Annual 2021 Spring KOPANA Seminar

# **Clinical Genomics in Surgical Pathology**

#### March 12, 2021

UT Southwestern Medical Center



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#### Education:

- Thomas Jefferson University (MD/PhD)
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#### **Genomic Medicine**



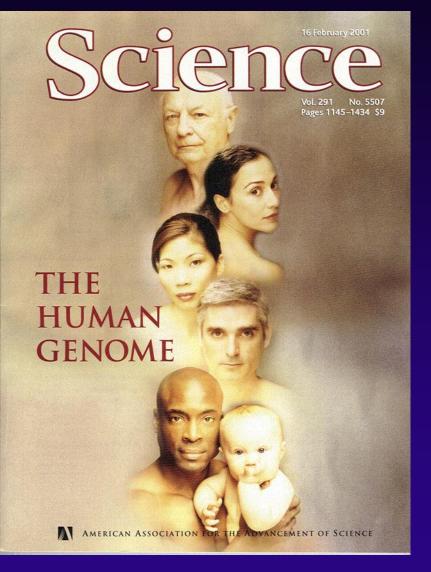
Patient

Specimen

DNA Testing of Many Genes

Diagnosis

Treatment/ Management



# **Genetic Variation**

- Each 'healthy' person (and their tumors!) has thousands of rare genetic variants
- Databases of variant significance
  - OncoKB (https://www.oncokb.org/)
  - My Cancer Genome (<u>http://www.mycancergenome.org/</u>)
  - ClinVar (<u>http://www.ncbi.nlm.nih.gov/clinvar/</u>)
  - Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)
  - The Human Gene Mutation Database (http://www.hgmd.org/)

