Annual 2021 Spring KOPANA Seminar

Clinical Genomics in Surgical Pathology

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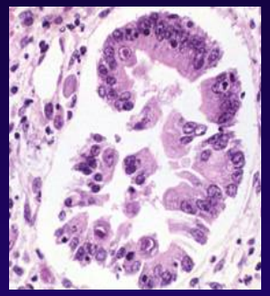
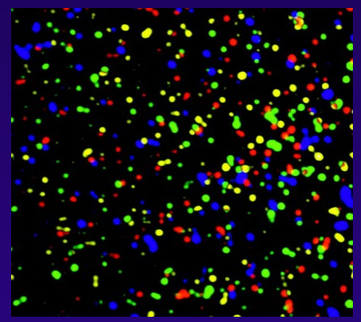
Genomic Medicine

Patient



Specimen

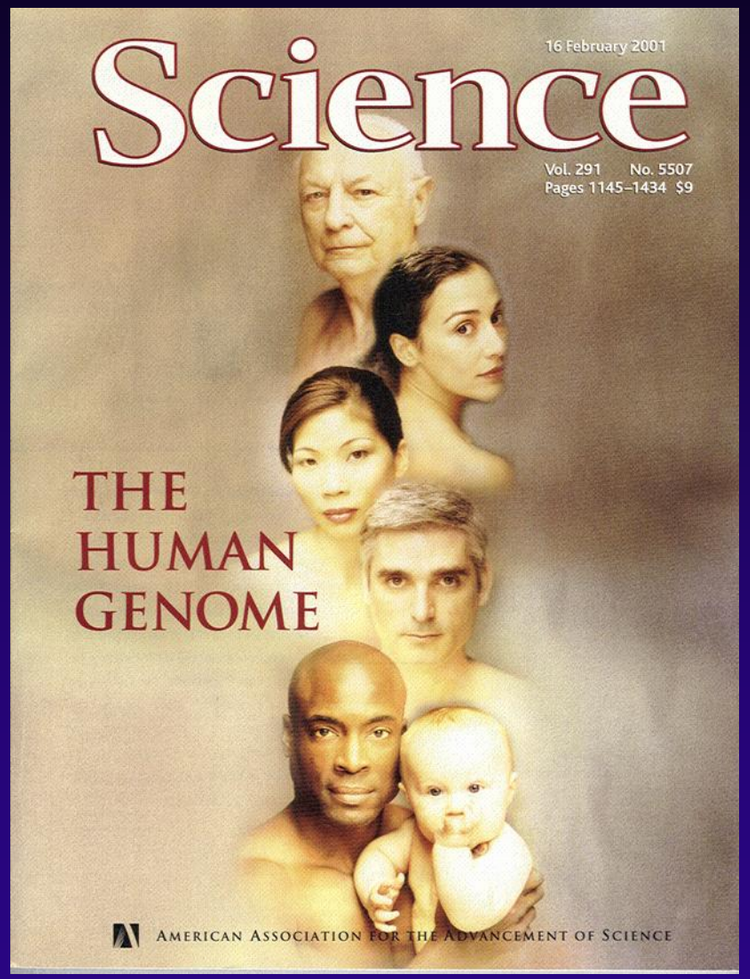


DNA Testing of Many Genes

Diagnosis

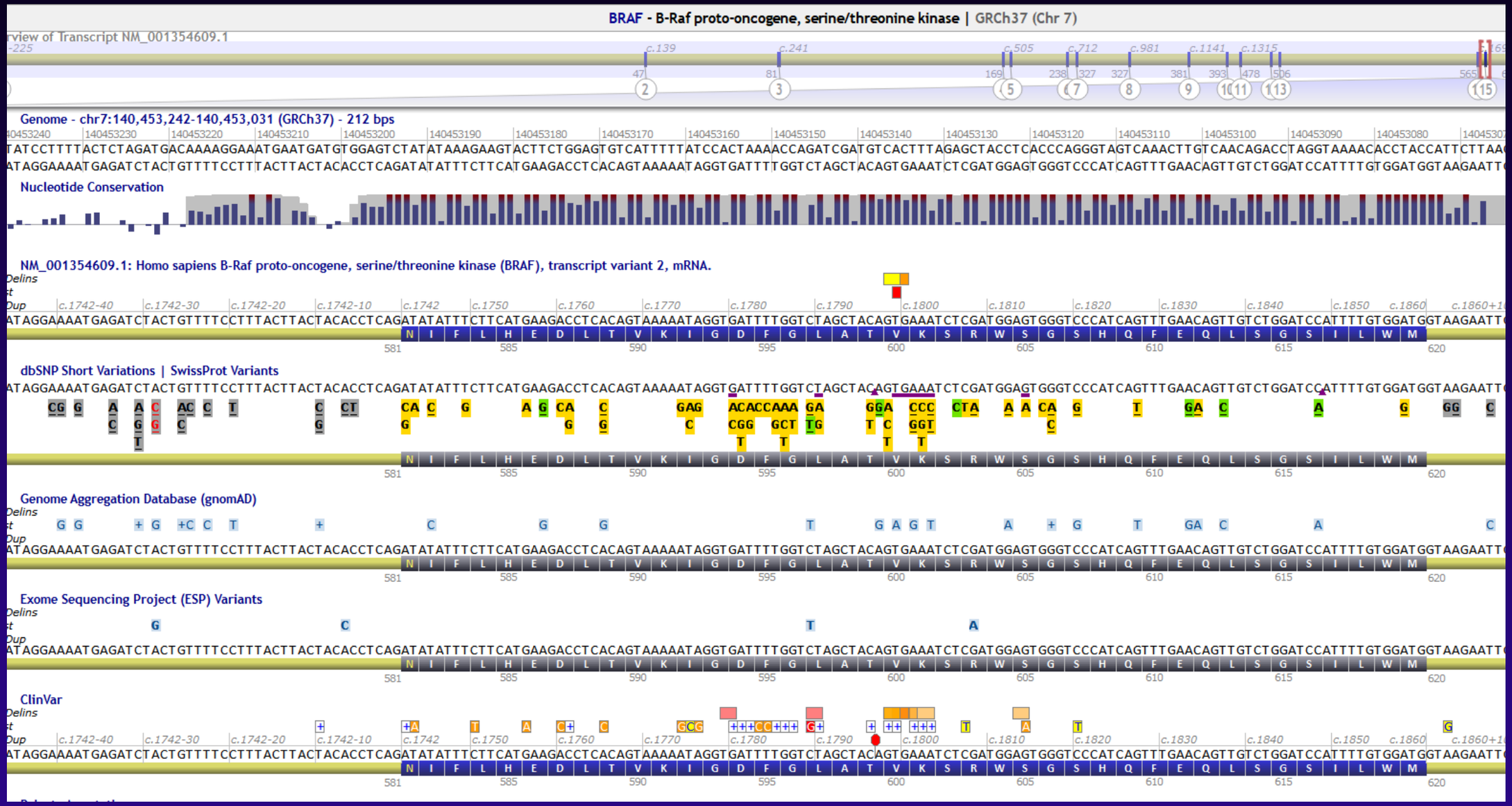
Treatment/ Management

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 (http://www.mycancergenome.org/)
  - ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/)
  - Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)
  - The Human Gene Mutation Database (http://www.hgmd.org/)

