

2019 KOPANA 18th Spring Seminar

Genomic Pathology for Surgical Pathologists

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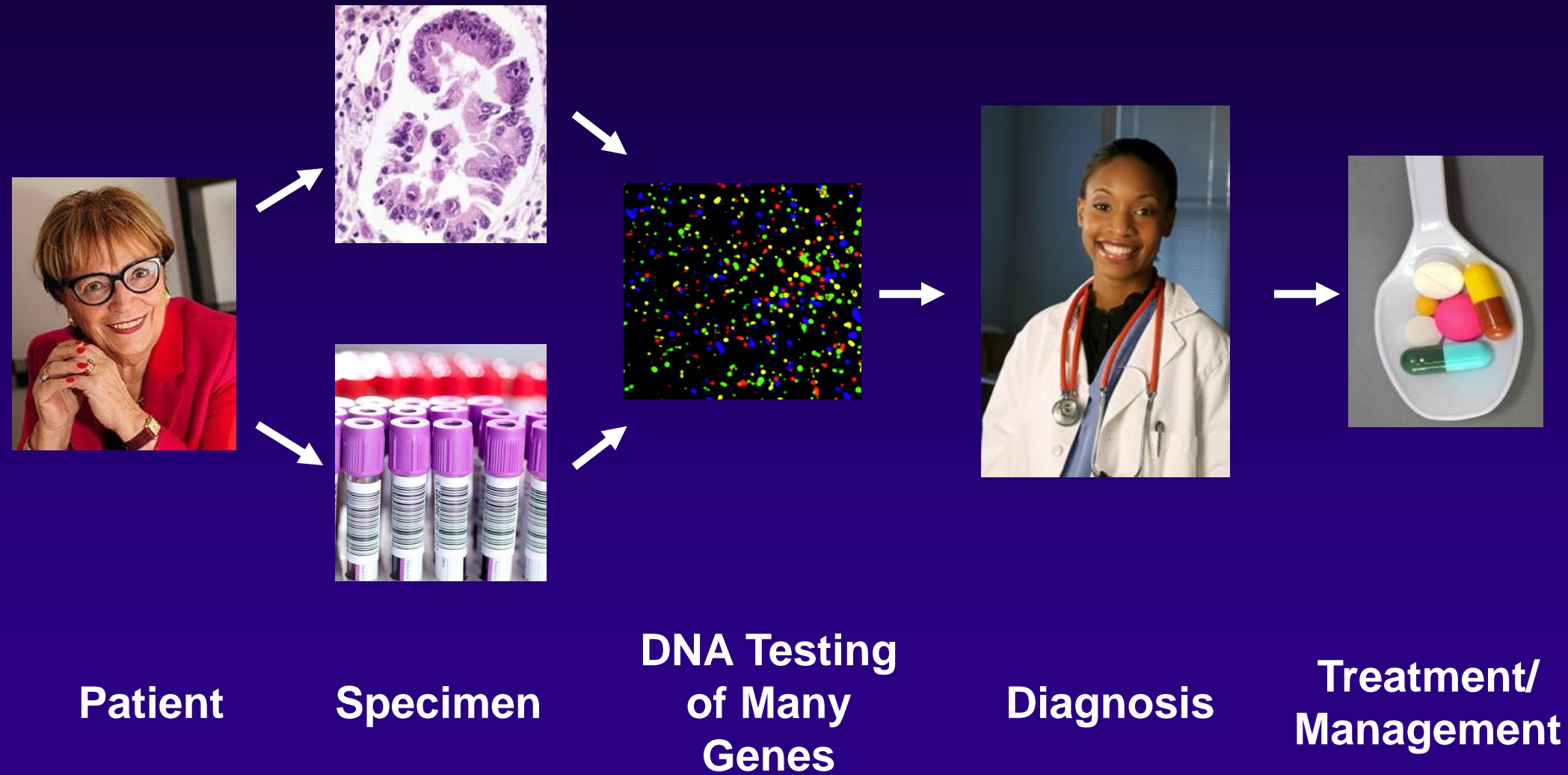
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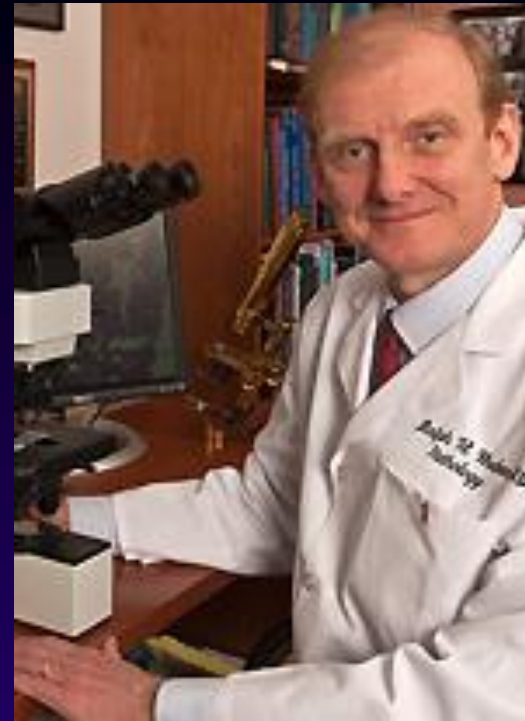
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Genomic Medicine