## **Dashboard Approach**

		BRAF - B-Raf proto	o-oncogene, serine/threonine kin	ase   GRCh37 (Chr 7)	
rview of Transcript NM_001354609.1 -225		<u>c.139</u>	<i>c.241</i>	c.505 c.712 c.981 c.1	1141 c.1315
		47	81		393 478 506 565 V e
)		2	3	5 (7 8 9	
Genome - chr7:140,453,242-140,453,031 (GRCh37)		40453180 140453170 1404531	160 140453150 140453140	140453130 140453120 140453110	140453100 140453090 140453080 1404530
TATCCTTTTACTCTAGATGACAAAAGGAAATGAATGAT	GTGGAGTCTATATAAAGAAGT	CTTCTGGAGTGTCATTTTTATCCA	ACT AAAACCAGAT CGAT GT CACT TT A	AGAGCTACCTCACCCAGGGTAGTCAAACTTG	TCAACAGACCTAGGTAAAACACCTACCATTCTTAA
AT AGGAAAAT GAGAT CT ACT GT TT T C C TT T A C T T A C T A C T A	CACCTCAGATATATTTCTTCAT	GAAGACCTCACAGTAAAAATAGGT	I GATTTTGGTCTAGCTACAGTGAAAT	TCTCGATGGAGTGGGTCCCATCAGTTTGAAC	AGTTGTCTGGATCCATTTTGTGGATGGTAAGAATT
Nucleotide Conservation					
			.11 11:11 11.11.111111111	11.11.11111.11.1111111111111.1	11111111.11.11.1.1.1111111111.1
	-				
NM_001354609.1: Homo sapiens B-Raf proto-oncoge	ene, serine/threonine kinase (BR	AF), transcript variant 2, mRNA.			
Delins t					
Dup         c.1742-40         c.1742-30         c.1742-20         c.1           ATAGGAAAATGAGATCTACTGTTTTCCTTTACTACTA         C<	1742-10 c.1742 c.1750	c.1760 c.1770	c.1780 c.1790 c.1800	c.1810 c.1820 c.1830	
	N I F L H	E D L T V K I G	D F G L A T V K	SRWSGSHQFE	Q L S G S I L W M
	581 585	590	595 600	605 610	615 620
dbSNP Short Variations   SwissProt Variants ATAGGAAAATGAGATCTACTGTTTTCCTTTACTTACTA		GAAGACCTCACAGTAAAAATAGGT	GATTITEGTCTAGCTACAGTGAAAI	TCTCCATCCACTCCCATCACTTCAAC	AGTIGICIGGATCCATITIGIGGATGGTAAGAATT
ATAGGAAAATGAGATCTACTOTTTCCTTTACTTACTA	CACCICAGATATATITCTICAT	UAAUACCI CACAUI AAAAAI AUUI			
CGGAACACCT C	CT CACG				
CEEAACCTCC CEEC	CT CACG G	A <mark>GCAC GAG</mark> G <u>G</u> C	ACACCAAA GA GGA CCC CGG GCT TG T C GGT		
CGGAACCT CGGCGC CGGC	CT CACG		ACACCAAA GA GGA CCC		
CEEAACCTC CEECC CEECC C	CT CA C G G N I F L H 581 585		ACACCAAA GA GGA CCC		
CG       G       A       C       AC       C       T       C         C       G       G       C       C       T       G         Genome Aggregation Database (gnomAD)	G N I F L H	AGCACGGC GGCC	ACACCAAA GA GGA CCC CGG GCT TG T C GGT T T T T D F G L A T V K	CTAAACAG T <u>GA</u> C S R W S G S H Q F E	A C A G G C Q L S G S I L W M
C G C G Genome Aggregation Database (gnomAD)	G N I F L H	AGCACGGC GGCC	ACACCAAA GA GGA CCC CGG GCT TG T C GGT T T T T D F G L A T V K 595 600	CTA     A     CA     G     T     GA       C     C     C     C     C     C       S     R     W     S     G     S     H     Q     F     E       605     610	A C A G G C Q L S G S I L W M
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C G C G Genome Aggregation Database (gnomAD)	G N I F L H S81 585 C ACACCTCAGATATATTTCTTCAT N I F L H	A       G       CA       C       GAG         G       G       G       C       C         E       D       L       T       V       K       I       G         590       G <t< td=""><td>ACACCAAA GA GGA CCC CGG GCT TG T C GGT T T T T T D F G L A T V K 595 600 T GATTTTGGTCTAGCTACAGTGAAAA D F G L A T V K</td><td>CTA       A       A       CA       G       T       GA         S       R       W       S       G       S       H       Q       F       E         605       610         A       +       G       T       GA         TCTCGAT GGAGT GGGT CCCAT CAGTTT GAAC.       S       R       W       S       G       S       H       Q       F       E</td><td>Q     L     S     G     S     I     L     W     M       615     615     620       Q     L     S     G     A     C       A     C     A     C     C     C       C     A     C     C     C       C     G     S     I     L     W     M       C     A     C     C       C     A     C     C       C     A     C       C     A     C       C     A     C       C     A     C</td></t<>	ACACCAAA GA GGA CCC CGG GCT TG T C GGT T T T T T D F G L A T V K 595 600 T GATTTTGGTCTAGCTACAGTGAAAA D F G L A T V K	CTA       A       A       CA       G       T       GA         S       R       W       S       G       S       H       Q       F       E         605       610         A       +       G       T       GA         TCTCGAT GGAGT GGGT CCCAT CAGTTT GAAC.       S       R       W       S       G       S       H       Q       F       E	Q     L     S     G     S     I     L     W     M       615     615     620       Q     L     S     G     A     C       A     C     A     C     C     C       C     A     C     C     C       C     G     S     I     L     W     M       C     A     C     C       C     A     C     C       C     A     C       C     A     C       C     A     C       C     A     C
G       G       C       G         Genome Aggregation Database (gnomAD)         Delins         t       G       G       + G       + C       T       +         Dup         ATAGGAAAATGAGATCTACTGTTTTCCTTTACTTACTA	G N I F L H 581 585 C	A       G       CA       C       GAG         G       G       G       C       C         E       D       L       T       V       K       I       G         590       G <t< td=""><td>ACACCAAA GA GGA CCC CGG GCT TG T C GGT T T T T D F G L A T V K 595 500 T G A G T</td><td>CTAAACAG TGA</td><td>Q     L     S     G     G     G     C       Q     L     S     G     S     I     L     W     M       615     615     620</td></t<>	ACACCAAA GA GGA CCC CGG GCT TG T C GGT T T T T D F G L A T V K 595 500 T G A G T	CTAAACAG TGA	Q     L     S     G     G     G     C       Q     L     S     G     S     I     L     W     M       615     615     620
