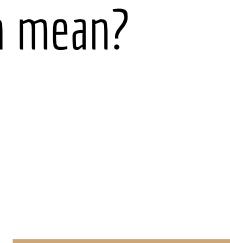
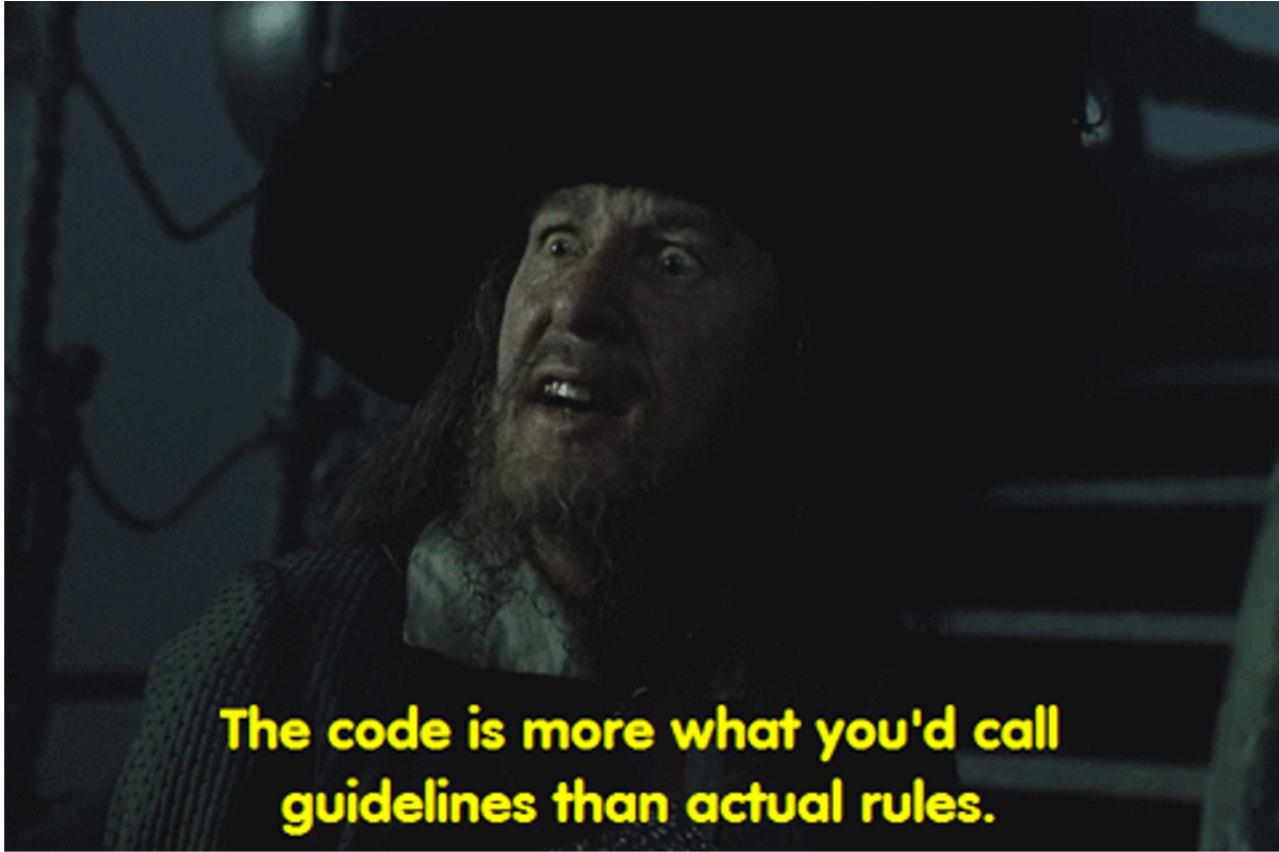


Variant Interpretation or Classification

aka: What does this even mean?





The code is more what you'd call
guidelines than actual rules.

Let's Talk about Guidelines for Variant Interpretation

- Why Guidelines?

Let's Talk about Guidelines for Variant Interpretation

- Why Guidelines?



ClinVar conflicting pathogenicity example

NM_000256.3(MYBPC3):c.3763G>A (p.Ala1255Thr)

Interpretation:	Conflicting interpretations of pathogenicity Pathogenic(1); Likely pathogenic(1); Uncertain significance(7); Benign(1); Likely benign(1)
Review status:	     criteria provided, conflicting interpretations
Submissions:	11
First in ClinVar:	Feb 27, 2016
Most recent Submission:	Oct 14, 2023
Last evaluated:	Aug 31, 2022
Accession:	VCV000164023.44
Variation ID:	164023
Description:	single nucleotide variant

<https://www.ncbi.nlm.nih.gov/clinvar/variation/164023/>

ClinVar conflicting pathogenicity example 2

NC_000023.10:g.(?_122318388)_(123505241_?)del

Interpretation:	Conflicting interpretations of pathogenicity Pathogenic(1); Uncertain significance(1)
Review status:	    criteria provided, conflicting interpretations
Submissions:	2
First in ClinVar:	Mar 28, 2022
Most recent Submission:	Feb 13, 2023
Last evaluated:	Dec 23, 2021
Accession:	VCV001455382.5
Variation ID:	1455382
Description:	1.2Mb deletion