Pathologist



**'Genomic
Pathologist'**



Test Selection

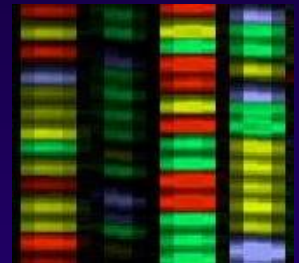
**Global Evaluation
(Large deletions)**

CGH Microarray



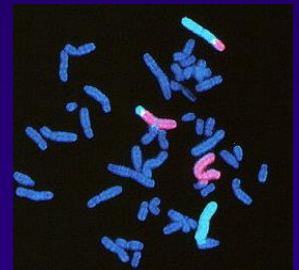
**Targeted Evaluation
(Mutation in a gene)**

Sanger Sequencing



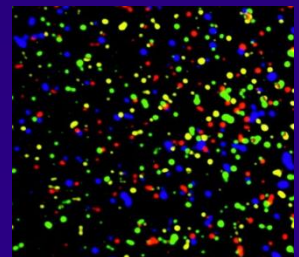
**Specific Rearrangement
(Translocations)**

FISH



**Genomic - Multiple Gene
Panels (Syndromic)**

**Next Generation
Sequencing**



DNA Variants

- **Single nucleotide variants (SNV)**

REFERENCE GGCCTTAACCC**C**CCGATTATCAG

PATIENT **GGCCTTAACCT**CCGATTATCAG

- **Small insertions/deletions (INDEL)**

REFERENCE GGCCTTAACCC**CCC**GATTATCAG

PATIENT **GGCCTTAACC**---GATTATCAG

- **Structural variants (Chromosomal)**

- Large insertions/deletions
- Copy number variants
- Translocations

Nucleotide change resulting in NO change in amino acid:

- Synonymous
- Silent

Nucleotide change resulting in CHANGE in amino acid:

- Non-Synonymous
- Missense

Question:

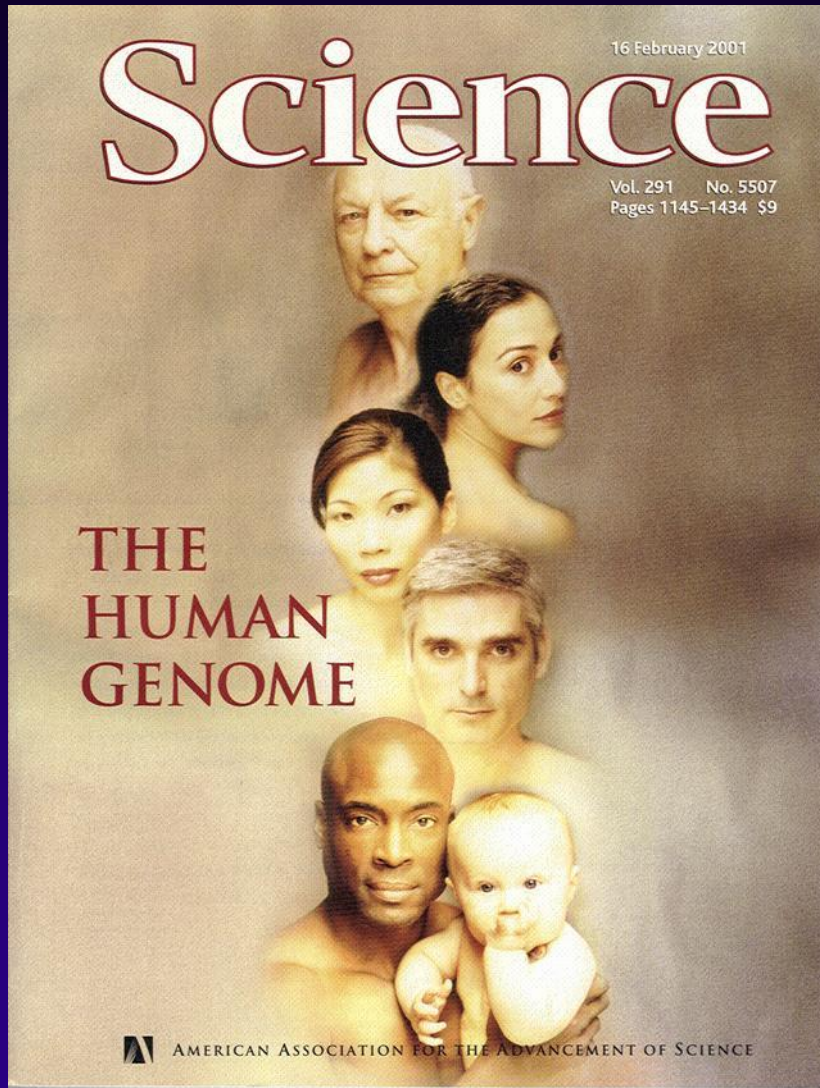
Is a non-synonymous variant (change in amino acid) a mutation?