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Genome Aggregation Database (gnomAD) Delins t G G + G + C T + Dup ATAGGAAAATGAGATCTACTGTTTTCCTTTACTTACTA Exome Sequencing Project (ESP) Variants Delins t G Dup ATAGGAAAATGAGATCTACTGTTTTCCTTTACTTACTA ClinVar	G N I F L H S81 585 C C C C C C C C C C C C C	A       G       CA       C       GAG         G       G       G       C       C         E       D       L       T       V       K       I       G         G       G       G       G       G       S90       S90         G       G       G       S90       K       I       G         G       D       L       T       V       K       I       G         S90       S90       S90       S90       S90       S90       S90	ACACCAAA       GA       GGA       CCC         CGG       GCT       TG       T       C       GGI         T       T       T       T       T       T       T         D       F       G       L       A       T       V       K         595       600       595       600       595       600         T       G       A       T       V       K         595       600       600       600       600         T       G       L       A       T       V       K         595       600       600       600       600       600         T       G       L       A       T       V       K         595       600       600       600       600       600         T       G       L       A       T       V       K         595       600       600       600       600       600         T       G       L       A       T       V       K         GATTTTGGTCTAGCTACAGTACAGTGAAAA       G       L       A       T       V         D       F	CTA       A       CA       G       I       GA         S       R       W       S       G       S       H       Q       F       E         S       R       W       S       G       S       H       Q       F       E         A       +       G       T       GA         CCCGAT GGAGT GGGGT CCCAT CAGTTT GAAC       S       R       W       S       G       S       H       Q       F       E         S       R       W       S       G       S       H       Q       F       E         605       605       610       610       610       610       610	Q     L     S     G     S     I     L     W     M       615     615     620       Q     L     S     G     S     I     L     W     M       615     620     620     620     620     620       Q     L     S     G     S     I     L     W     M       C     A     C     C     C     C     C       C     G     S     I     L     W     M       G15     G20     620     620       C     G     S     I     L     W     M       C     G     S     I     L     W     M       C     G     S     I     L     W     M
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Genome Aggregation Database (gnomAD) Delins st G G + G + C T + Dup ATAGGAAAAT GAGAT CT ACT GTTTT CCTTT ACTT AC	G N I F L H 581 585 C C C C C C C C C C C C C	A       G       CA       C       GAG         C       G       G       C       C         E       D       L       T       V       K       I       G         G       G       G       G       G       G       G       G         G       G       G       G       G       G       G       G         G       G       G       S90       K       I       G         G       D       L       T       V       K       I       G         GAAGACCTCACAGTAAAAAATAGGT       590       S90       S90       G       G       G         GAAGACCTCACAGTAAAAAATAGGT       S90       G       S90       G       G       S90       G         M       C       C       G	ACACCAAA       GA       GGA       CCC         CGG       GCT       TG       T       C       GGI         T       T       T       T       T       T         D       F       G       L       A       T       V       K         S95       595       600       595       600       595       600         T       G       A       T       V       K       595       600         T       G       L       A       T       V       K       595       600         T       G       L       A       T       V       K       595       600         T       GATTTTGGTCTAGCTACAGTGAAAT       D       F       G       L       A       T       V       K         595       600       595       600       600       600       600       600         T       F       G       L       A       T       V       K       600         F       G       L       A       T       V       K       600       600         H       H       H       H       H       H       H	CTA       A       A       CA       G       I       GA         S       R       W       S       G       S       H       Q       F       E         A       H       G       T       GA         A       H       G       T       GA         TCTCGAT GGAGT GGGT CCCAT CAGTTT GAAC       S       R       W       S       G       S       H       Q       F       E         A       H       G       S       R       Q       F       E       605       610         A       H       G       S       H       Q       F       E       605       610         A       S       G       S       H       Q       F       E       605       610         A       S       G       S       H       Q       F       E       605       610         A       S       G       S       H       Q       F       E       605       610         C       C.1810       C.1820       C.1830       C.1830       C.1830       C.1830       C.1830	Q     L     S     G     S     I     L     W     M       Q     L     S     G     S     I     L     W     M       615     615     620         Q     L     S     G     S     I     L     W     M       615     615     620         C     A     C       C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     A     C         C     B     I     L     W     M         C     G     S     I     L     W     M       G15     G20     C     C         C     I     L     W     M         C     I     L     W     M