ClinVar conflicting pathogenicity example 2

Interpretation (Last evaluated)	Review status (Assertion criteria)	Condition (Inheritance)	Submitter	More information
Uncertain significance (Dec 23, 2021)	criteria provided, single submitter (Inviteae Variant Classification Sherloc (09022015)) Method: clinical testing	- not provided Affected status: unknown Allele origin: germline	Inviteae Accession: SCV003794377.1 First in ClinVar: Feb 13, 2023 Last updated: Feb 13, 2023	Publications: PubMed (1) Comment: A gross deletion of the genomic region encompassing the full coding sequence of the GRIA3 gene has been identified. The current clinical and genetic evidence ... (more)
Pathogenic (Sep 15, 2021)	criteria provided, single submitter (Inviteae Variant Classification Sherloc (09022015)) Method: clinical testing	- X-linked lymphoproliferative disease due to XIAP deficiency Affected status: unknown Allele origin: germline	Inviteae Accession: SCV002228824.2 First in ClinVar: Mar 28, 2022 Last updated: Feb 13, 2023	Publications: PubMed (4) Comment: A gross deletion of the genomic region encompassing the full coding sequence of the XIAP gene has been identified. Loss-of-function variants in XIAP are known ... (more)

Germline Interpretation/Classification

Germline

← Benign → ← Pathogenic →

	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in <i>trans</i> with a dominant variant BP2 Observed in <i>cis</i> with a pathogenic variant BP2		For recessive disorders, detected in <i>trans</i> with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Population Data

- PS4: Prevalence in affected individuals statistically increased over controls
 - Ex. *FLCN* p.Tyr463Ter - Variant found in 5+ affected families (PMID: 12204536, 15852235, 18234728)
- PM2: Absent in population databases
- BA1: Allele frequency is >5% in gnomAD or other population databases
- BS1: Allele frequency is greater than expected for disorder
- BS2: Seen in healthy adult for recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, full penetrance expected at an early age

Computational & Predictive Data

- PVS1: Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease
- PS1: Same AA as previously described pathogenic variant
 - Ex. c.34G>C (p.Val12Leu) & c.34G>T (p.Val12Leu)
- PM5: Novel missense change at an AA where a different missense change determined to be pathogenic has been seen before
 - Ex. Arg156His is pathogenic; now you observe Arg156Cys
- PM4: Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants
- PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

Computational & Predictive Data (Benign)

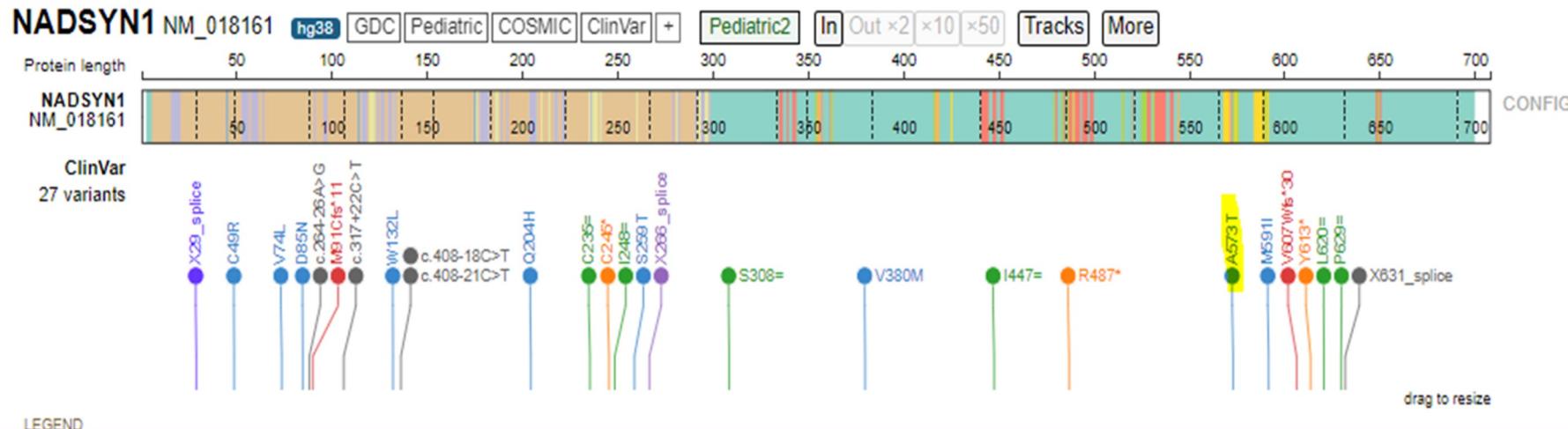
- BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease
- BP3: In-frame deletions/insertions in a repetitive region without a known function
- BP4: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)
- BP7: A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