Answer:

Only some are pathogenic (mutation)

Each non-synonymous variant is interpreted by using databases and literature

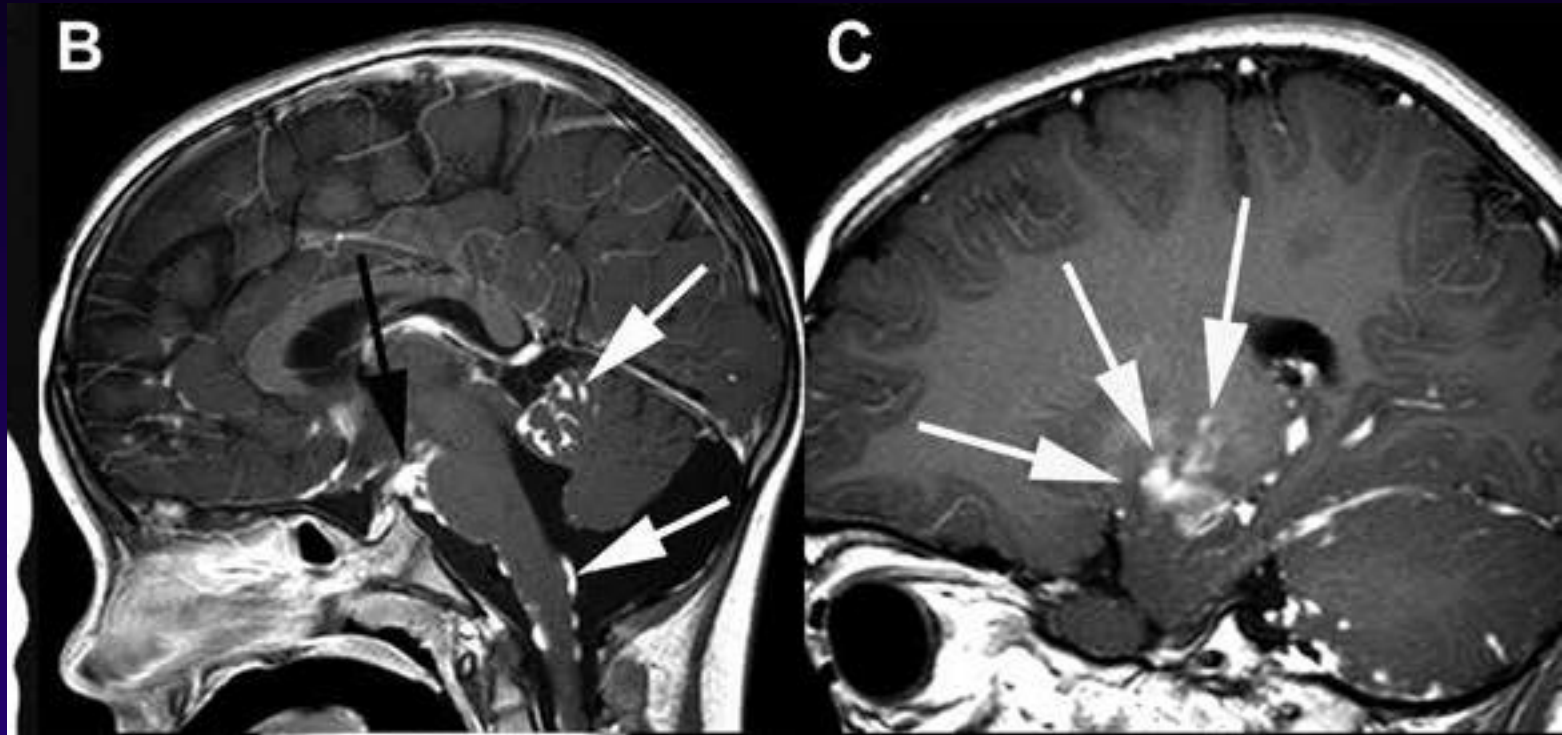
Genetic Diversity



Each 'disease-free' person has:

- Thousands of rare or unique DNA variants that change the amino acids at a single position
- Hundreds of rare frameshift DNA variants that result in truncated proteins

Clinical Case 1



- 13 month-old female presented with nystagmus; imaging showed an optic-hypothalamic mass
- Surgically debulked, pathology showed *pilocytic astrocytoma*
- 4 chemotherapy regimens over 3 years
- No response