Alamut, Interactive Biosoftware

#### **Pathologists as Genomic Physicians**

#### 3. Pathologist



1. Patient

2. Provider





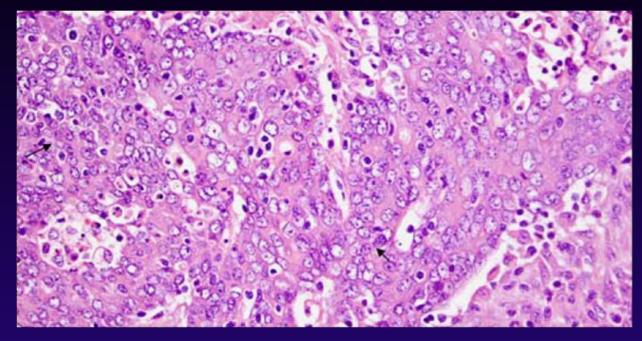
**Determine if variant is clinically significant** Pathogenic / Likely Pathogenic vs Uncertain

#### Change in clinical management Screening Surgery Treatment

#### Manage changes in variant significance Uncertain upgrade to Pathogenic Pathogenic downgrade to Benign New treatment modalities for existing variant

# **Genomic Case Studies**

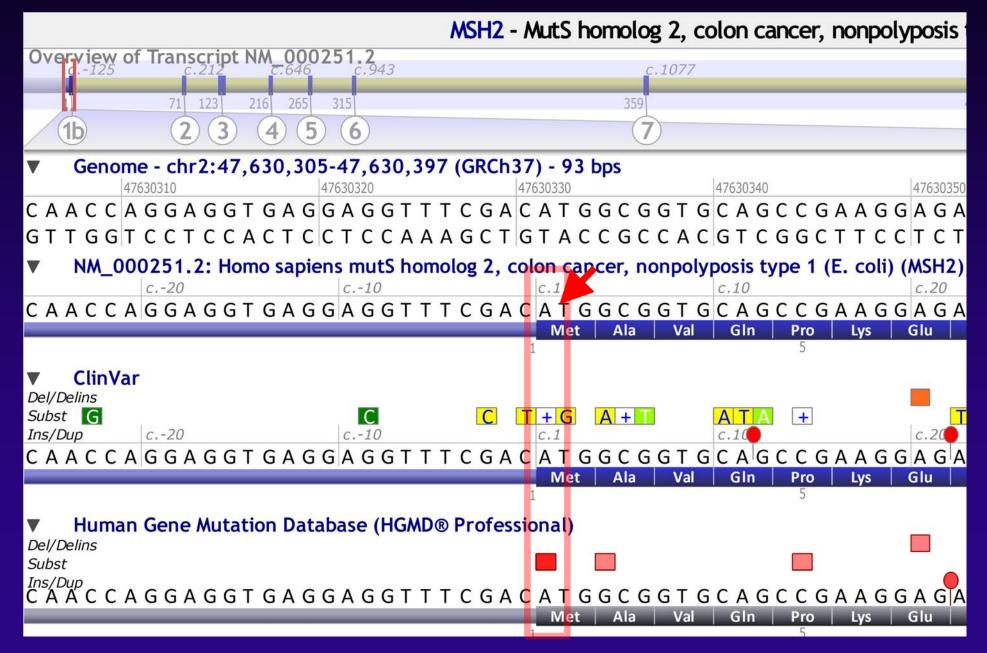
#### Case Study 1:



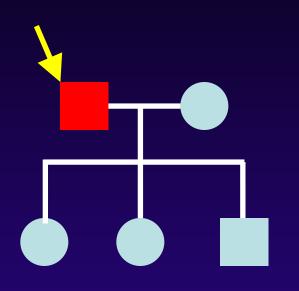
- 60-year-old man with colon adenocarcinoma identified as mismatch repair deficient (MSH2/MSH6 loss by immunohistochemistry)
- MSH2 gene sequencing identifies: c.1A>C (p.Met1\_Gly25del)

Truta et al 2008 Familial Cancer 7:267-74 Rosenthal et al 2015 Clin Genet 88:533-541

## MSH2 c.1A>C (p.Met1\_Gly25del) mutS homolog 2



#### Lynch Syndrome Familial Testing



- Patient's *MSH2* c.1A>C reported as likely pathogenic
- *MSH2* is one of the DNA mismatch repair genes (*MLH1*, *MSH6*, and *PMS2*) associated with Lynch Syndrome
- Lynch syndrome is an autosomal dominant cancer syndrome associated with early colorectal, endometrial and ovarian cancers

Question: If all children are tested and two are positive, which of the following are important next steps? A.Follow-up screening for both gene test positive and negative patients B.Follow-up screening only for gene test positive patients C.Follow-up screening only for gene test positive patients but also keep up-to-date records of gene test negative patients