Functional Data

- PS3: Well-established functional studies show a deleterious effect
- PM1: Mutational hot spot or well-studied functional domain without benign variation

Functional Data

PM1



Functional Data

- PS3: Well-established functional studies show a deleterious effect
- PM1: Mutational hot spot or well-studied functional domain without benign variation
- PP2: Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease

Segregation Data

- PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease
 - Ex. Segregates with disease in multiple families (PMIDs: 18234728, 15852235)
- BS4: Lack of segregation in affected members of a family

De novo data

- PS2: De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
- PM6: Assumed de novo, but without confirmation of paternity and maternity

Allelic Data

- PM3: For recessive disorders, detected in trans with a pathogenic variant
 - Ex. One pathogenic variant inherited from Mother and one pathogenic variant inherited from Father
- BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder OR in cis with a pathogenic variant in any inheritance pattern

Other Databases

- PP5: Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation
- BP6: Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation

Other Data

- PP4: Patient's phenotype or family history is highly specific for a disease with a single genetic etiology
 - Ex. Patient has a variant in *ABCA4*, the patient and family members have been diagnosed with Stargardt Macular Degeneration
- BP5: Variant found in a case with an alternate molecular basis for disease
 - Ex. Patient has been diagnosed with Stargardt Macular Degeneration and has a variant in *ABCA4*, another variant was found in *CFTR* and is clearly not the cause of disease in this patient

Overall Classification Rules

Pathogenic	(i) 1 Very strong (PVS1) AND (a) ≥ 1 Strong (PS1–PS4) OR (b) ≥ 2 Moderate (PM1–PM6) OR (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) OR (d) ≥ 2 Supporting (PP1–PP5) (ii) ≥ 2 Strong (PS1–PS4) OR (iii) 1 Strong (PS1–PS4) AND (a) ≥ 3 Moderate (PM1–PM6) OR (b) 2 Moderate (PM1–PM6) AND ≥ 2 Supporting (PP1–PP5) OR (c) 1 Moderate (PM1–PM6) AND ≥ 4 supporting (PP1–PP5)	Likely pathogenic	(i) 1 Very strong (PVS1) AND 1 moderate (PM1–PM6) OR (ii) 1 Strong (PS1–PS4) AND 1–2 moderate (PM1–PM6) OR (iii) 1 Strong (PS1–PS4) AND ≥ 2 supporting (PP1–PP5) OR (iv) ≥ 3 Moderate (PM1–PM6) OR (v) 2 Moderate (PM1–PM6) AND ≥ 2 supporting (PP1–PP5) OR (vi) 1 Moderate (PM1–PM6) AND ≥ 4 supporting (PP1–PP5)
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Overall Classification Rules

Benign	(i) 1 Stand-alone (BA1) <i>OR</i> (ii) ≥ 2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) <i>OR</i> (ii) ≥ 2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met <i>OR</i> (ii) the criteria for benign and pathogenic are contradictory

Germline Classification Example

Phenotype

- Global developmental delay
- Congenital nystagmus
- Dysmorphic facial features
- Short stature
- Microcephaly
- Ventriculomegaly
- Hypotonia



Previous Testing

- Chromosomal microarray
- Fragile X
- Methylation for PWS/AS

All tests were negative

Variants of Interest

Disease Gene (Transcript ID)	Genomic Change (GRCh37)	Nucleotide Change	Zygosity/ Inheritance	Predicted Protein Change	Associated Disease/ Condition
<i>THOC6</i> (NM_024339.3)	chr16:3076141 T>A	c.298T>A	Het/Mat	p.Trp100Arg	
<i>THOC6</i> (NM_024339.3)	chr16:3077171 G>C	c.700G>C	Het/Mat	p.Val234Leu	(AR) Beaulieu-Boycott-Innes syndrome (OMIM: 613680)
<i>THOC6</i> (NM_024339.3)	chr16:3077380 G>A	c.824G>A	Het/Mat	p.Gly275Asp	
<i>THOC6</i> (NM_024339.3)	chr16:3076762 A>G	c.566A>G	Het/Pat	p.Asp189Gly	(AR) Beaulieu-Boycott-Innes syndrome (OMIM: 613680)

THOC6



Encodes for subunit of multi-protein THO complex

Involved in coordination between transcription and mRNA processing



Associated with Beaulieu-Boycott-Innes Syndrome (Autosomal Recessive)

OMIM

THO COMPLEX, SUBUNIT 6; THOC6

Alternative titles; symbols

FUNCTIONAL SPLICEROSOME-ASSOCIATED PROTEIN, 35-KD; FSAP35

*HGNC Approved Gene Symbol: THOC6**Cytogenetic location: 16p13.3 Genomic coordinates (GRCh38): 16:3,024,034-3,027,749 (from NCBI)*

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key
16p13.3	Beaulieu-Boycott-Innes syndrome	613680	AR	3

PheneGene Graphics ▾



INHERITANCE

- Autosomal recessive

HEAD & NECK

Head

- Microcephaly

Face

- Tall forehead
- High anterior hairline

Eyes

- Deep-set eyes
- Short palpebral fissures
- Upslanting palpebral fissures
- Myopia

