How to do a lot with a little in cancer biomarker testing

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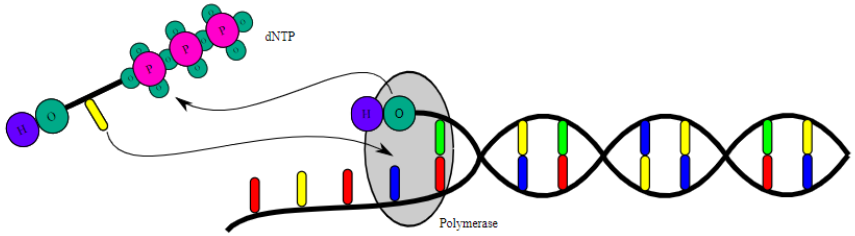
The problem

- Patients with advanced cancer need testing of the most recent tissue
- Biomarker-driven decisions are time-sensitive
- Testing should be done rapidly
- 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

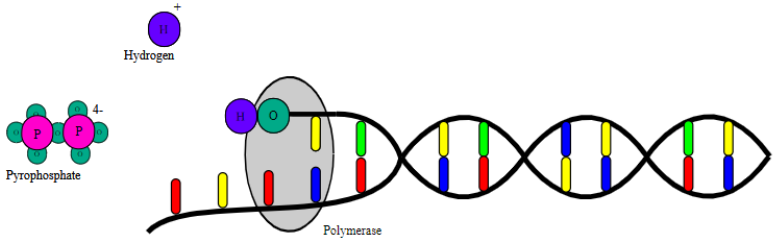
The solution

- 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

Semi-conductor sequencing



Polymerase integrates a nucleotide.



Hydrogen and pyrophosphate are released.

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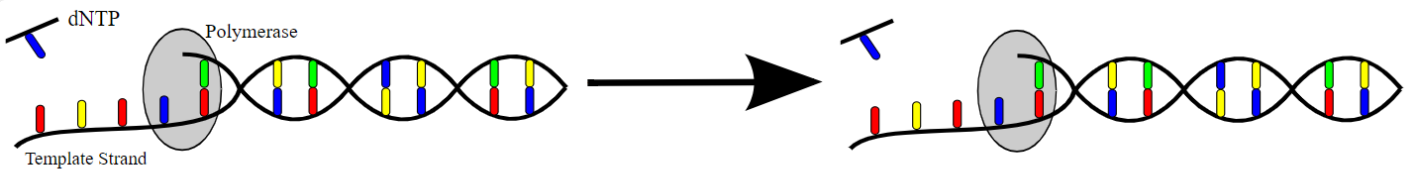
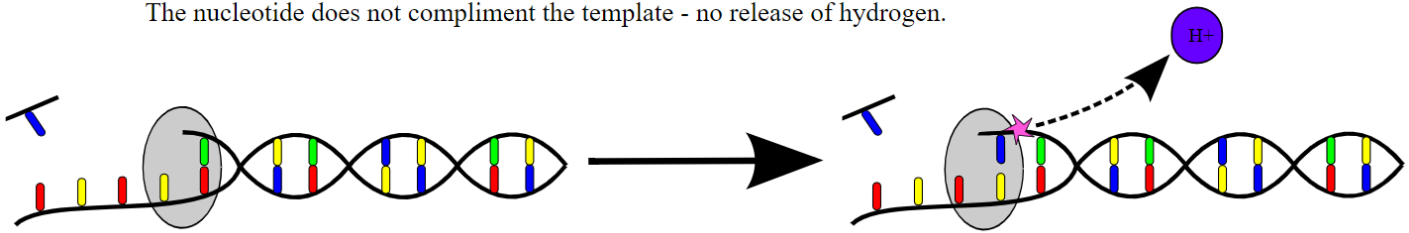
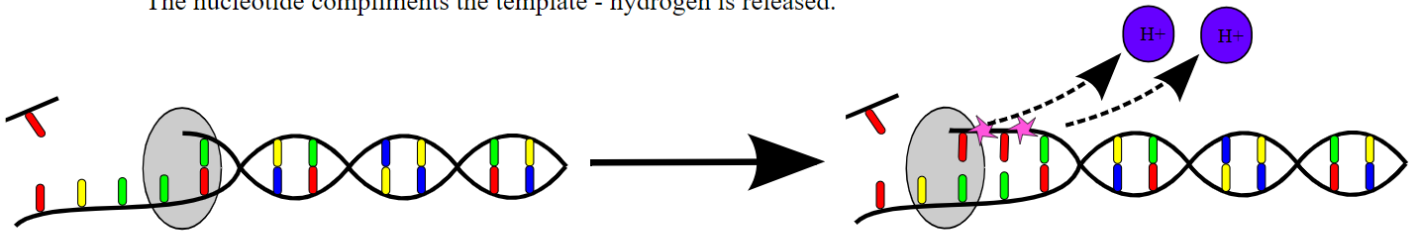


The nucleotide does not compliment the template - no release of hydrogen.



The nucleotide compliments the template - hydrogen is released.



The nucleotide compliments several bases in a row - multiple hydrogen ions are released.