## Two Years Later...Revised Classification: c.1A>C is no longer considered "likely pathogenic"

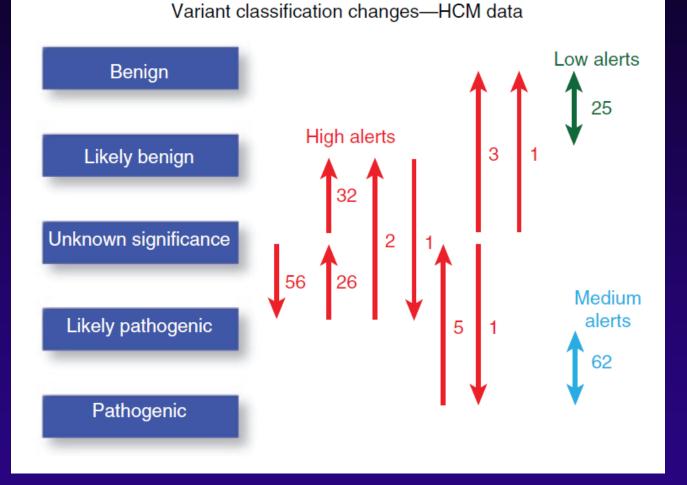
- Clinical genetics laboratory noted new evidence
  - Multiple cases of same variant seen
  - -Alternative start codon is proven
  - Low penetrance with an inheritance pattern inconsistent with pathogenicity
- New interpretation: <u>variant of uncertain significance</u>

Question: If all children are tested and two are positive, which of the following are important next steps?

> Revised Answer: Counsel both gene test positive & negative

#### **Genomic Test Results are Dynamic**

- •New discoveries require revised/ corrected reports
- •Laboratory needs to provide dynamic reporting
- •Example:
  - Harvard Partners noted
     214 changes over 7 years
  - >100 corrected reports



JAMA | Original Investigation

# Prevalence of Variant Reclassification Following Hereditary Cancer Genetic Testing

JAMA. 2018;320(12):1266-1274. doi:10.1001/jama.2018.13152

- •UT Southwestern study of 1.45 mil patients tested at Myriad Genetics (2006-2016)
- •Hereditary cancer genetic testing (56.6% with personal history)
- •59,955 amended reports because of variant reclassification
- •Overall, 6.4% of variants were reclassified
- 24.9% of Uncertain (VUS) variants were changed

#### **Emerging Diagnostic Split Decisions**

Clinical labs interpret variants differently
Example: Hereditary Cancer-Predisposing Syndrome Genetics

**Other Labs** 

#### **Diagnoses from one laboratory**

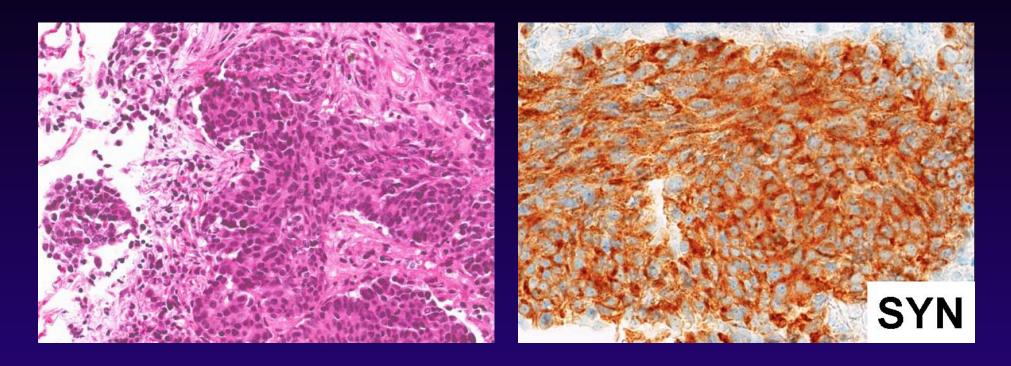
	pathogenic	likely pathogenic	uncertain significance	likely benign	benign
pathogenic	0	595	93	3	0
likely pathogenic	266	0	246	1	1
uncertain significance	39	97	0	1019	227
likely benign	3	1	2149	2	1883
benign	0	1	411	1012	0

https://clinvarminer.genetics.utah.edu/

#### **Case 1 Summary**

- Genetic Variation *≠* Pathogenicity
  - Variants may have no clinical significance
  - Significance of variants may change over time
- Who is responsible for re-interpreting previous genetic tests?
- Is re-interpretation better managed by the patient?