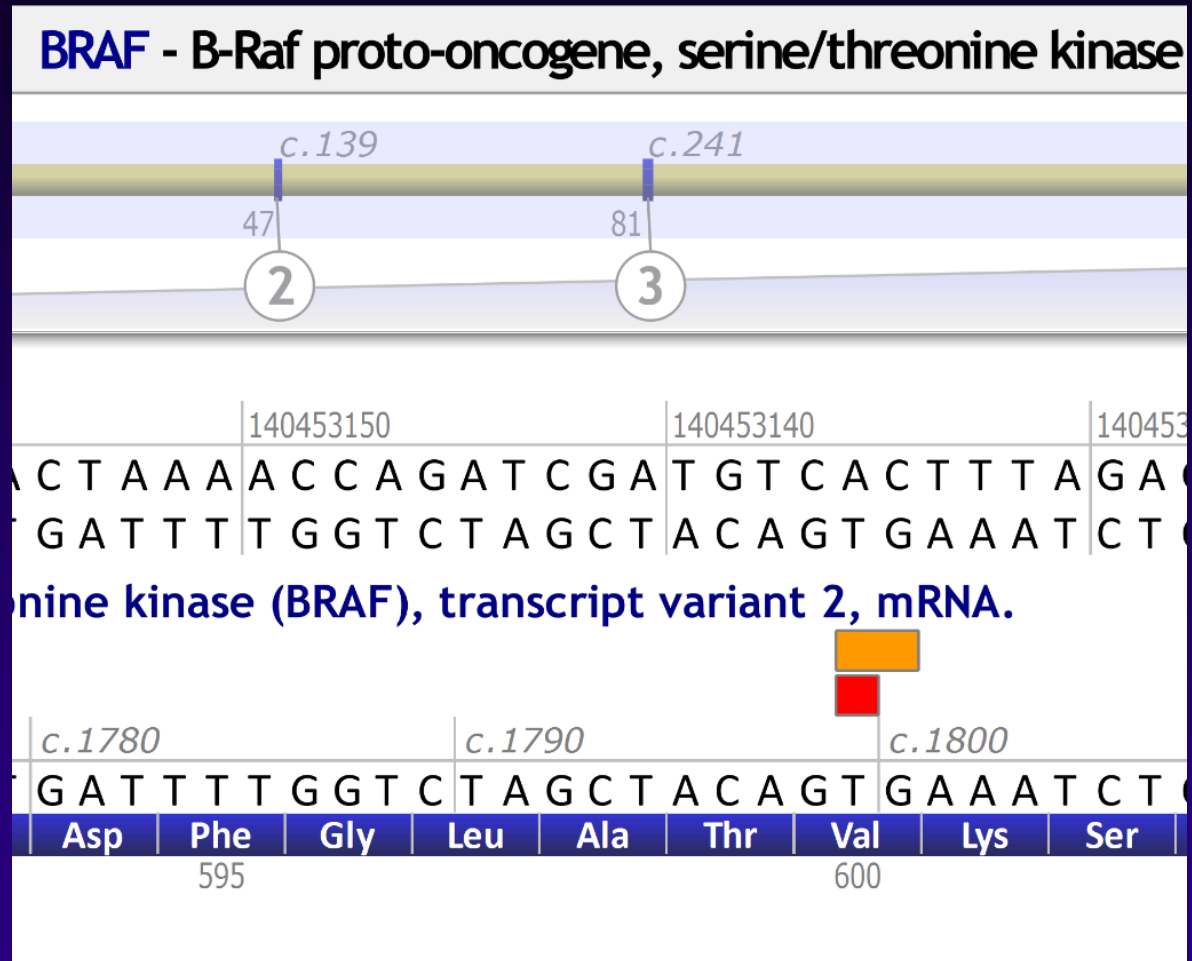
Further Progression

- Radiation therapy recommended
- Family declined radiation due to long-term morbidity concerns
- BRAF molecular testing
 - *BRAF-KIAA1549* fusion
NEGATIVE (FISH)
 - *BRAF V600E* NEGATIVE (IHC)



Identified: *BRAF* c.1799_1800delinsAT, p.Val600Asp

- V600D, rare variant not previously described in LGG (2015)
- Melanoma cell line data indicated V600D inhibited similarly as V600E (Gentilcore 2013)
- Subsequent studies show V600D in infantile ganglioglioma/astrocytoma