Nose

- Long nose
- Low-hanging columella

Mouth

- Thick vermillion of upper and lower lips

Teeth

- Dental malocclusion
- Caries

CARDIOVASCULAR

Heart

- Ventricular septal defect, membranous or muscular

Vascular

- Patent ductus arteriosus

GENITOURINARY

Internal Genitalia (Female)

- Ovarian failure, premature
- Endometriosis

Kidneys

THOC6-related developmental delay-microcephaly-facial dysmorphism syndrome

 Suggest an update

Disease definition

A rare, autosomal recessive, syndromic intellectual disability disorder characterized by global development delay, mild microcephaly, mild to severe intellectual disability and non-specific facial dysmorphism in association with variable multiple congenital anomalies including congenital heart defects, dental anomalies, cryptorchidism, renal and cerebral malformations. Short stature is frequent.

ORPHA:363444

Classification level: Disorder

Synonym(s):

BBIS

Beaulieu-Boycott-Innes syndrome

Inheritance: Autosomal recessive

Age of onset: Infancy, Neonatal

ICD-10: Q87.0

UMLS: -

MeSH: -

GARD: -

Prevalence: <1/1 000 000

OMIM: [613680](#)

MedDRA: -

PM2

Absent from controls (or at extremely low frequency if recessive)

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
▶ European (non-Finnish)	36	129008	0	0.0002791
▶ Other	2	7212	0	0.0002773
▶ European (Finnish)	3	25102	0	0.0001195
▶ Latino	4	35434	0	0.0001129
▶ African	2	24934	0	0.00008021
▶ East Asian	1	19950	0	0.00005013
▶ South Asian	1	30616	0	0.00003266
▶ Ashkenazi Jewish	0	10352	0	0.000
Male	31	153282	0	0.0002022
Female	18	129326	0	0.0001392
Total	49	282608	0	0.0001734
Include: <input checked="" type="checkbox"/> Exomes <input checked="" type="checkbox"/> Genomes				