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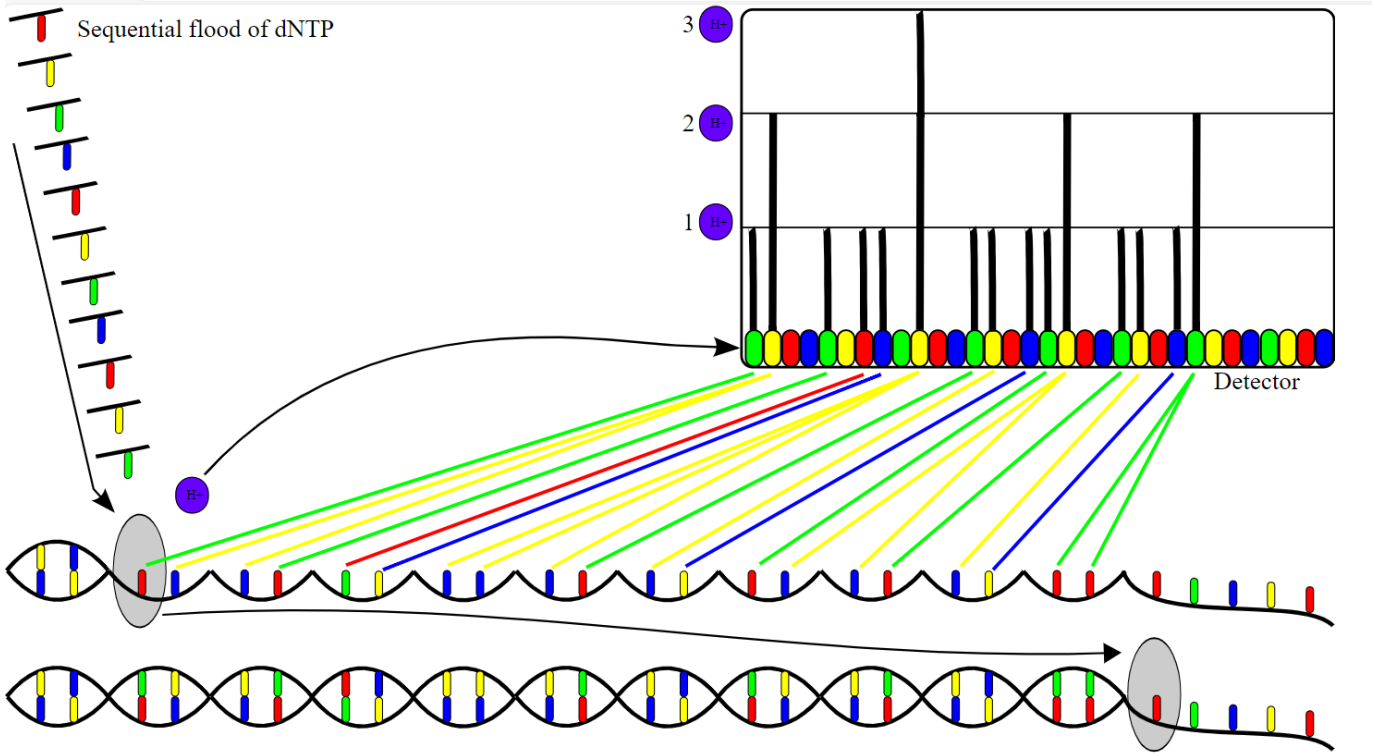

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Semi-Conductor Sequencing

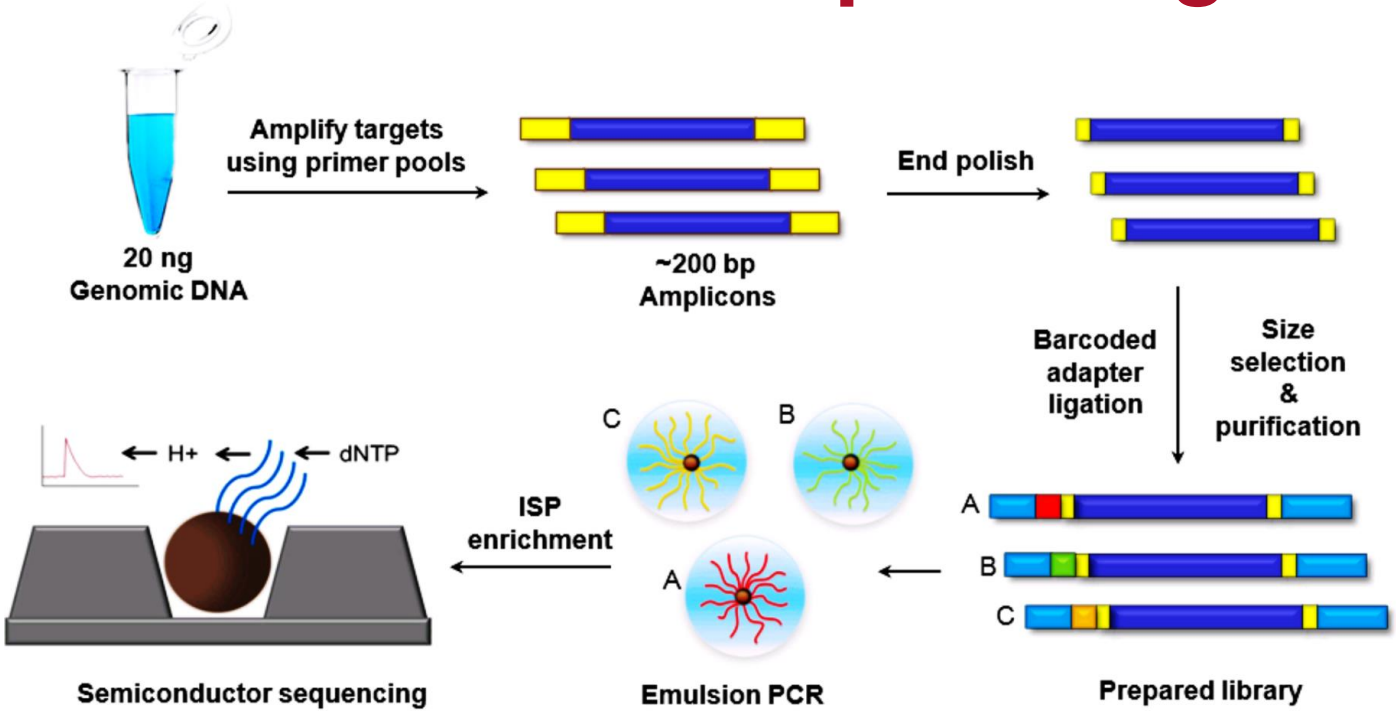


Figure 1. Li, Z., Huang, J., Zhao, J. *et al.* Rapid molecular genetic diagnosis of hypertrophic cardiomyopathy by semiconductor sequencing. *J Transl Med* **12**, 173 (2014). https://doi.org/10.1186/1479-5876-12-173. This article is published under license to BioMed Central Ltd. This is an Open Access article is distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (https://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Assay Discussed Today