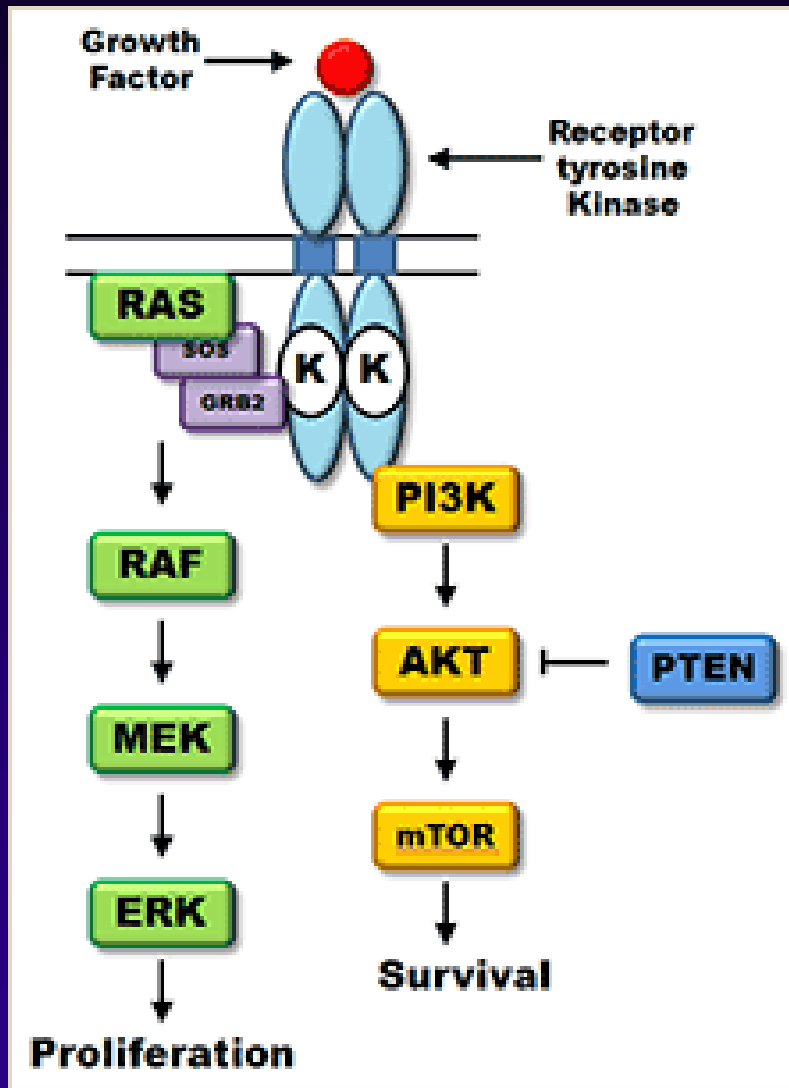


Gentilcore G et al 2013 BMC Cancer
Greer A et al 2017 Pediatr Blood Cancer
Wang AC et al 2018 Mol Cancer Res

Frequent *BRAF* gene alterations in pediatric high and low grade gliomas

	<i>BRAF-KIAA1549</i> fusion	<i>BRAF V600E</i>
Pilocytic astrocytoma	78%	6%
Pilomyxoid astrocytoma	63%	5%
Pleomorphic xanthoastrocytoma	50%	50%
Ganglioglioma	26%	21%
Diffuse fibrillary astrocytoma	3%	8%
Anaplastic astrocytoma	0%	16%
Glioblastoma multiforme	0%	9%

BRAF signaling



- RAF kinase which effects:
 - growth factor signaling
 - cell cycle progression
 - proliferation
- Part of the mitogen activated protein kinase (MAPK) pathway

Question: How should the *BRAF* variant be interpreted?

- A. Variant of Strong Clinical Significance
- B. Variant of Potential Clinical Significance
- C. Variant of Unknown Clinical Significance
- D. Benign or Likely Benign Variant

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

AMP, ASCO, CAP (2017)

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Level A Evidence

FDA-approved therapy
Included in professional guidelines

Level B Evidence

Well-powered studies with consensus from experts in the field

Tier II: Variants of Potential Clinical Significance

Therapeutic, prognostic & diagnostic

Level C Evidence

FDA-approved therapies for different tumor types or investigational therapies
Multiple small published studies with some consensus

Level D Evidence

Preclinical trials or a few case reports without consensus

Tier III: Variants of Unknown Clinical Significance

Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases

No convincing published evidence of cancer association

Tier IV: Benign or Likely Benign Variants

Observed at significant allele frequency in the general or specific subpopulation databases

No existing published evidence of cancer association

Question: How should the *BRAF* variant be interpreted?

- A. Tier 1: Strong Clinical Significance
- B. Tier 2: Potential Clinical Significance
- C. Tier 3: Unknown Clinical Significance
- D. Tier 4: Benign or Likely Benign Variant

Answer: B, Tier 2 Potential Clinical Significance

- BRAF V600D variant is biologically significant
- FDA approved therapies in other cancers
- No data on BRAF or MEK inhibitors for V600D in LGG (2015)

Question: Should off-label therapy be recommended?

PROS

- No other therapy options (family resistant to radiation therapy)
- BRAF V600E responds to targeted inhibition

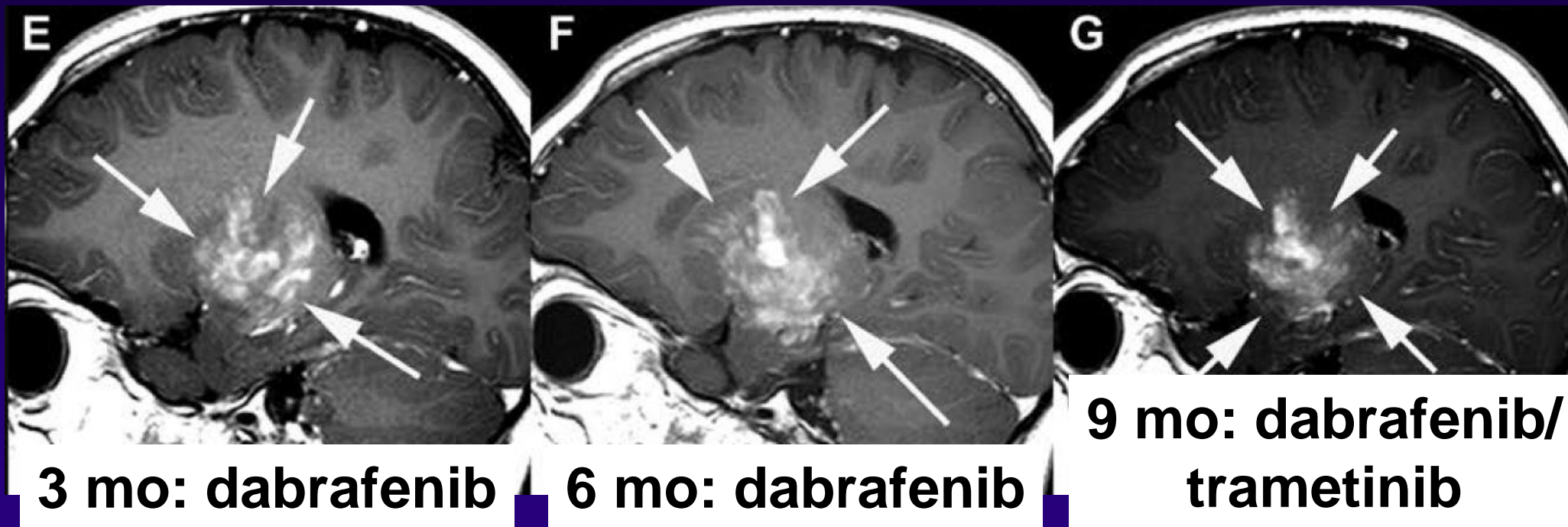
CONS

- Limited data on V600E (no data on V600D)
- BRAF inhibitors have high rate of toxicity in adult studies of melanoma

Question: Should off-label therapy be recommended?