#### Case Study 2:



- 52-year-old male presents with acute and chronic back pain
- MRI (and subsequent CT) reveals multiple lesions in chest (1.2 cm) and brain (4.3 cm)
- CT-guided FNA of chest lesion reveals neuroendocrine carcinoma

- No response to systemic chemotherapy
- Too many brain metastases for radiotherapy (gamma knife)
- Minimal neurologic impairment for total brain irradiation
- Insufficient FNA tissue for molecular testing (targeted therapy)

Question: What are the next options for molecular testing?

A. Biopsy of brain lesion

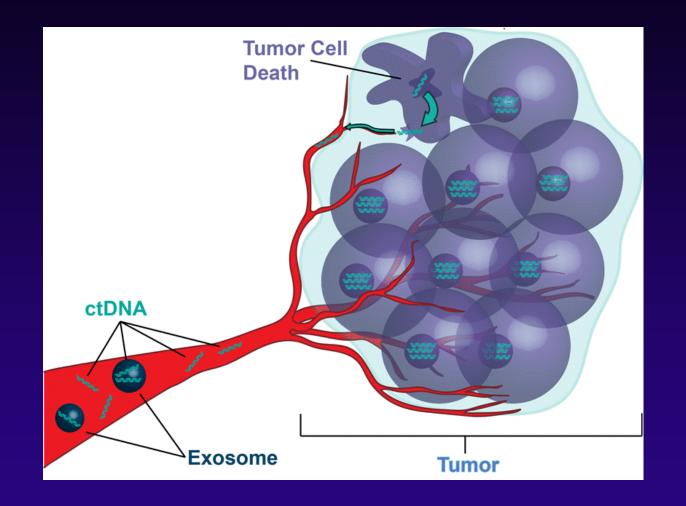
- B. Biopsy of lung lesion
- C. Liquid biopsy

D. None, watch and wait

**Question: What is the next option for** molecular testing? A. Biopsy of brain lesion B. Biopsy of lung lesion C. Liquid biopsy D. None, watch and wait

Answer in this case: C. Liquid biopsy

### **Elevated Cell Free DNA (cfDNA) in Many Settings**



• Pregnancy (NIPT)

Cancer

- Traumatized tissue
- Inflamed tissue
- Transplantation

- ctDNA circulating tumor DNA either in <u>intact cells (CTC)</u> or <u>cell free</u> (cfDNA)
- Exosomes cell fragments which contain both DNA and cellular protein

## cfDNA Compared to Tissue Biopsy

	cfDNA	Tissue
Risk to patient	Very low	Biopsy risk
Serial measurement	Easy	Limited by risk
Representative	Better Survey	Limited by sampling
Cost	Cheap	Very expensive
Diagnostic Value	Emerging	Gold Standard

#### **False Positives in Cell-Free DNA**

False positive rate of <0.5% with PPV of ~40% (Noninvasive prenatal testing)

- Germline variations (CNV/SNV)
- Mosaicism (germline, placental)
- Clonal Hematopoiesis
- Chimerism
- Analytical and bioinformatic issues

# Back to the Case Study

Cell-Free DNA (cfDNA) Liquid Biopsy

- Patient's peripheral blood sent for cfDNA genomic assay
- 62 genes: SNVs & fusions
- Detected:

#### –Fusion <u>SMC5:ALK</u>

- Not previously reported
- ELM4:ALK in 3-7% of NSCLC
  - -CR 29%
  - -PR 24%
  - -Stable 38%

ALK Inhibitors for NSCLC (FDA approved) Crizotinib

Alectinib

Ceritnib

# Question: How should the ALK fusion by interpreted?

- A. Variant of Strong Clinical SignificanceB. Variant of Potential Clinical
  - Significance
- C. Variant of Uncertain Clinical Significance
- D. Benign or Likely Benign Variant

#### Answer:

- SMC5:ALK fusion has not been previously reported
  - Because it has not been reported no *in vitro* or *in vivo* therapeutic evidence
- Theoretically it could behave like ELM4:ALK
- Are fusions from cfDNA always the same as from tissue?
- Best to report as <u>Variant of Uncertain Clinical</u> <u>Significance</u>