Dashboard Approach



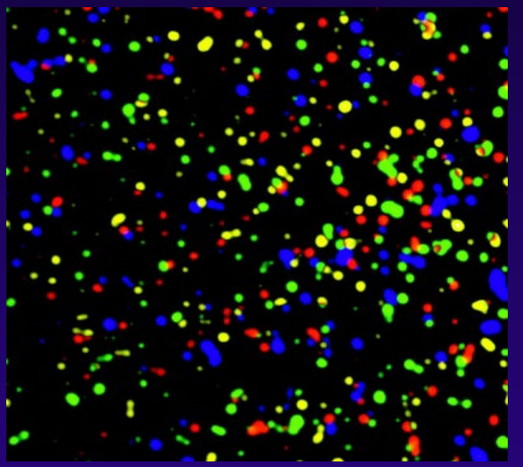
Alamut, Interactive Biosoftware

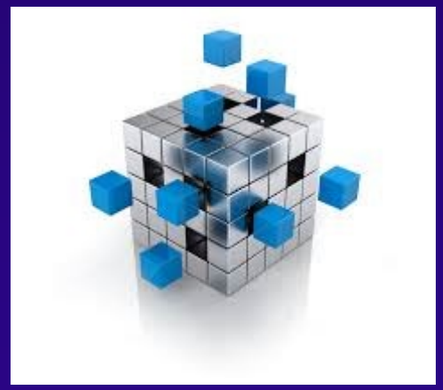
Pathologists as Genomic Physicians

1. Patient

2. Provider

3. Pathologist





Determine if variant is clinically significant

Pathogenic / Likely Pathogenic vs Uncertain

Change in clinical management

Screening

Surgery

Treatment

Manage changes in variant significance

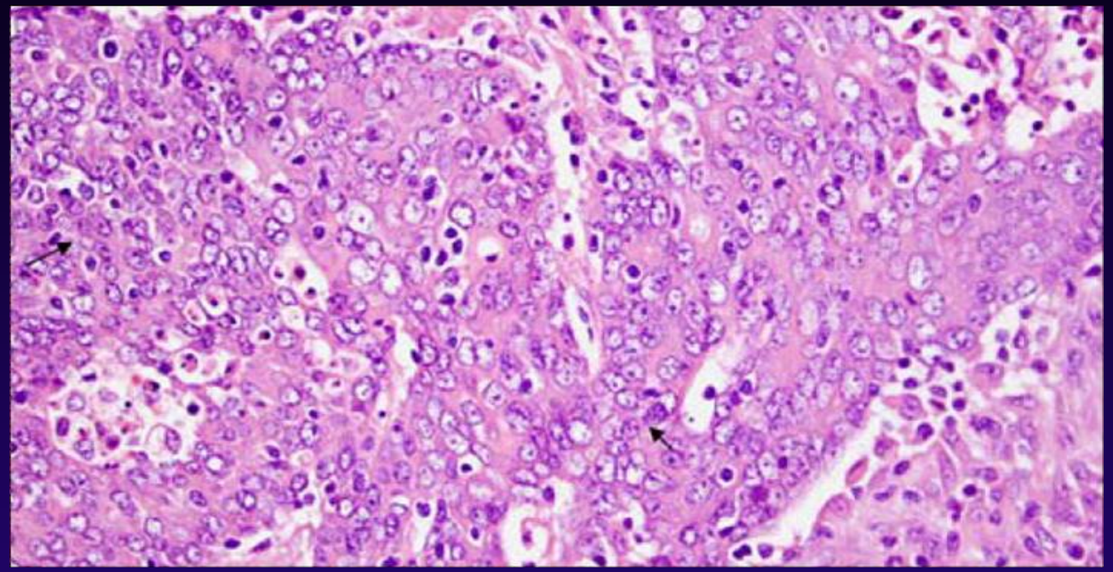
Uncertain upgrade to Pathogenic

Pathogenic downgrade to Benign

New treatment modalities for existing variant

Genomic Case Studies

Case Study 1:



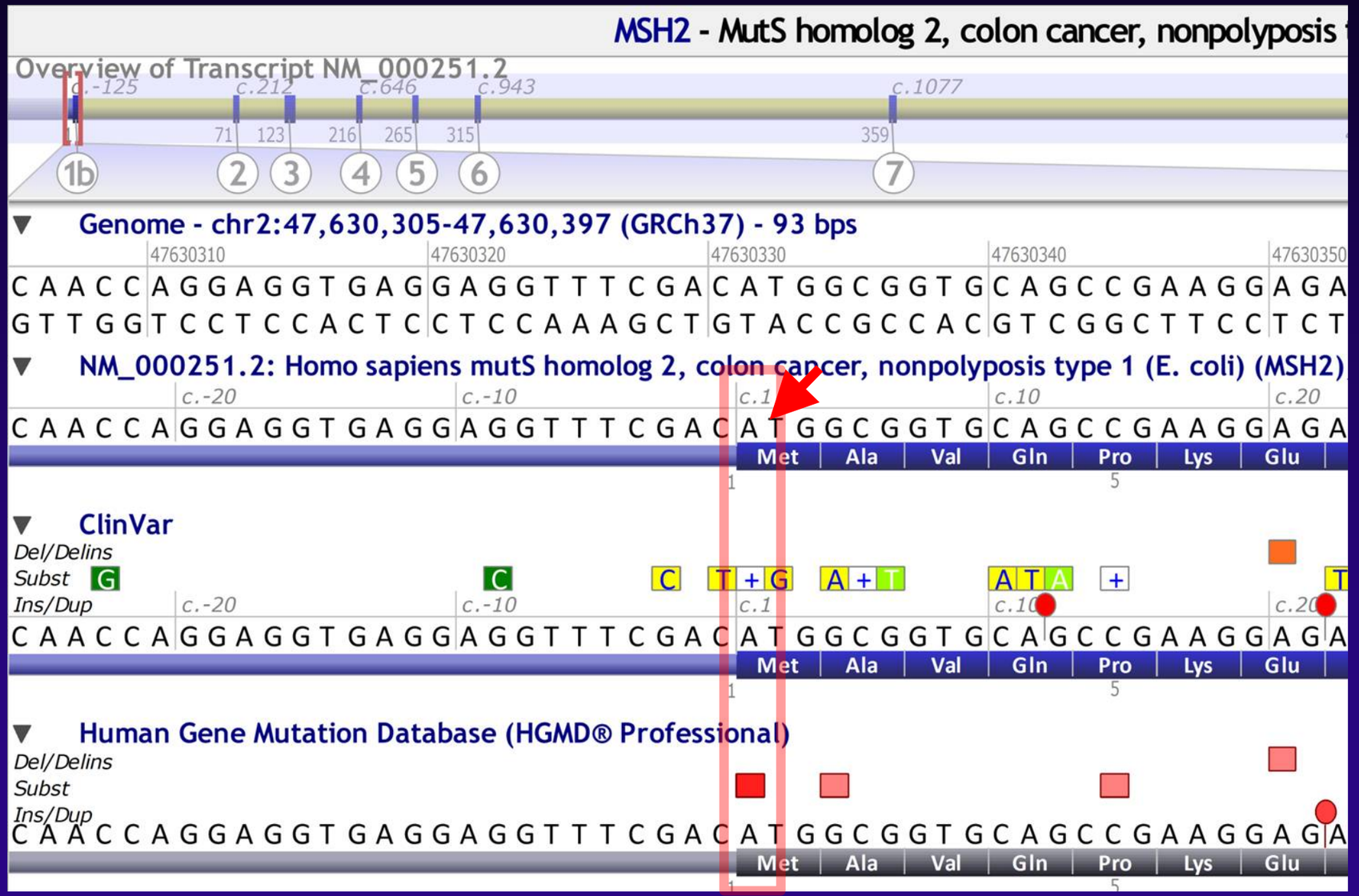
- 60-year-old man with colon adenocarcinoma identified as mismatch repair deficient (MSH2/MSH6 loss by immunohistochemistry)
- *MSH2* gene sequencing identifies:

  c.1A>C (p.Met1_Gly25del)

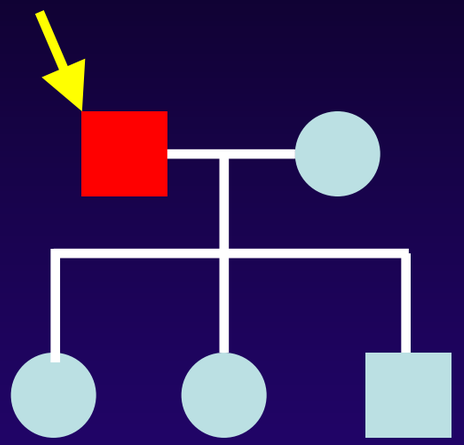
Truta et al 2008 Familial Cancer 7:267-74
Rosenthal et al 2015 Clin Genet 88:533-541