Extraction of FFPE by automated platforms

- Silica membrane column extraction
- Magnetic bead technology
- Extraction of both DNA and RNA
- Fluorometry measurement required

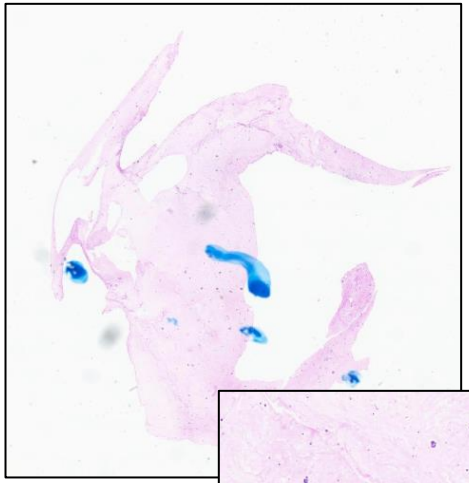
Assay that is used (minimum input of 10 ng DNA and RNA):

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*					

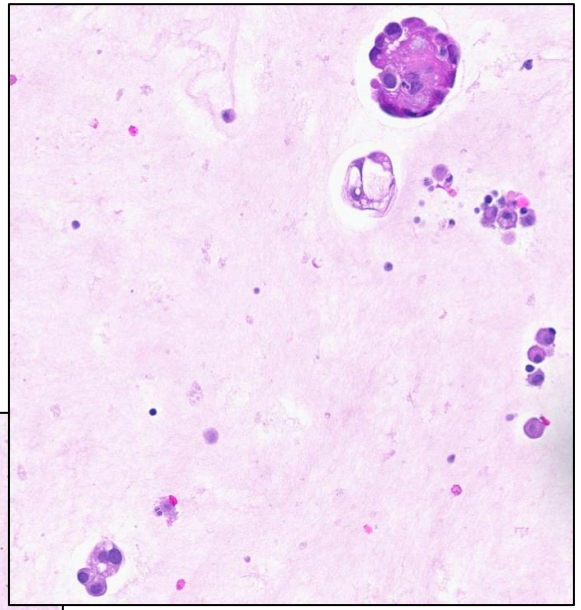
Lung Cancer Cases

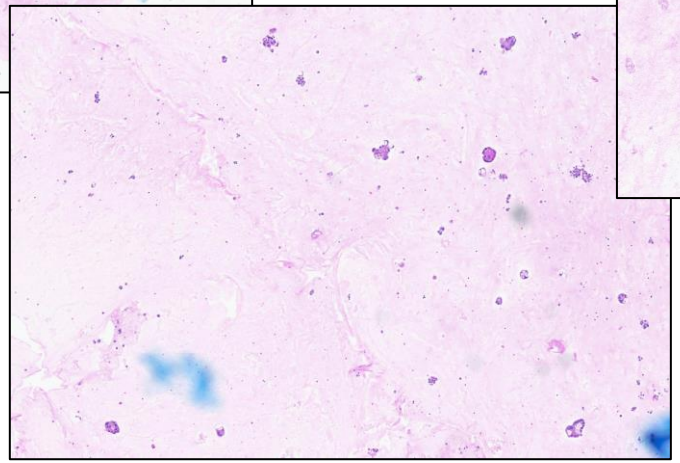
Case 1 Clinical History

- 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

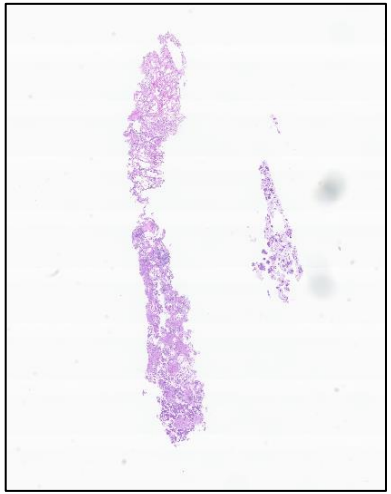
Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Afatinib	Primary sensitivity	EGFR Exon 19 Deletion (E746_A750del)	Non-Small Cell Lung Cancer	Single agent (FDA, NCCN), or may be considered in combination with cetuximab after progression on afatinib, 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

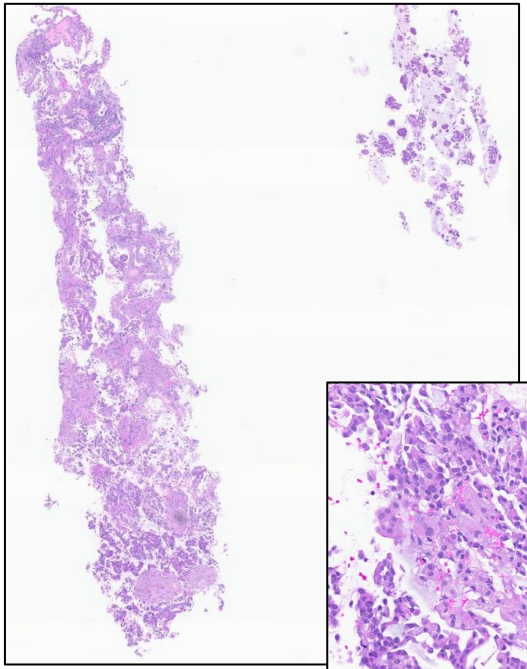
- No surgical intervention
- Continued reduction or stabilization of lesions and effusion
- Effect of TP53 variant[1] ?