Answer:

- Off-label dabrafenib was initiated



- >40 months stable disease on combination dabrafenib/trametinib

Clinical Case Summary

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Tier II: Variants of Potential Clinical Significance

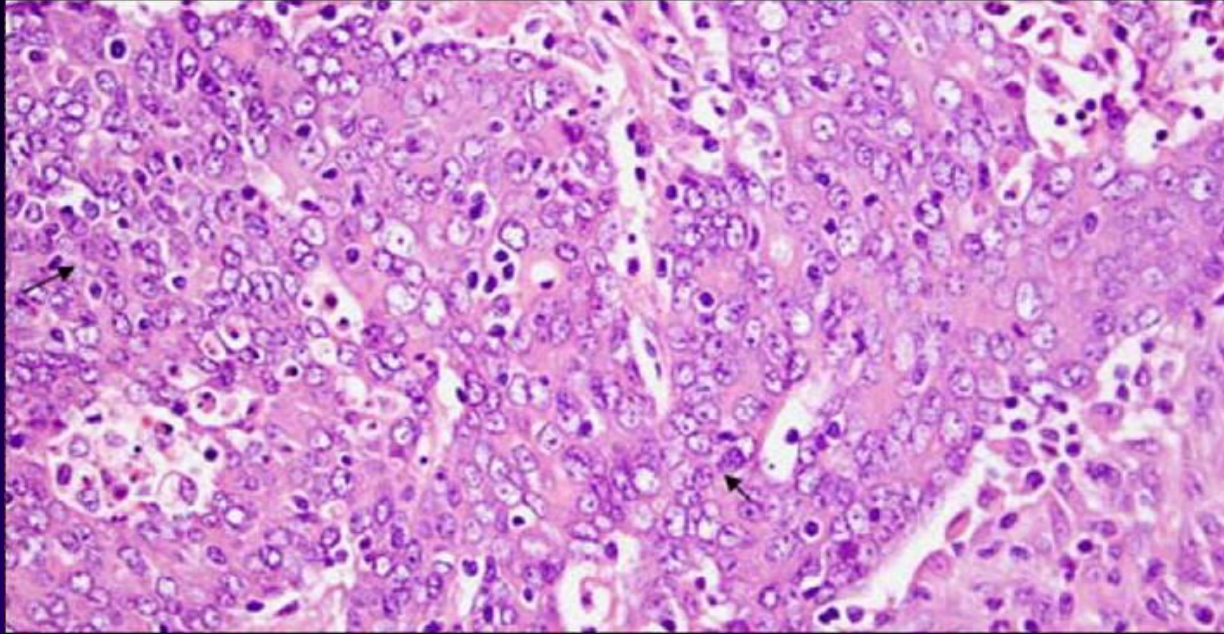
Therapeutic, prognostic & diagnostic

Tier III: Variants of Unknown Clinical Significance

Tier IV: Benign or Likely Benign Variants

- Patient with low grade glioma and novel BRAF variant (BRAF V600D)
- 2017 somatic interpretation guideline (CAP/ASCO/AMP) is a useful framework
- Uncertain whether V600D response will be broadly applicable
- LGG routine testing of *BRAF* fusions and V600 variants is recommended

Clinical Case 2



- 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)

MSH2 c.1A>C (p.Met1_Gly25del) mutS homolog 2

MSH2 - MutS homolog 2, colon cancer, nonpolyposis

Overview of Transcript NM_000251.2



Genome - chr2:47,630,305-47,630,397 (GRCh37) - 93 bps

47630310	47630320	47630330	47630340	47630350
C A A C C A G G A G G T G A G G A G G T T T C G A C A T G G C G G T G C A G C C G A A G G A G A				
G T T G G T C C T C C A C T C C T C C A A A G C T G T A C C G C C A C G T C G G C T T C C T C T				

NM_000251.2: Homo sapiens mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2)

c.-20	c.-10	c.1	c.10	c.20				
C A A C C A G G A G G T G A G G A G G T T T C G A C A T G G C G G T G C A G C C G A A G G A G A								
		Met	Ala	Val	Gln	Pro	Lys	Glu
		1				5		

ClinVar

Del/Delins

Subst **G**

Ins/Dup

c.-20	c.-10	c.1	c.10	c.20				
C A A C C A G G A G G T G A G G A G G T T T C G A C A T G G C G G T G C A G C C G A A G G A G A								
		Met	Ala	Val	Gln	Pro	Lys	Glu
		1				5		

Human Gene Mutation Database (HGMD® Professional)