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: variant of uncertain significance

Rosenthal et al 2015 Clin Genet 88:533-541

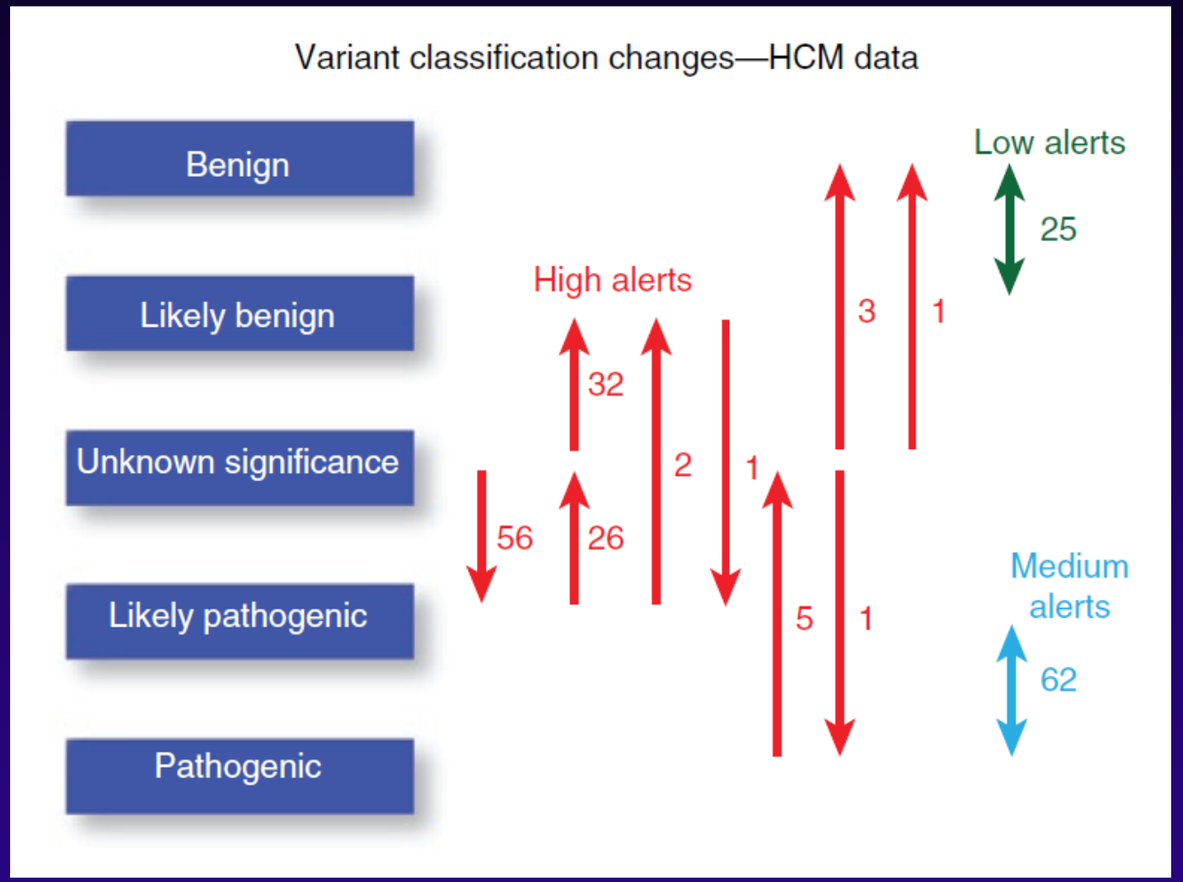
Question: If all children are tested and two are positive, which of the following are important next steps?

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



Aronson 2012 Genet Med

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

Diagnoses from one laboratory

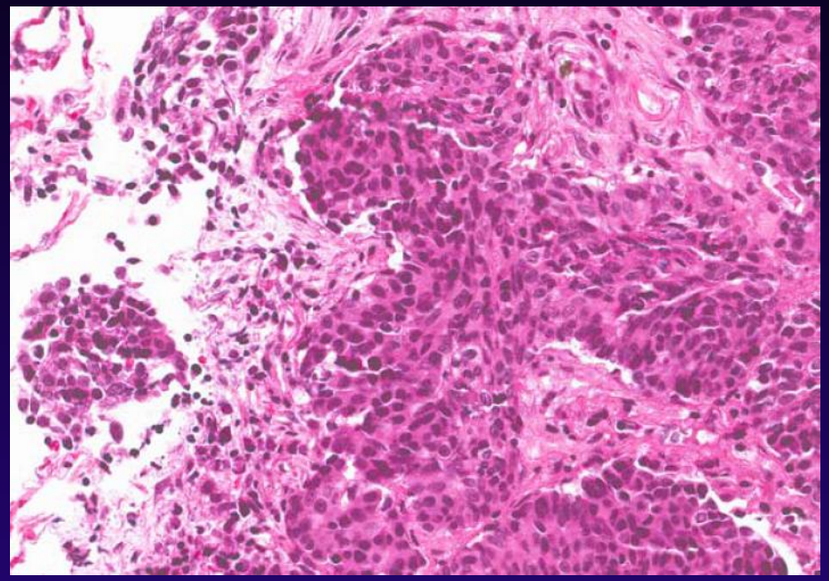
Other Labs	pathogenic	likely pathogenic	uncertain significance	likely benign	benign
pathogenic	0	595	93	3	0
likely pathogenic	266	0	246	1	1
uncertain significance	39	97	0	1019	227
likely benign	3	1	2149	2	1883
benign	0	1	411	1012	0