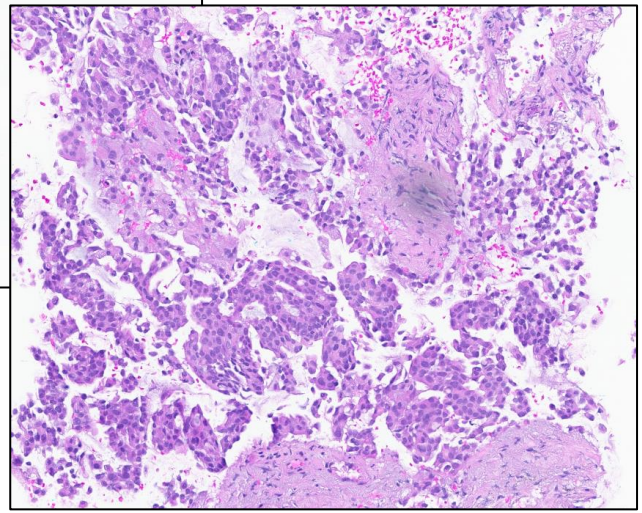
[1]The Role of *TP53* Mutations in *EGFR*-Mutated Non-Small-Cell Lung Cancer: Clinical Significance and Implications for Therapy. Cancers (Basel). 2022 Feb 23;14(5):1143.

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







<u>Right middle lobe lung biopsy</u>
Adenocarcinoma, focal papillary features
TTF-1 +
Napsin +

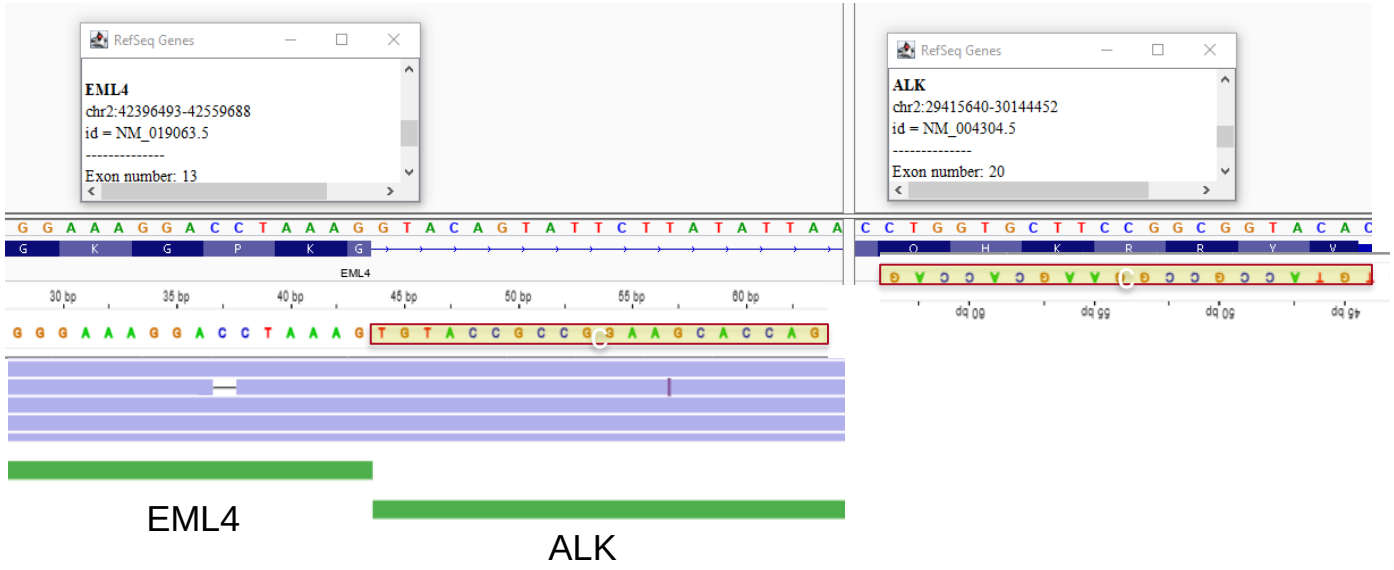
DNA concentration: 0.9 ng/µl
RNA concentration: 2.7 ng/µl

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)



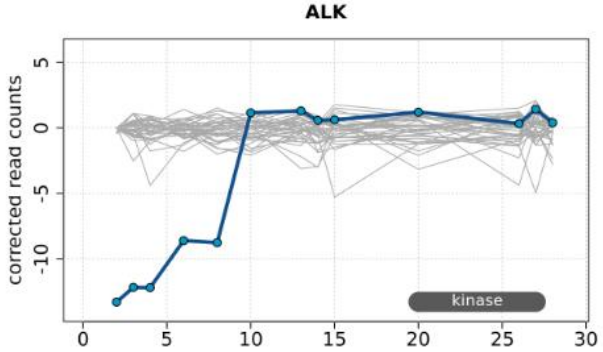
Follow up treatment

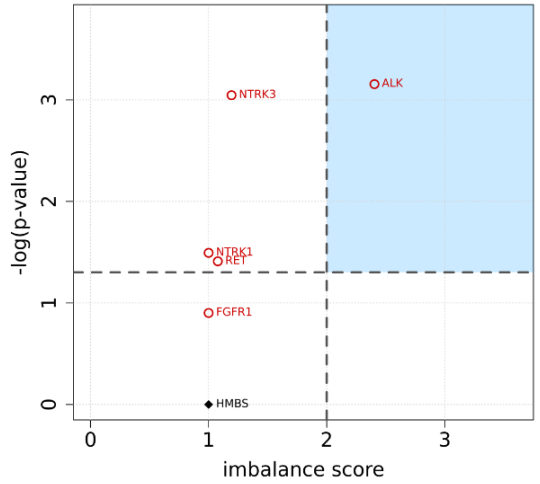
- Referred to an outside oncologist
- Other co-morbidities being addressed
- Lost to follow up