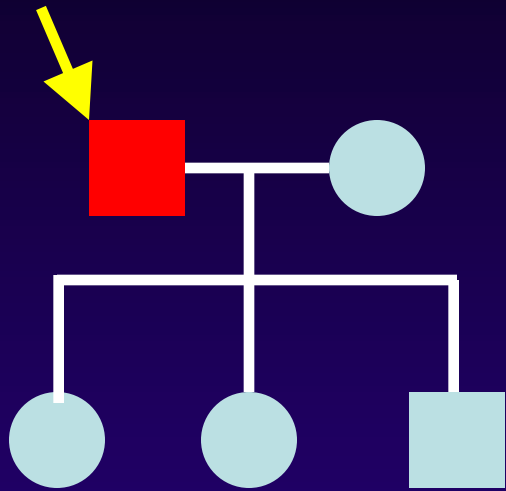
Del/Delins

Subst

Ins/Dup

C A A C C A G G A G G T G A G G A G G T T T C G A C A T G G C G G T G C A G C C G A A G G A G A								
		Met	Ala	Val	Gln	Pro	Lys	Glu
		1				5		

Lynch Syndrome Familial Testing

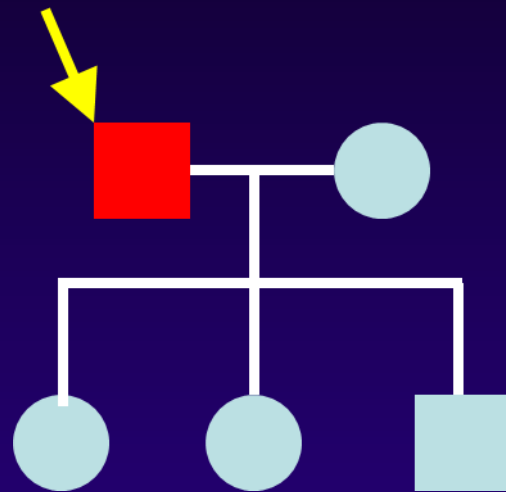


- Patient's *MSH2* c.1A>C reported as likely pathogenic
- *MSH2* is one of the DNA mismatch repair genes (*MLH1*, *MSH6*, and *PMS2*) associated with Lynch Syndrome
- Lynch syndrome is an autosomal dominant cancer syndrome associated with early colorectal, endometrial and ovarian cancers
- Patient's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

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

- Clinical genetics laboratories 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: variant of uncertain significance