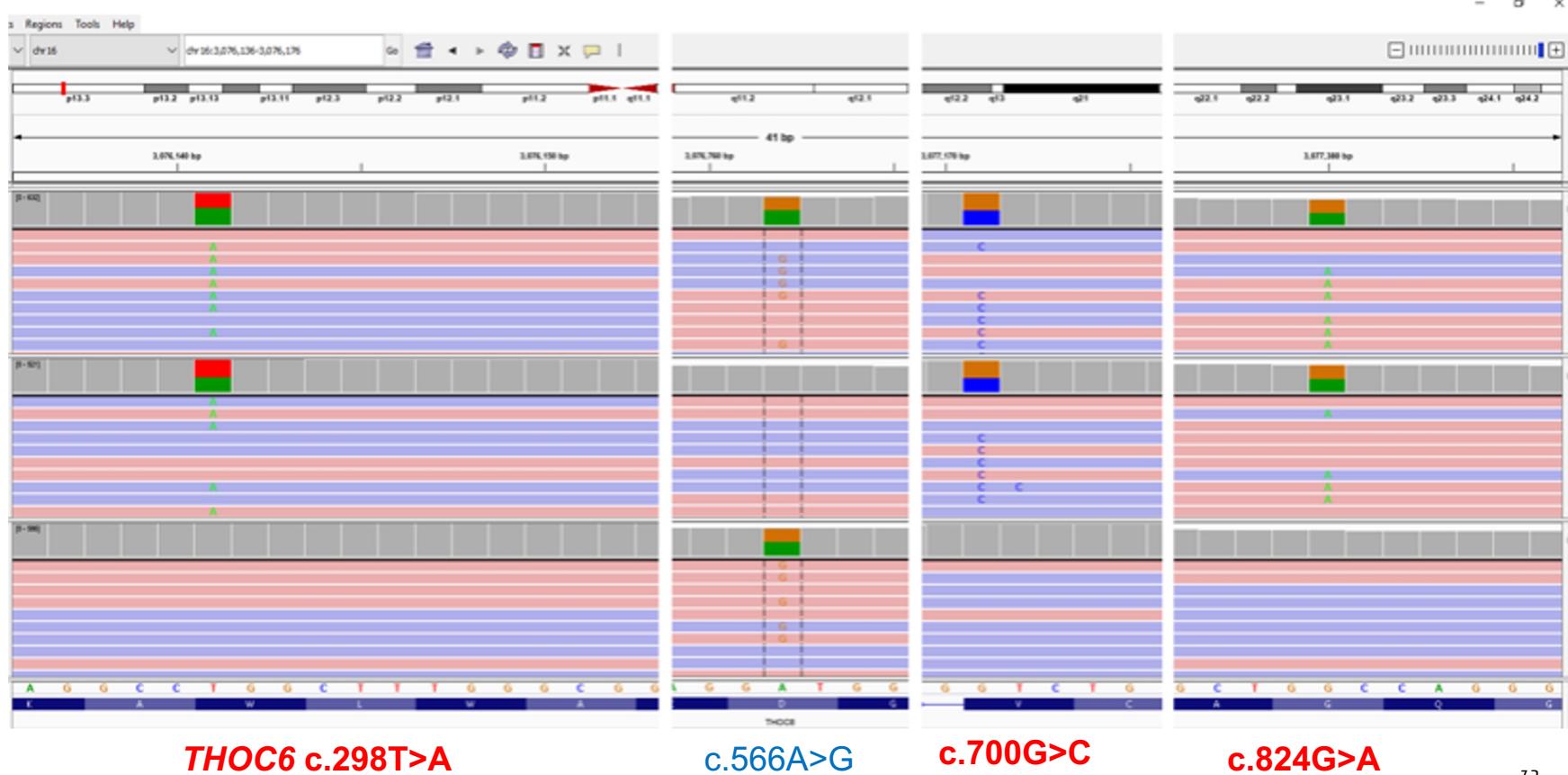
Haplotype

- Group of variants usually inherited together
- Paternal ancestry:
- Mixed
- European/Irish/Cherokee
- Maternal ancestry:
- Irish/German/French/Welsh

The haplotype c.(298 T>A; 700 G>C; 824 G>C) is recurrent in the European population

The haplotype composed of the three substitutions, c.(298 T>A; 700 G>C; 824 G>C), was recently described at the homozygous state in another individual presenting a BBIS form of ID (7). These three variants are also reported at the heterozygous state in the GnomAD database at the same MAF in the different subpopulations, suggesting they are in total linkage disequilibrium (Supplementary Material, Table S1). The highest frequency is obtained in the European population. In the 1,000 Genomes database, the variants are present in only one European individual with a British origin. Recent data from UK10K indicate a MAF of 0.001 for these variants in the population from United Kingdom ($n = 3,577$). Together, these data suggest that this haplotype may originate from this specific region. Interestingly, the three individuals with BBIS with this haplotype also have a Northern European origin: Patient 1 is from north of France, Patient 2 is with likely a Northern European descent (surname sounding English) and the patient reported by Casey et al. (7) is of Irish traveler origin. We compared the clinical

Investigation of the BAM files with IGV - PM3



ClinVar

- PP2 Calculation

- $\bullet \frac{1}{10} = 0.1$

- BP1 Calculation

- $\bullet \frac{9}{13} = 0.692$

- Select “Missense”
$$\frac{B+LB}{B+LB+VUS+LP+P}$$
- <10% is considered low rate of benign missense

- Select “LP + P”

- $$\frac{FS+NS+SS}{FS+NS+SS+Missense}$$

- >90% is considered LOF MOD

*Sample size < 20;
too low, do not apply criteria

NM_024339.5(THOC6):c.298T>A (p.Trp100Arg)

ClinVar - PP5

Interpretation:	Pathogenic/Likely pathogenic
Review status:	★★☆☆ criteria provided, multiple submitters, no conflicts
Submissions:	3 (Most recent: May 29, 2019)
Last evaluated:	Feb 2, 2019
Accession:	VCV000521347.4
Variation ID:	521347
Description:	single nucleotide variant

Submitted interpretations and evidence



Interpretation (Last evaluated)	Review status (Assertion criteria)	Condition (Inheritance)	Submitter	Supporting information (See all)
Likely pathogenic (Nov 14, 2018)	criteria provided, single submitter (ambry_reporting_categories_2017) Method: clinical testing	Hereditary disease Allele origin: germline	Ambry Genetics Accession: SCV000741882.1 Submitted: (Feb 08, 2018)	Evidence details Publications PubMed (7) Comment: Lines of evidence used in support of classification: LIKELY POSITIVE: Relevant Alteration(s) Detected
Pathogenic (Feb 02, 2019)	criteria provided, single submitter (ACMG Guidelines, 2015) Method: research	Beaulieu-Boycott-Innes syndrome (Autosomal recessive inheritance) Allele origin: germline	Medgenome Labs Pvt Ltd Accession: SCV000920786.1 Submitted: (May 29, 2019) Comment: The haplotype c. [298T>A; 700G>C; 824G>A] in the parents were heterozygous.	Evidence details
Likely pathogenic (Oct 12, 2018)	criteria provided, single submitter (ACMG Guidelines, 2015) Method: clinical testing	Beaulieu-Boycott-Innes syndrome Allele origin: germline	Baylor Genetics Accession: SCV000992695.1 Submitted: (Mar 14, 2019)	Evidence details Publications PubMed (1)