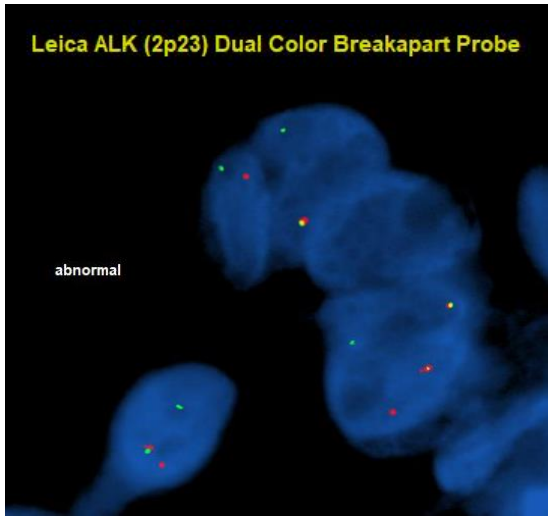
Drugs Associated with Sensitivity for Patient's Tumor Type, Based on Genomic Analysis

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)







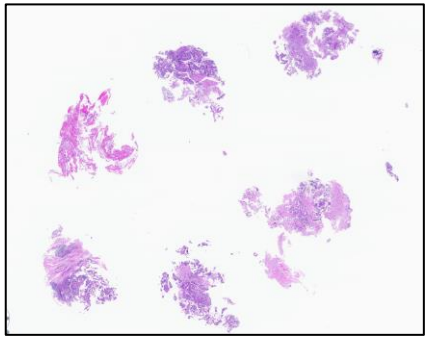
Positive for rearrangement of the *ALK* (2p23) locus (92%)

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

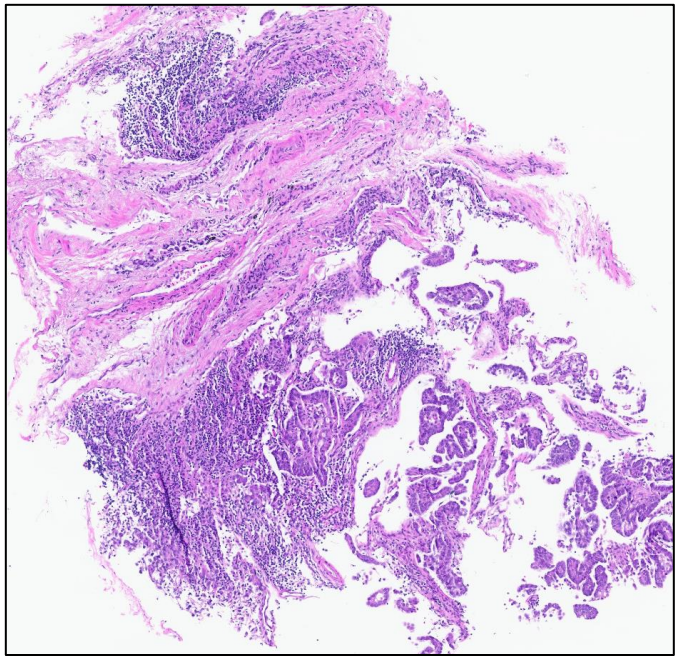
Adenocarcinoma

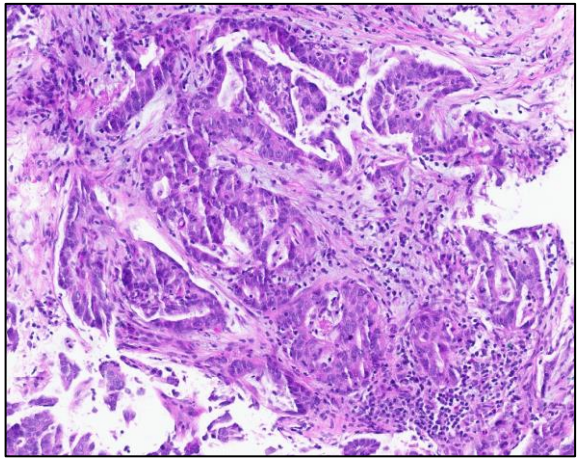
Papillary and micropapillary type

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

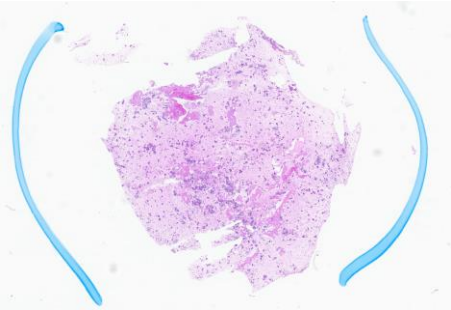
Drugs Associated with Sensitivity for Patient's Tumor Type, Based on Genomic Analysis

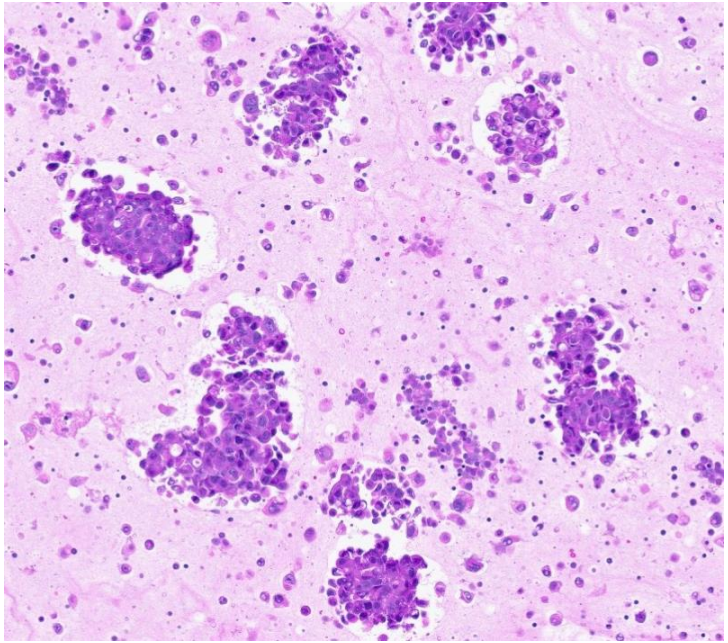
Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Cabozantinib	Primary sensitivity	RET Fusion	Non-Small Cell Lung Cancer	Recommended by NCCN under Category 2A.	Metastatic	NCCN
Pralsetinib	Primary sensitivity	RET Fusion	Non-Small Cell Lung Cancer	Indicated 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