# Question: Should off-label therapy be recommended?

#### PROS

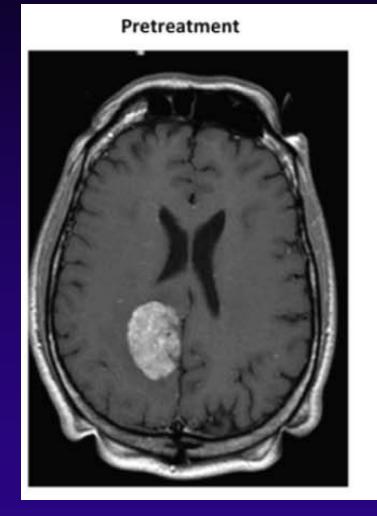
- No other therapy options
- ALK inhibitors in NSCLC show response

#### CONS

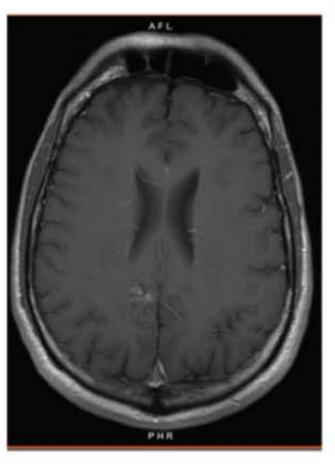
- Specific ALK fusion not previously identified
- Limited data of ALK inhibitors in neuroendocrine carcinomas
- ALK inhibitors have high rates of gastrointestinal toxicity

## **Partial Response to ALK Inhibitor**

- Patient started on Alectinib (ALK inhibitor)
- Partial response
  - -60% decrease in main brain mass
  - –Decrease in other lesions
- Stable 5 months after therapy



Posttreatment 3 months

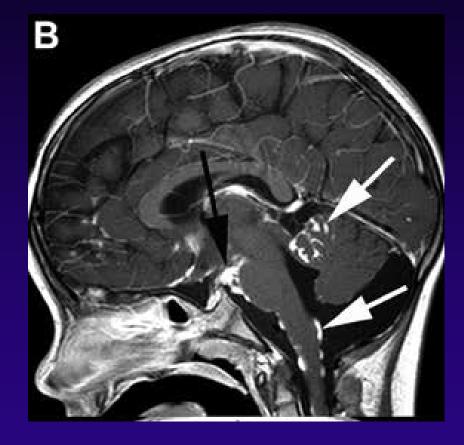


#### **Case 2 Summary**

- New technologies in oncologic surgical pathology are rapidly emerging and outpace guidelines
   – Targeted mutation, NGS, cfDNA
- Uncertain <u>when</u> responses in case reports will be more broadly applicable

## Case Study 3

- 1 year old female presented with nystagmus
- MRI showed a large optichypothalamic tumor
- Tumor consistent with a pilocytic astrocytoma
- Underwent surgical debulking received multiple chemotherapy regimens



#### **Tumor Next-Generation Sequencing**

- Tumor burden increased despite chemotherapy
- Residual tumor (FFPE) submitted for sequencing on a 25 gene panel
- POSITIVE for a pathogenic variant in BRAF:

NM\_001354609.1(BRAF): c.1799\_1800delinsAT, p.(Val600Asp)

## BRAF c.1799\_1800delinsAT, p.(Val600Asp)





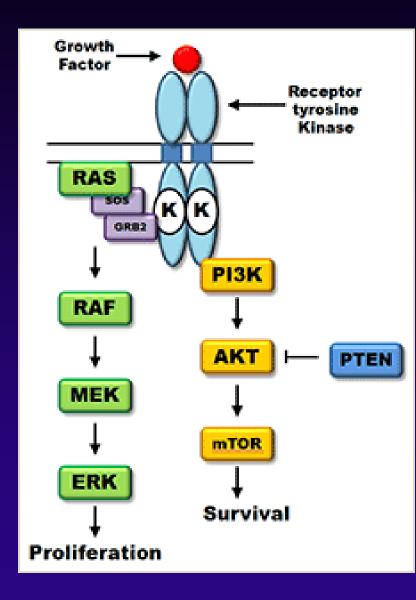
OncoK	( <b>B</b> -	evels of Evidence	Actionable Genes	Cancer Genes	API Access	About T	<b>Team</b>	News	Terms	FAQ	
	Oncoge		unction  • Level			ncers amon	a others				
	BRAF, an intracellular kinase, is frequently mutated in melanoma, thyroid and lung cancers among others. The BRAF V600D mutation is known to be oncogenic. Select a cancer type										
	Therap	eutic Diagnostic									
	Level 🔷	Alterations		Level-associa	ited cancer types	S 🚯		Drugs			Citations
	0	V600		Erdheim-Che	ster Disease		,	Vemurafe	enib		2
	0	V600		Melanoma			,	Vemurafe	enib + Ate	zolizumab + Cobim	etinib 1
	2	V600		Melanoma				Encorafe	nib + Bini	metinib	1
	2	V600		Melanoma				Dabrafen	ib + Tram	etinib	10
	2	V600		Melanoma			,	Vemurafe	enib + Col	bimetinib	2
	2	V600		Anaplastic Th	yroid Cancer			Dabrafen	ib + Tram	etinib	1
	34	Oncogenic Mutatio	ns	Histiocytosis				Cobimeti	nib		3