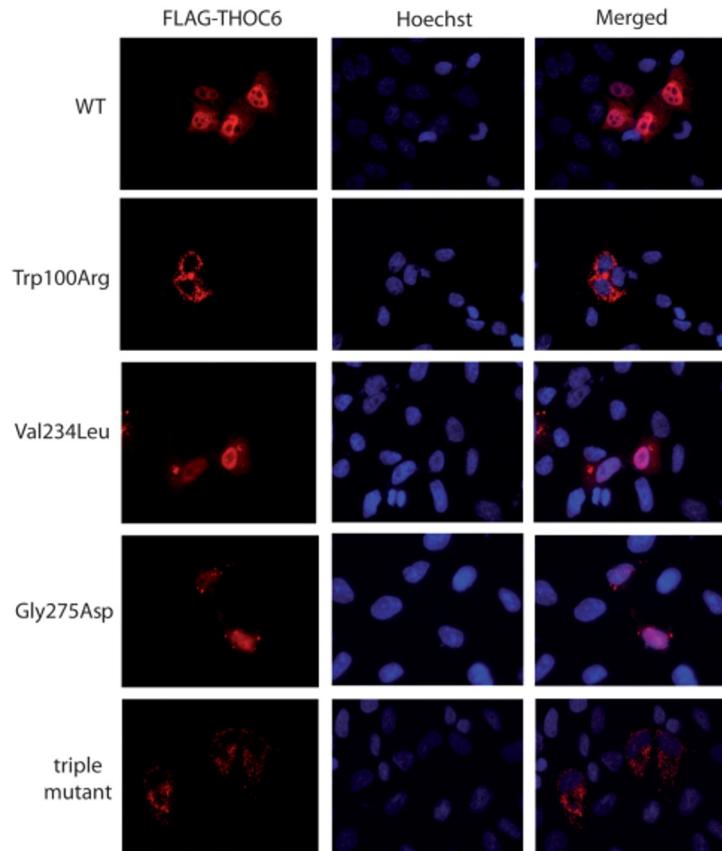
Varsome - Computational Evidence

Pathogenicity Scores				
	12	9	0	
BayesDel noAF dbNSFP version 4.2	noAF prediction Damaging	noAF score 0.1203	noAF rankscore 0.7825	
CADD  version 1.6	Score 29.3999			
DANN  version 2014	Score 0.9963			
EIGEN dbNSFP version 4.2	prediction Pathogenic	raw coding 0.5614	raw coding rankscore 0.7078	
EIGEN PC dbNSFP version 4.2	prediction Pathogenic	PC raw coding score 0.5506	PC raw coding rankscore 0.7144	PC phred coding score 5.6553
FATHMM-MKL  dbNSFP version 4.2	coding prediction Damaging	coding score 0.8963	coding rankscore 0.5022	
FATHMM-XF dbNSFP version 4.2	coding prediction Damaging	coding score 0.7164	coding rankscore 0.6682	
LRT  dbNSFP version 4.2	prediction Deleterious	score 0	converted rankscore 0.8433	Omega 0
Mutation assessor  dbNSFP version 4.2	prediction Medium	score 2.22	rankscore 0.6291	
MutationTaster  dbNSFP version 4.2	Prediction  Disease causing	Accuracy  0.9999	converted rankscore 0.5876	
PROVEAN  dbNSFP version 4.2	prediction Damaging	score -9.79, -9.32	converted rankscore 0.9866	
Polyphen2 HDIV  dbNSFP version 4.2	prediction Damaging	score 1	rankscore 0.9058	
Polyphen2 HVAR  dbNSFP version 4.2	prediction Damaging	score 0.992, 0.99	rankscore 0.8044	
SIFT4G dbNSFP version 4.2	prediction Damaging	score 0	converted rankscore 0.9282	

BayesDel addAF dbNSFP version 4.2	addAF prediction Tolerated	addAF score 0.03923	addAF rankscore 0.5695
DEGEN2 dbNSFP version 4.2	prediction Tolerated	score 0.3223	rankscore 0.6933
FATHMM  dbNSFP version 4.2	prediction Tolerated	score 1.01	converted rankscore 0.4106
LIST-S2 dbNSFP version 4.2	prediction Tolerated	score 0.7796	rankscore 0.4137
MVP dbNSFP version 4.2	prediction Benign	score 0.6046	rankscore 0.6015
MetaLR  dbNSFP version 4.2	prediction Tolerated	score 0.2405	rankscore 0.6084
MetaSVM  dbNSFP version 4.2	prediction Tolerated	score -0.6474	rankscore 0.6283
PrimateAI dbNSFP version 4.2	prediction Tolerated	score 0.7519	rankscore 0.7473
REVEL dbNSFP version 4.2	prediction Benign	score 0.3939	rankscore 0.7123