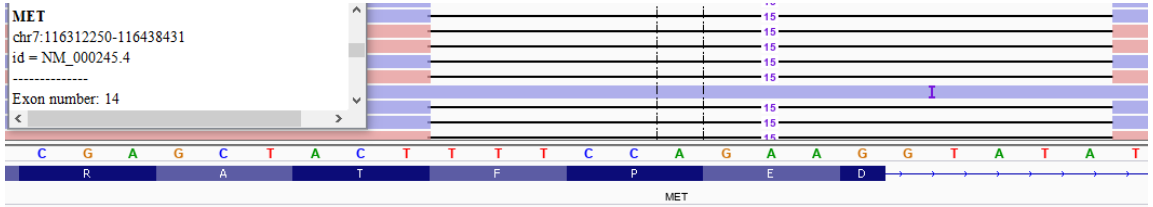




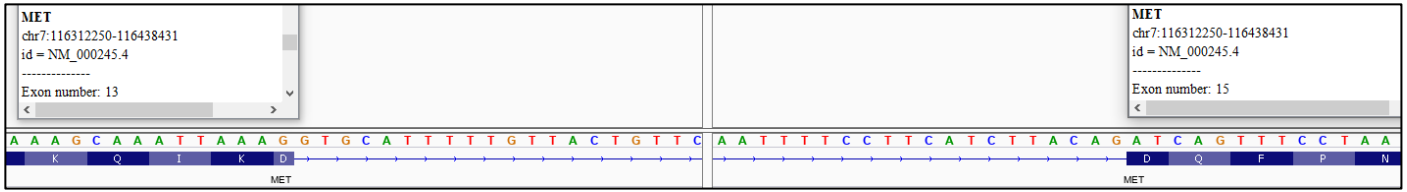
Pleural Fluid
Adenocarcinoma
TTF1 +
Napsin A +
PDL1 – TPS score 90%

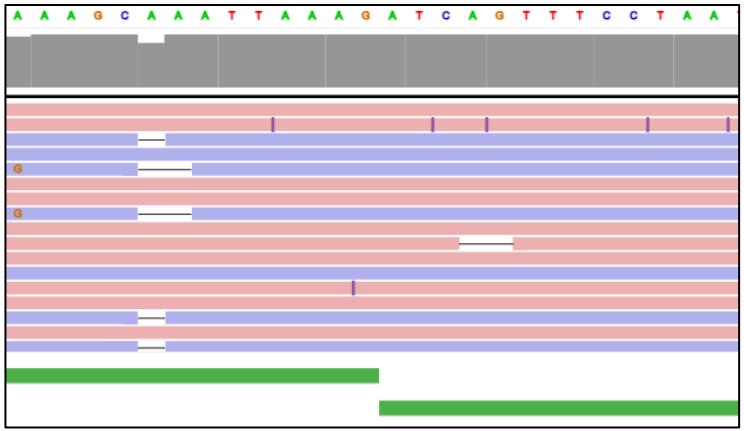
DNA concentration:
16.8 ng/µl
RNA concentration:
21.5 ng/µl

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

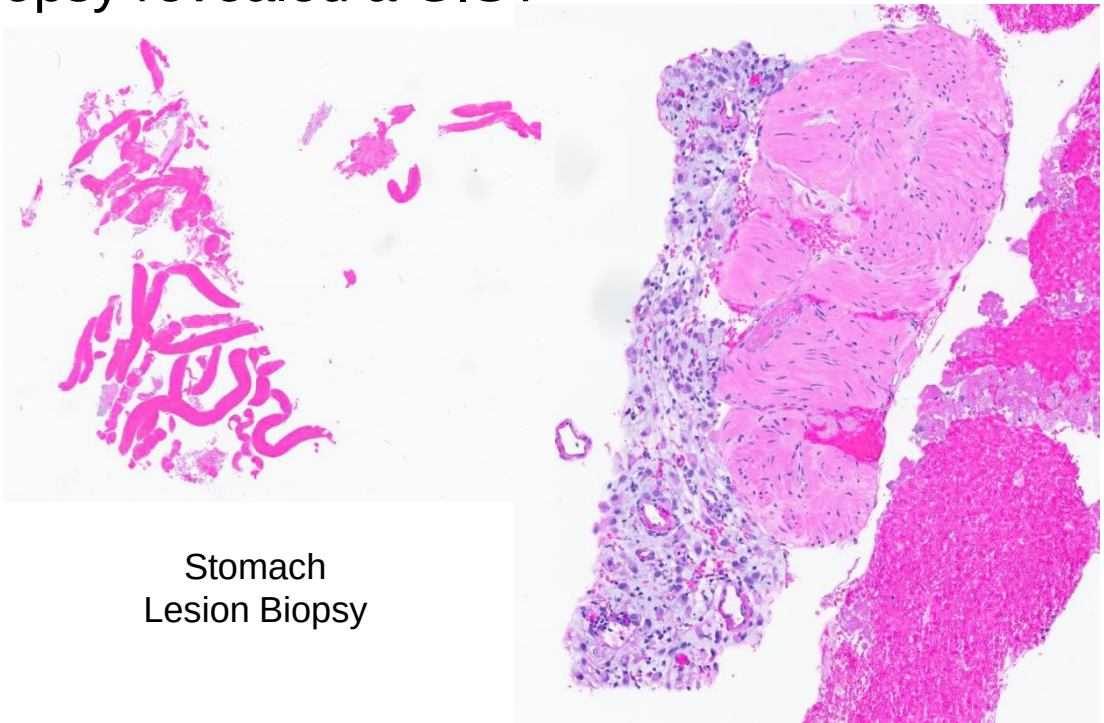
- Started on Capmatinib 400 mg BID (oral)
- Reduction of most lesions within two months
- Tolerating treatment well