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

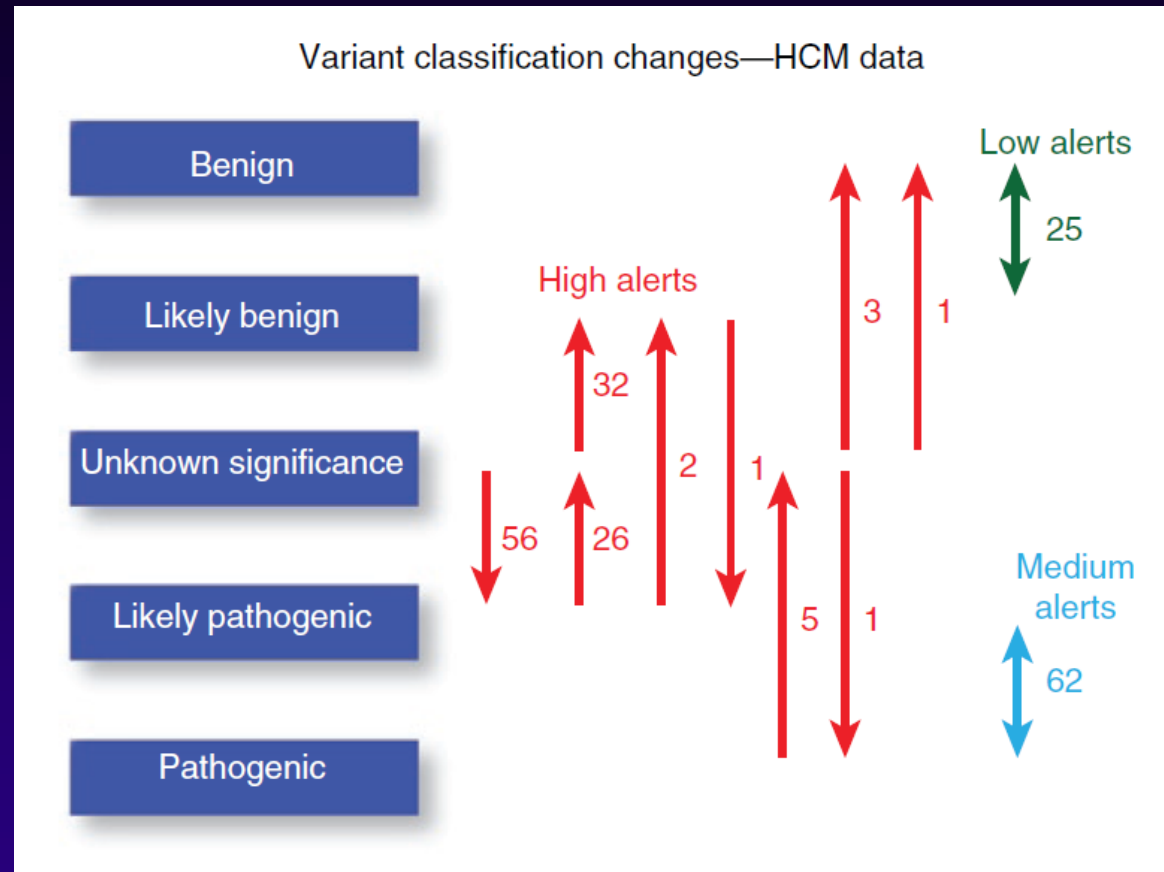


**Previous Answer: Counsel/screen only
gene test positive**

**Revised Answer: Counsel/screen all
gene test positive and negative**

Genomic Test Results are *Dynamic*

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



Medical Literature is Constantly Growing

- 27% of mutations cited in literature (2011) were found to be common population variants or incorrectly reported
- Traditional human genetic studies (pre-2010) used 100s of control genomes to define a novel or rare disease variant
 - High rate of calling benign variants as mutations
- Recent studies use >100,000 control genomes (gnomAD, 1000 Genomes)

Prevalence of Variant Reclassification Following Hereditary Cancer Genetic Testing

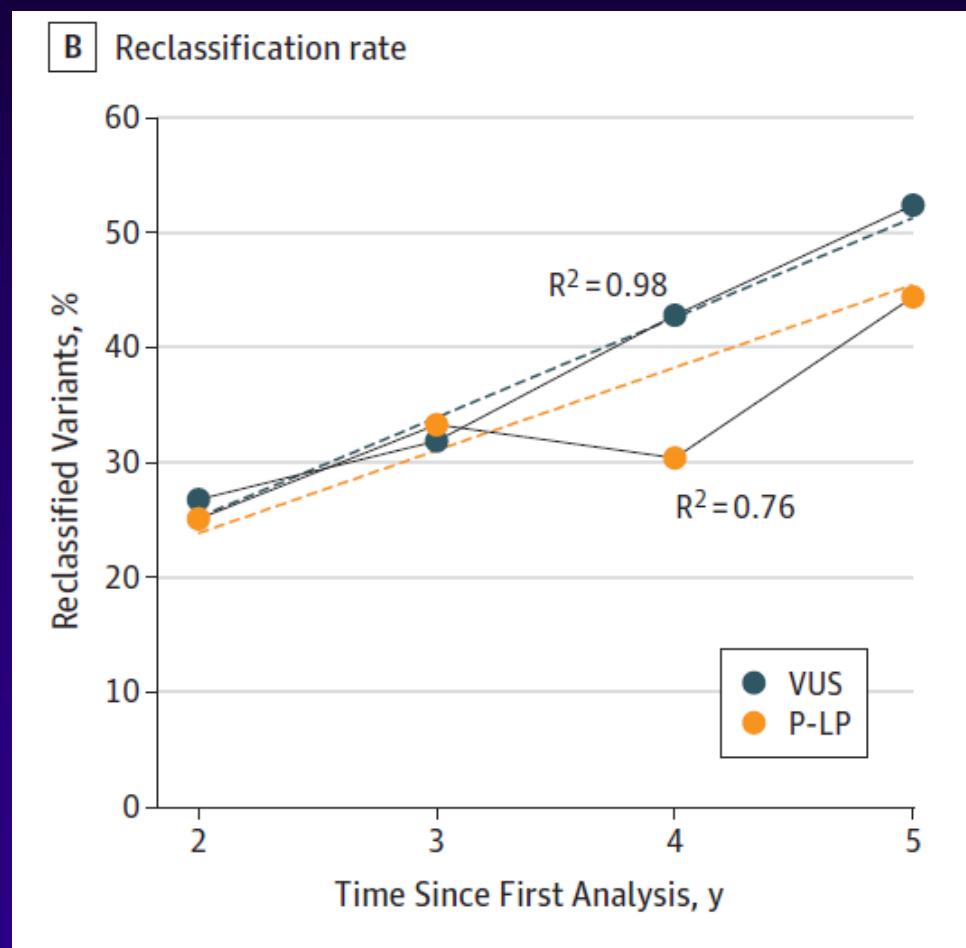
JAMA. 2018;320(12):1266-1274. doi:10.1001/jama.2018.13152

- 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

Clinical Utility of Reinterpreting Previously Reported Genomic Epilepsy Test Results for Pediatric Patients

JAMA Pediatr. doi:10.1001/jamapediatrics.2018.2302

- UT Southwestern study of 309 patients tested at GeneDx (2012-2015)
- Genomic pediatric epilepsy testing
- Overall, 36.2% of patients had variants reclassified
- 31.3% of reclassified variant changed the diagnosis



Emerging Diagnostic Split Decisions

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

Diagnoses from one laboratory

Other Labs

	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

Final Question: How do you manage patients with the same genetic change, but tested by different labs with different interpretations?

- A. Create internal consensus opinion and treat the patients the same
- B. Treat patients based on the different lab interpretations
- C. Share discordant information with patients and let them decide
- D. I don't know

Summary

- Genomic testing and interpretation are critical for advanced malignancies
- Genomic test interpretations are dynamic over time
- The practice of genomic medicine requires pragmatic judgment despite diagnostic uncertainty (i.e., surgical pathologist)