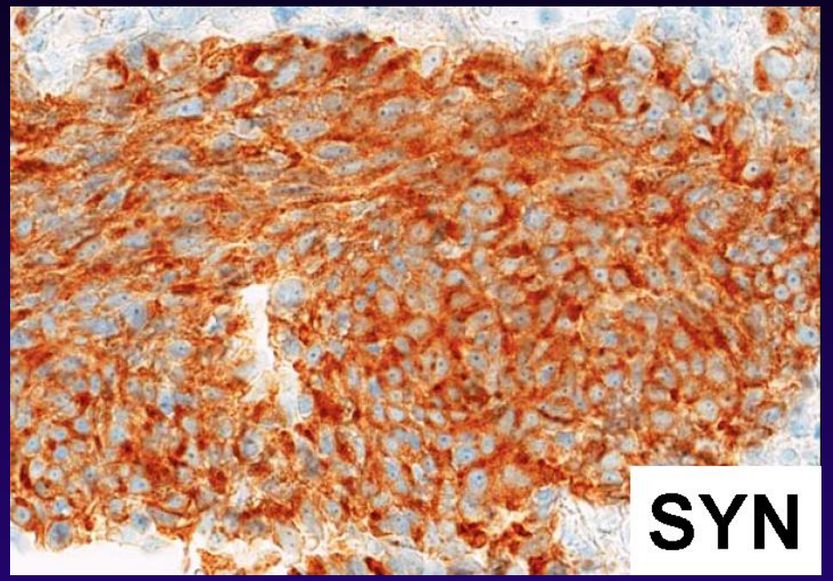
https://clinvarminer.genetics.utah.edu/

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

Wang et al 2017 Oncologist 22:768-773

- 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)

Wang et al 2017 Oncologist 22:768-773

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

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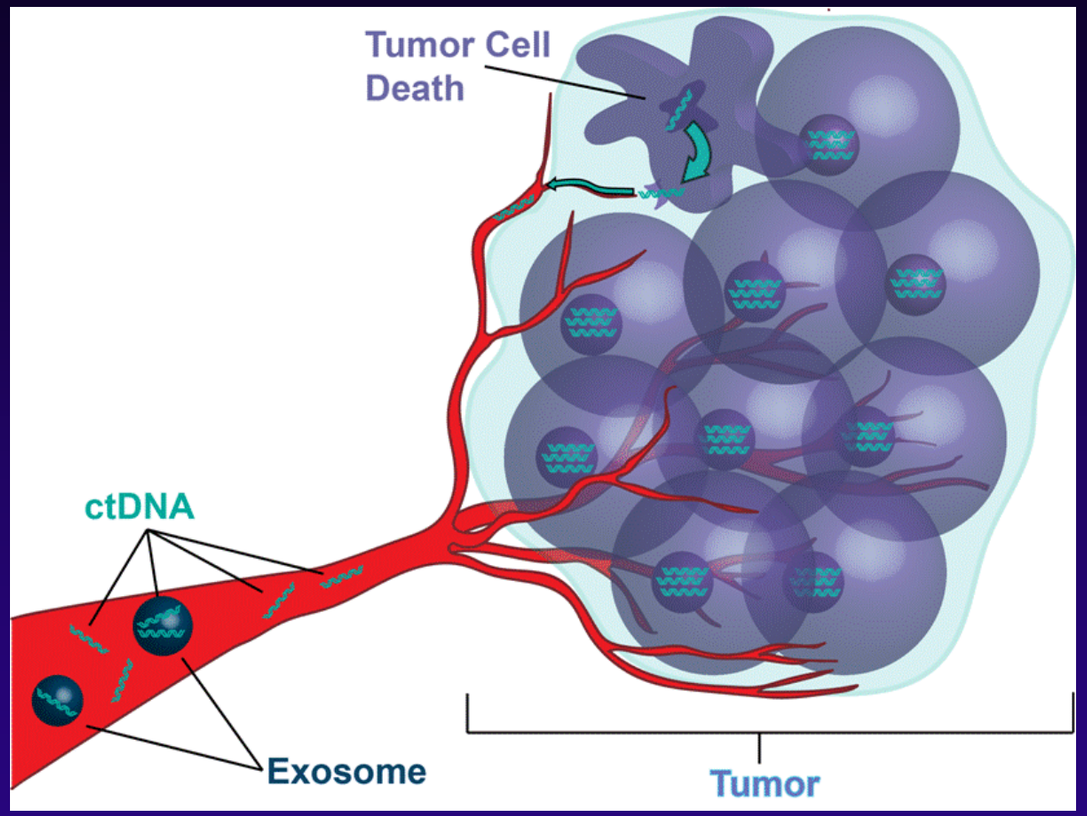
D. None, watch and wait

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 intact cells (CTC) or cell free (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% (Non-invasive prenatal testing)

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

Snyder MW et al 2015 N Engl J Med 372:1639-45

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 ***SMC5:ALK***
- 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

Wang et al 2017 Oncologist 22:768-773

Question: How should the *ALK* fusion by interpreted?

A. Variant of Strong Clinical Significance

B. 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 **Variant of Uncertain Clinical Significance**

Question: Should off-label therapy be recommended?