Our institution has recently implemented up-front panel testing on all newly diagnosed NSCLC by amplicon based sequencing

Non-Lung Cancer Cases

Gastrointestinal Stromal Tumor (GIST)-Case 1

- 50 year-old female admitted to ER for gastric mass
- Biopsy revealed a GIST



Stomach
Lesion Biopsy

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

Gastrointestinal Stromal Tumor (GIST)-Case 1

			VAF	Coverage
PDGFRA	c.2525A>T;p.D842V	Tier 1A - Pathogenic	7%	5368

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
Avapritinib	Primary sensitivity	PDGFRA Exon 18 Mutation (D842V)	GIST	Indicated for unresectable or metastatic GIST harboring a PDGFRA exon 18 mutation, including D842V.	Metastatic	FDA, NCCN

Drugs Associated with Resistance, Based on Genomic Analysis

Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information	Line of Therapy	Source
Imatinib	Primary resistance	PDGFRA D842V	GIST	Per NCCN, GIST with PDGFRA D842V is unlikely to respond to imatinib.	Metastatic	NCCN

- Started on Avapritinib neoadjuvantly
- Partial response to treatment
- Resected with clear margins– tumor with noted therapy effect

Gastrointestinal Stromal Tumor (GIST)-Case 2

- 73 year-old with abdominal distension and 40 cm mass from diaphragm to pubis – GIST
- Partial response to neoadjuvant treatment with imatinib, followed by surgery followed by adjuvant imatinib switched to sunitinib
- GIST panel ordered

BRAF - No variant was identified in the regions covered by this panel.
KIT - Variant(s) detected. See results below for detected variant(s).
PDGFRA - No variant was identified in the regions covered by this panel.

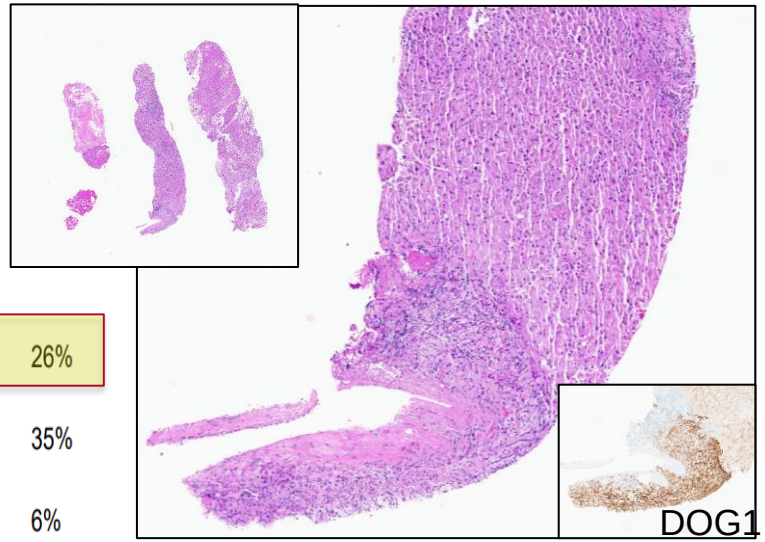
KIT p.W557_V559delinsF (VAF: 93.0%)

- 2 years later – liver lesions
- Switched to regorafenib (due to side effects and progression)

Gastrointestinal Stromal Tumor (GIST)-Patient 2

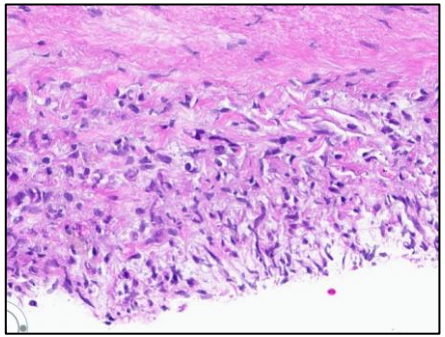
Liver Biopsy

BRAF	c.1799T>A;p.V600E	Tier 1A - Pathogenic	26%
KIT	c.1670_1675delGGAAGG;p.W557_V559delinsF	Tier 1A - Pathogenic	35%
FGFR4	c.1651G>C;p.E551Q	Tier 2C - Likely Pathogenic	6%



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

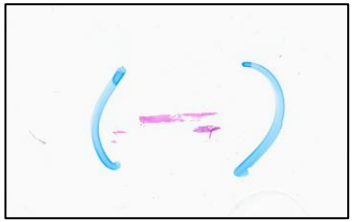
Drug	Response to Drug	Alteration Detected	Condition	Other Relevant Information
Imatinib	Primary sensitivity	KIT Exon 11 Mutation (W557_V559delinsF)	GIST	NCCN recommended as first line therapy for unresectable, progressive, or metastatic GIST with KIT exon 11 mutation. FDA approved regardless of mutation status, for KIT-positive GIST.
Trametinib, Dabrafenib	Primary sensitivity	BRAF V600E	GIST	Indicated for unresectable/metastatic solid tumors (except colorectal cancer) with BRAF V600E, with progression after prior treatment and no satisfactory alternative options.



