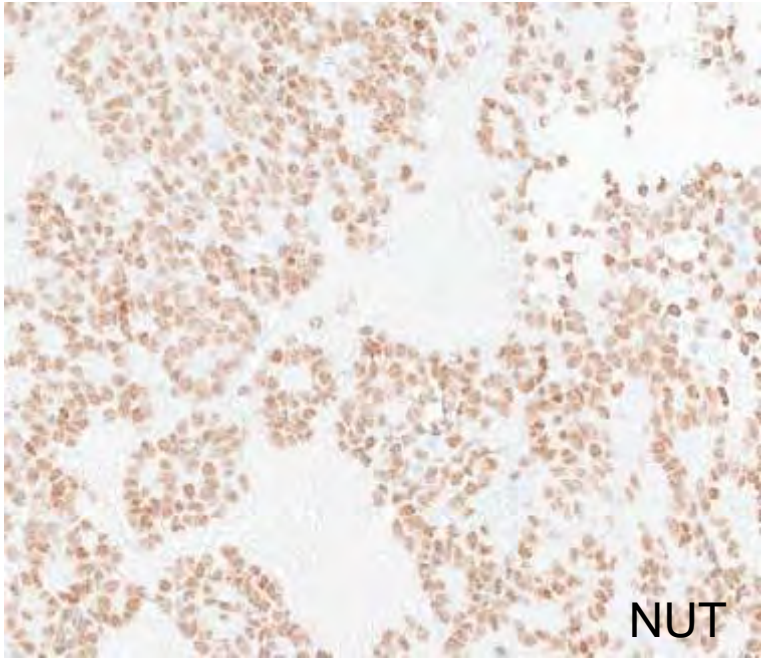
- Follow up care at Harvard with a NUT-carcinoma specialist
- Neoadjuvant chemotherapy and immunotherapy and resection
- Adjuvant chemotherapy
- Developed colitis
- Interval CT scan revealed a chest wall/breast mass
- Biopsy performed

Metastatic Breast Lesion:
FISH Positive *NUTM1::BRD4* fusion

NGS:
STPP Panel NM: Negative

Harvard (previous specimen):
Intergenic rearrangement BRD4 exon 10 (Tier 4)



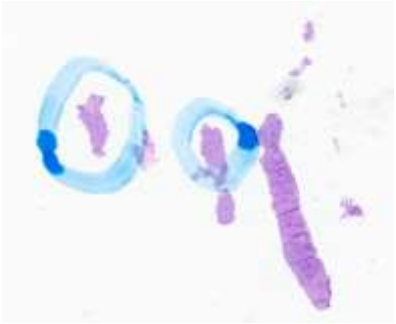


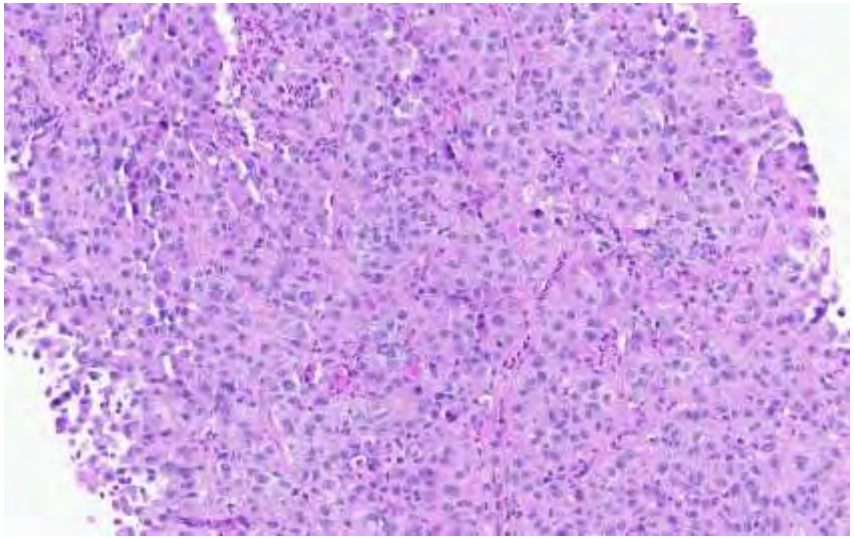
Take Home: No test is 100% sensitive and multimodality testing may be needed in certain cases

Case 7

- 67 year old never smoker male presenting with 2 weeks of non-productive cough
- Bilateral lung masses on CT scanning and hilar lymphadenopathy
- Normal CXR two years prior
- Renal mass also identified
- Biopsy performed by IR of right lung nodule
- Poorly differentiated carcinoma with necrosis (PDL1 >80%)
  - Possible kidney (PAX8+), but necrosis interfering
- Started ipilimumab + nivolumab, then single nivolumab with initial response then some progression

Biopsy of Axillary Lymph Node for Hybrid capture- based NGS (Large Panel-POP300)





Single Nucleotide Variations and/or Small Insertions/Deletions (Pathogenic or Likely Pathogenic):

Gene	Alteration	Classification	VAF
ATM	c.7882dupA;p.I2628fs	Tier 2C - Likely Pathogenic	28%
BRCA1	c.3049G>T;p.E1017*	Tier 2C - Pathogenic	25%
FBXW7	c.1480_1481insA;p.L494fs	Tier 2C - Likely Pathogenic	25%
MET	c.3328G>A;p.V1110I	Tier 2C - Pathogenic	42%
TERT	c.-146C>T	Tier 2C - Pathogenic	23%

Copy Number Variations:

Gene	Alteration	Classification
None		

Structural Variations:

Genes	Alteration	Classification
None		

Biomarker Summary:

Microsatellite Stable (MSS) (2.61 PercentageUnstableSites) | TMB-Low (5.5 muts/Mb)

- Added cabozantinib (broad TKI) with nivolumab with treatment response
- Inherited Testing negative

Conclusions

- Semiconductor Sequencing using Amplicon-Based NGS is useful for small specimens
- Allows for actionable results in a timely manner (our data)
  - 99% signed out ≤ 8 business days from specimen receipt
  - 80% signed out ≤ 8 business days from order
  - Can rapidly screen newly diagnosed cancers as per recommendations
- Prevents additional procedures or liquid biopsy
- Our institution has recently implemented up-front panel testing on all newly diagnosed NSCLC by amplicon-based sequencing

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