Conservation scores			
GERP  version 2010	NR  5.55	RS  5.55	

Mastermind - PM_PS3



Downgraded to moderate due to lack of technical replicates

Final Assessment

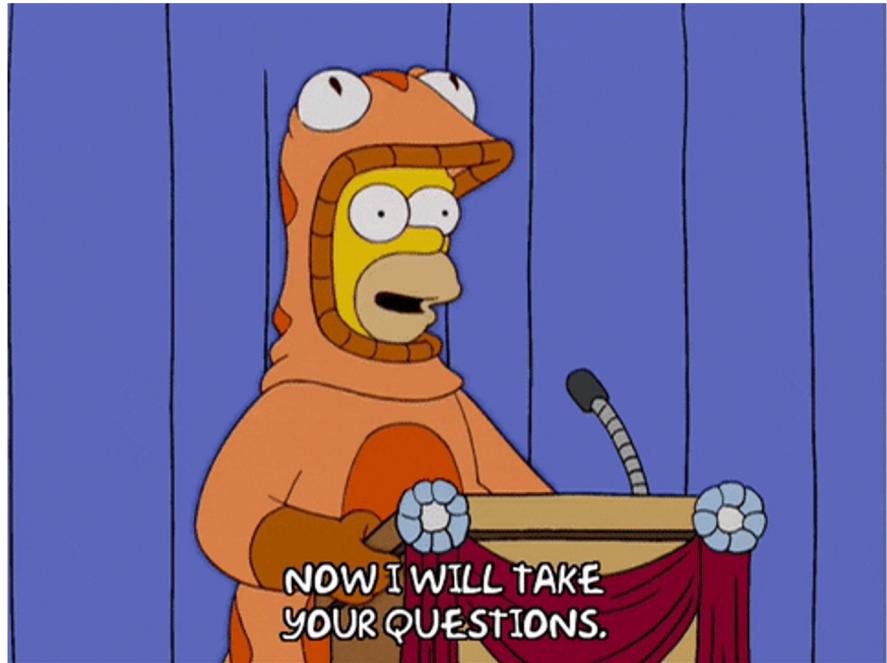
- PM2 - Absent from controls (or at extremely low frequency if recessive)
- PM3 - For recessive disorders, detected in trans with a pathogenic variant
- PM_PS3 - Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product (modulated to Moderate strength)
- PP5 - Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

Likely Pathogenic

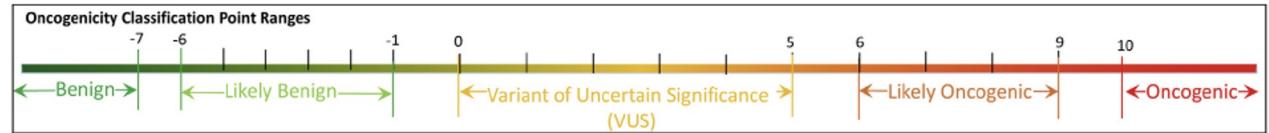


Stand Up and Stretch

Any questions?



Somatic



Evidence Strength	Benign			Oncogenic			
	Very Strong	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
POINTS	-8	-4	-1	+1	+2	+4	+8
Population Data	MAF is >5%	MAF is >1%		Absent in population databases			
Functional Data		Well-established functional studies show no oncogenic effects				Well-established functional studies supportive of an oncogenic effect	
Predictive Data			Silent mutation (no predicted impact on splicing)		Missense change at an amino acid residue where a different missense change determined to be oncogenic has been documented	Same amino acid change as a previously established oncogenic mutation	Null variant in tumor suppressor
Cancer Hotspots				Cancer hotspots with low frequency of recurrence	Cancer hotspot with moderate frequency of recurrence	Cancer hotspot with high frequency of recurrence	
Computational Evidence			All utilized lines of computational evidence suggest no impact of a variant	All utilized lines of computational evidence support oncogenicity			

Figure 1 Somatic standard operating procedure evidence framework overview (all criteria are not listed). MAF, minor allele frequency.

ClinGen/CGC/VICC Oncogenicity Guidelines (2022)

Variant
Identification

Variant
Annotation

Clinical
Significance

Oncogenicity
Classification

Clinical
Decision

Population Data

- OP4: Absent from controls (or at an extremely low frequency) in Genome Aggregation Database (gnomAD).
- SBVS1: Minor allele frequency is >5% in Genome Aggregation Database (gnomAD) in any of 5 general continental populations: African, East Asian, European (Non-Finnish), Latino, and South Asian.
- SBS1: Minor allele frequency is >1% in Genome Aggregation Database (gnomAD) in any of 5 general continental populations: African, East Asian, European (Non-Finnish), Latino, and South Asian.

Functional Data

- OS2: Well-established in vitro or in vivo functional studies supportive of an oncogenic effect of the variant. Note: Functional studies that have been shown to be reproducible and robust are considered the most well established.
- SBS2: Well-established in vitro or in vivo functional studies show no oncogenic effects.

Predictive Data

- OVS1: Null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a bona fide tumor suppressor gene.
- OS1: Same amino acid change as a previously established oncogenic variant (using this standard) regardless of nucleotide change.
 - Ex. Val→Leu caused by either G>C or G>T in the same codon
- OM4: Missense variant at an amino acid residue where a different missense variant determined to be oncogenic (using this standard) has been documented.
 - Ex. p.Arg156His is oncogenic; now you observe p.Arg156Cys.
- SBP2: A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Mutational Hotspot Data

- OS3: Located in one of the hotspots in cancerhotspots.org with at least 50 samples with a somatic variant at the same amino acid position, and the same amino acid change count in cancerhotspots.org in at least 10 samples.
- OM3: Located in one of the hotspots in cancerhotspots.org with less than 50 samples with a somatic variant at the same amino acid position, and the same amino acid change count in cancerhotspots.org is at least 10.
- OP3: Located in one of the hotspots in cancerhotspots.org and the particular amino acid change count in cancerhotspots.org is below 10.