- Started dabrafenib and trametinib based on these findings
- 1.5 months later developed significant bleeding episode
- Deceased

Case 7

- 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

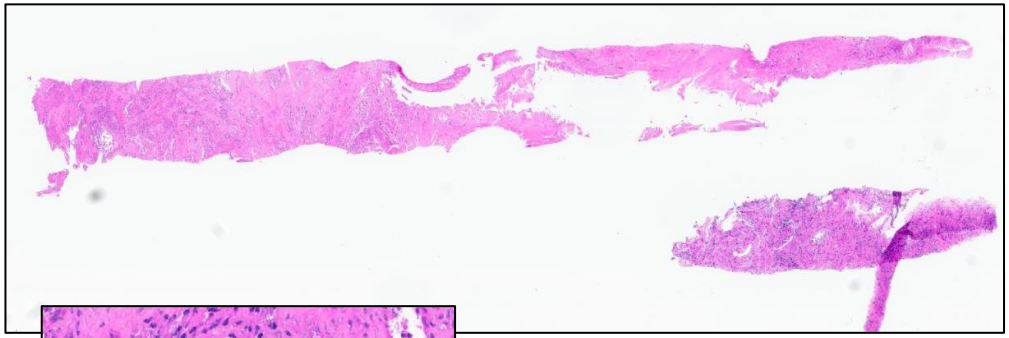


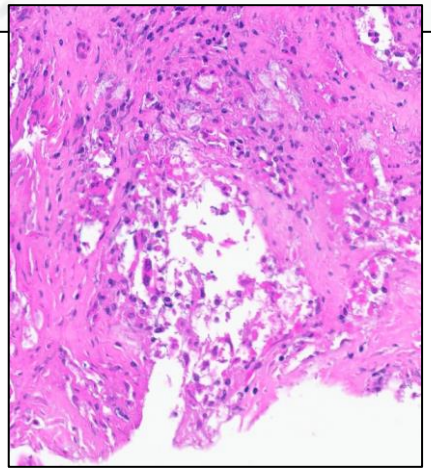
Left Lung Biopsy

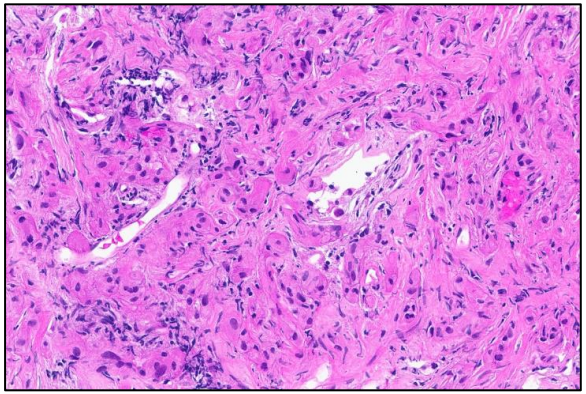
Poorly differentiated neoplasm, c/w melanoma

SOX10 +

S100 +



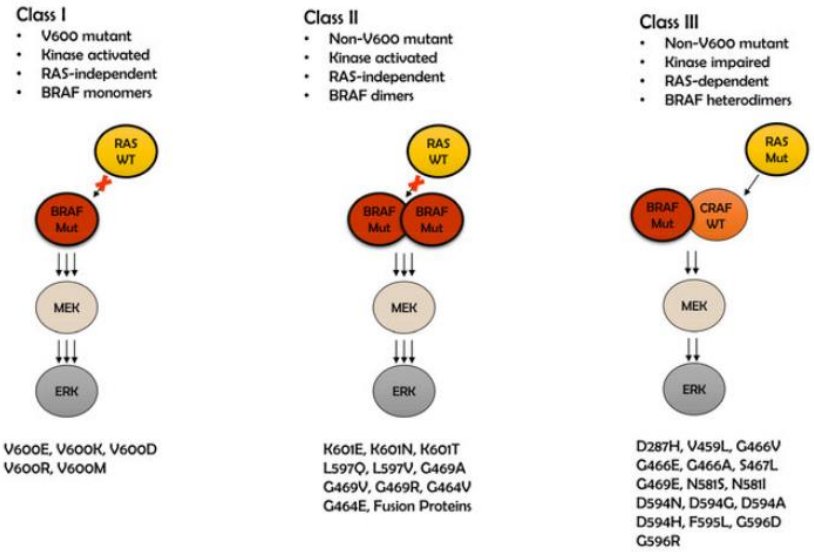




BRAF non-V600 variants

Clinically Significant Alterations

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



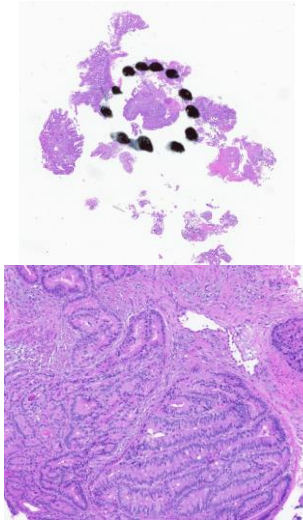
BRAF and MEK inhibition currently not recommended for non-V600E variants in exons 11 or 15 per NCCN 2.2024

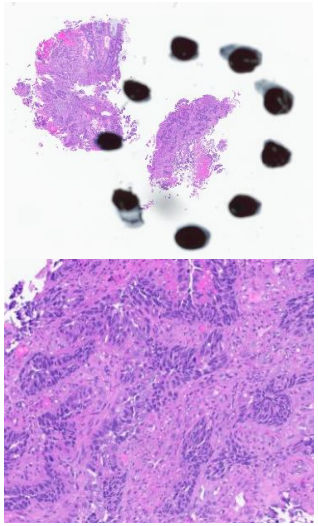
Śmiech M, Leszczyński P, Kono H, Wardell C, Taniguchi H. Emerging *BRAF* Mutations in Cancer Progression and Their Possible Effects on Transcriptional Networks. Genes (Basel). 2020 Nov 12;11(11):1342. doi: 10.3390/genes11111342. PMID: 33198372; PMCID: PMC7697059. Licensed by Creative Commons version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

Final Case -Colorectal Cancer with Synonymous lesions

Are these the same or different tumor?

Both Mismatch Repair Proficient by IHC





	Distal Transverse Colon	Sigmoid Colon
TP53 p.R175H	VAF: 62%	Not Detected
TP53 p.R248W	Not detected	VAF: 47%
MET Copy Number	Increased (CN: 7.68)	Increased (CN: 6.28)
EGFR Copy Number	Increased (CN: 4.96)	Not increased
ERBB2 Amplification	Not Amplified	Amplified (CN: 118.24)

Due to different profiles for these lesions, testing of any advanced/metastatic lesions in the future is recommended for targeted therapy.

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
- Updated technology has reduced manual laboratory steps
  - Allows for time for new test development

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