PROS

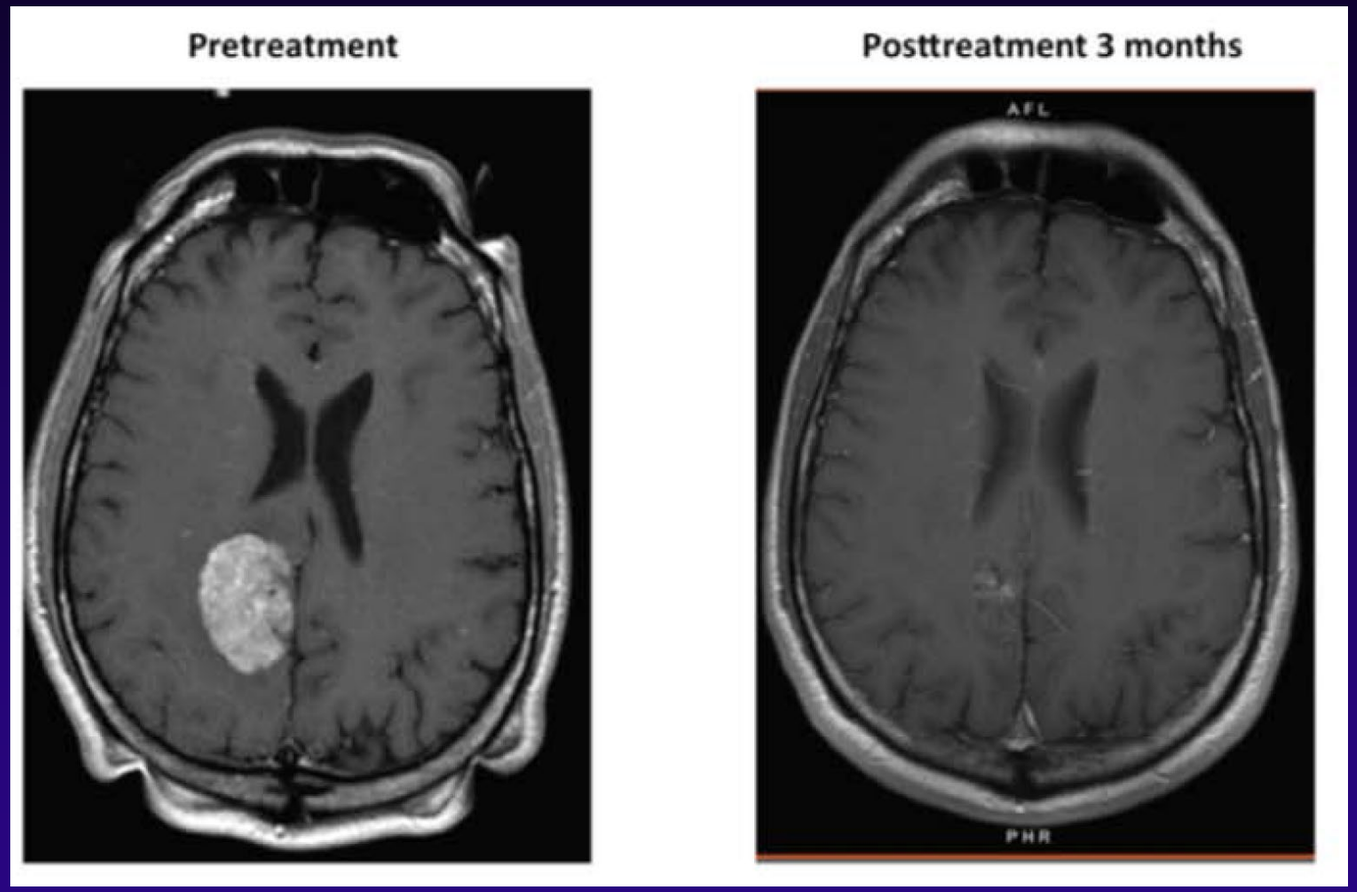
- No other therapy options
- ALK inhibitors in NSCLC show response