#### **ClinVar**

# https://www.ncbi.nlm.nih.gov/clinvar/

NM_004333.6	Cite this record				
Interpretation:	Pathogenic			0	
Review status: Submissions: Last evaluated: Accession: Variation ID: Description:	☆ ☆ ☆ ☆ no assert 1 (Most recent: Jul 1 Jul 14, 2015 VCV000375939.2 375939 2bp indel	ion criteria provided 8, 2016)			
Variant details	NM 004333 6(BRAE):c 17	99_1800delinsAT (p.Val600Asp)			0
Conditions	Allele ID:	362818			
	Variant type:	Indel			
Gene(s)	Variant length:	2 bp			
	Cytogenetic location:	7q34			
	Genomic location:	7: 140753335-140753336 (GRCh38) GRCh38 U	CSC		
		7: 140453135-140453136 (GRCh37) GRCh37 U	CSC		
	HGVS:	Nucleotide	Protein	Molecular consequence	
		NC_000007.13:g.140453135_140453136delinsAT			
		NC_000007.14:g.140753335_140753336delinsAT		.	
		NM_001354609.2:c.1799_1800delinsAT more HGVS	NP_001341538.1:p.Val600Asp	missense	
	Protein change:	V600D			
		10000			

# **BRAF Inhibition**

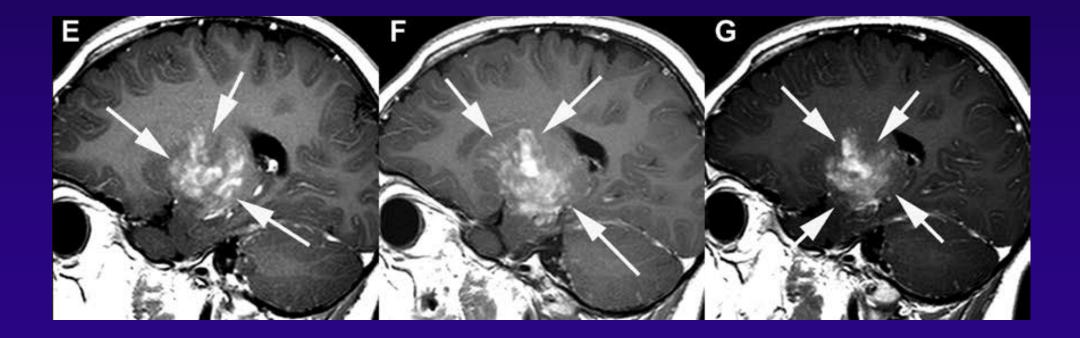


- Mitogen activated protein kinase (MAPK) pathway key to initiation and maintenance of many tumors
- BRAF is one of the 3 RAF kinases
- Targeted therapies exist for BRAF and MEK

https://www.mycancergenome.org/content/disease/melanoma/braf/54/

# Targeted Therapy

- Patient started on dabrafenib; stable at 3 months (E)
- Progression at 6 months post-dabrafenib (F)
- Combination dabrafenib (BRAF) + trametinib (MEK) initiated
- 4 years post combination, disease is stable (G)



Drobysheva et al 2017 J Natl Comp Canc Netw

#### **Case 3 Summary**

- Genomic testing can identify clinically significant variants associated with therapy
- Off-label use of targeted therapy can result in long term (>4 year) sustained control (?cure) of malignancies

# Conclusions

Genomic testing and interpretation are critical for the diagnosis and management of oncologic diseases
Genomic test results are dynamic over time
Genomic technologies continues to advance
Pathologists are genomic physicians