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



Wang et al 2017 Oncologist 22:768-773

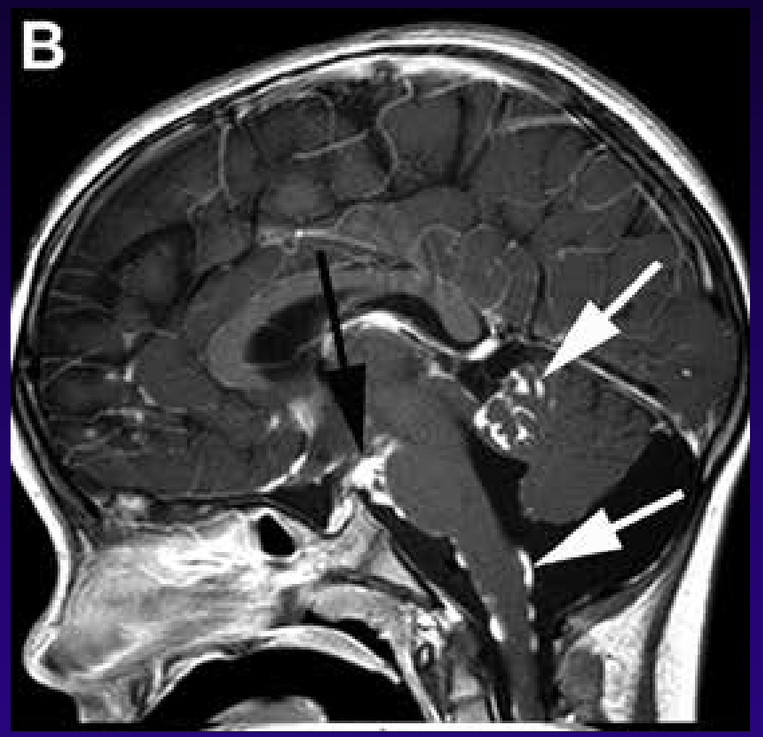
Case 2 Summary

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

Li et al 2017 J Mol Diagn 19:4-23

Case Study 3

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



Drobysheva et al 2017 J Natl Comp Canc Netw

Sequencing Results

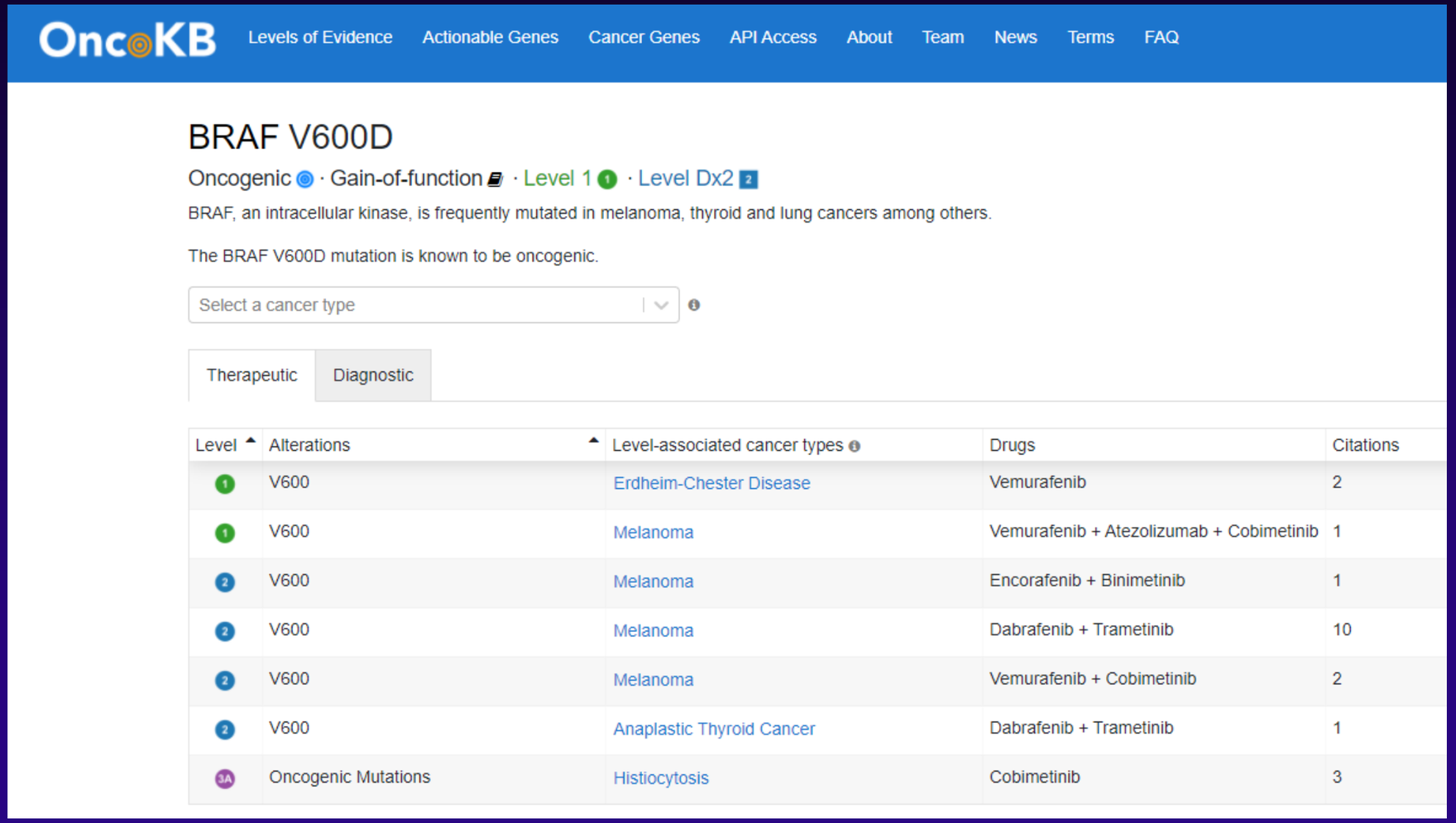
Tumor Next-Generation Sequencing

- 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)

OncoKB
https://www.oncokb.org/



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

NM_004333.6(BRAF):c.1799_1800delinsAT (p.Val600Asp) Cite this record

Interpretation: Pathogenic

Review status: ☆☆☆☆ no assertion criteria provided
Submissions: 1 (Most recent: Jul 18, 2016)
Last evaluated: Jul 14, 2015
Accession: VCV000375939.2
Variation ID: 375939
Description: 2bp indel

Variant details
Conditions
Gene(s)

NM_004333.6(BRAF):c.1799_1800delinsAT (p.Val600Asp)

Allele ID: 362818
Variant type: Indel
Variant length: 2 bp
Cytogenetic location: 7q34
Genomic location: 7: 140753335-140753336 (GRCh38) GRCh38 UCSC
7: 140453135-140453136 (GRCh37) GRCh37 UCSC

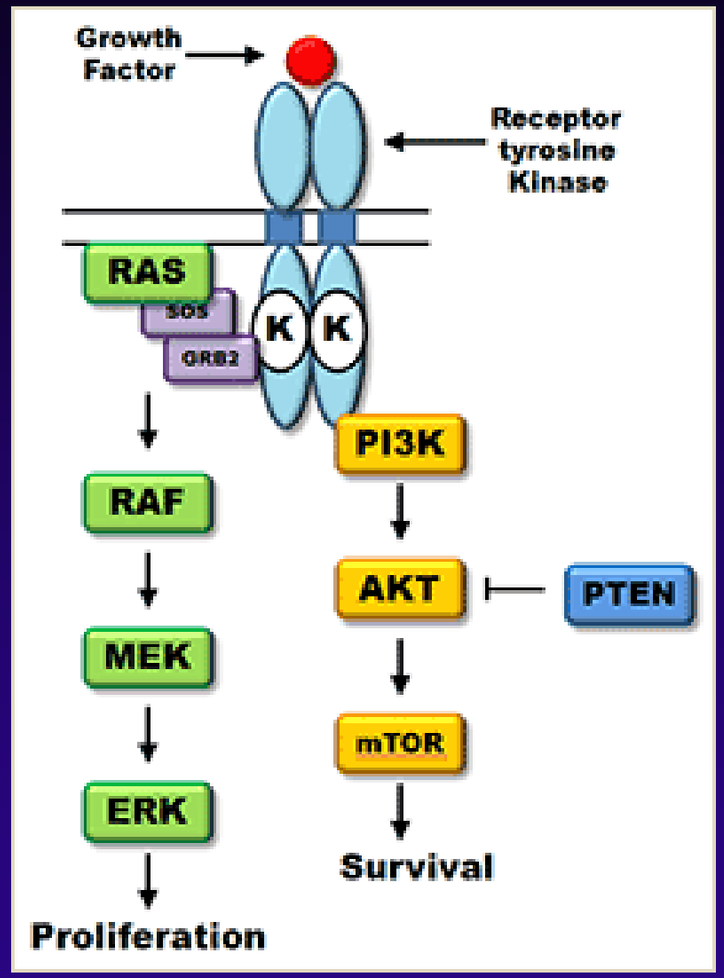
HGVS:

Nucleotide	Protein	Molecular consequence
NC_000007.13:g.140453135_140453136delinsAT		
NC_000007.14:g.140753335_140753336delinsAT		
NM_001354609.2:c.1799_1800delinsAT	NP_001341538.1:p.Val600Asp	missense

... more HGVS

Protein change: V600D

BRAF Inhibition

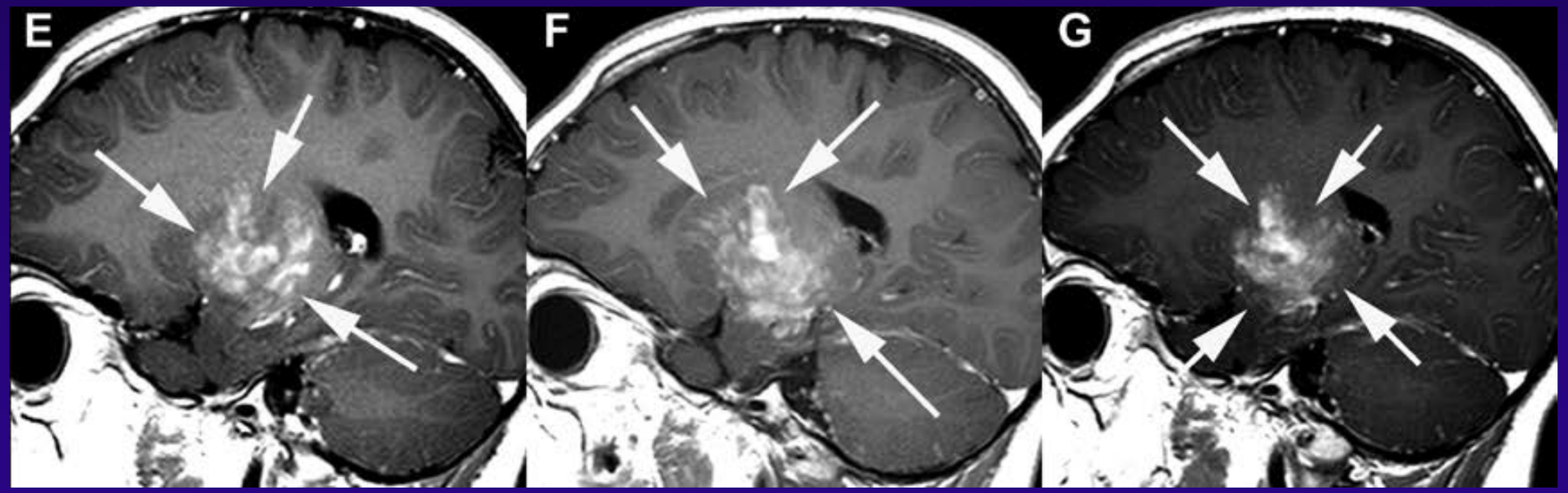


- Mitogen activated protein kinase (MAPK) pathway key to initiation and maintenance of many tumors
- BRAF is one of the 3 RAF kinases
- Targeted therapies exist for BRAF and MEK

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