Computational Evidence

- OP1: All utilized lines of computational evidence support an oncogenic effect of a variant (conservation/evolutionary, splicing impact, etc.).
- SBP1: All utilized lines of computational evidence suggest no impact of a variant (conservation/ evolutionary, splicing impact, etc.).

Other Data

- OM1: Located in a critical and well-established part of a functional domain.
 - Ex. active site of an enzyme, or in protein binding domain
- OP2: Somatic variant in a gene in a malignancy with a single genetic etiology.
 - Ex. retinoblastoma is caused by bi-allelic RB1 inactivation.

Example Assessment

- Tumor type: Neoplasm
- Variant: chr7:g.140453155C>T (*BRAF* p.Asp594Asn)

BRAF p.Asp594Asn (Neoplasm)

- Is this a null variant (OVS1)?
 - NO
- Is this the same amino acid change as a previously established oncogenic variant (**using this standard**) regardless of nucleotide change (OS1)?
 - NO - there is very little data online utilizing this standard at this point, so by extension we also can't apply OM4 at this point...
- Any well-established studies (OS2)?
 - Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS
 - YES (+4) - PMID: 28783719 (loss of kinase activity, signaling through CRAF), class 3 BRAF variant

Total score: 4

BRAF p.Asp594Asn (Neoplasm)

- Mutational Hotspot data (OS3)?
 - [Cancer Hotspots](#)
 - YES (+4) - AA 594 change count in cancerhotspots.org is ≥ 50 (51), p.Asp594Asn count 24
- Located in a functional domain (OM1)?
 - [cBioPortal](#)
 - YES BUT... This rule cannot be used if OS1 or OS3 is applicable.
- Is this an in-frame indel in a known oncogene or tumor suppressor, or a stop-loss in a tumor suppressor (OM2)?
 - NO
- Does computational evidence support an oncogenic effect (OP1)?
 - YES (+1) - REVEL score = 0.974

Total score = 4+4+1 (9)

BRAF p.Asp594Asn (Neoplasm)

- Is this a somatic variant in a gene in a malignancy with a single genetic etiology (OP2)?
 - NO
- Is this absent from controls (or at an extremely low frequency) in gnomAD (OP4)?
 - YES (+1) Absent from controls

Total score = 4+4+1+1 (10)

Final Assessment = 10, Oncogenic

Any questions about the Oncogenicity Guidelines?



Somatic

AMP/ASCO/CAP Guidelines

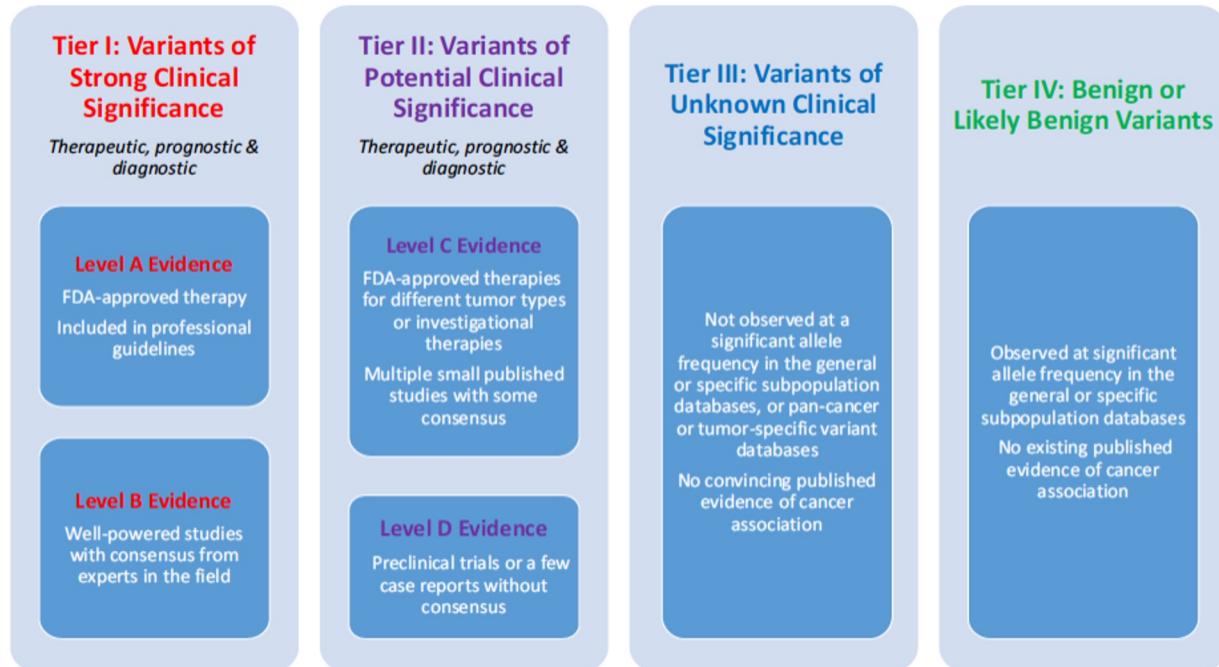
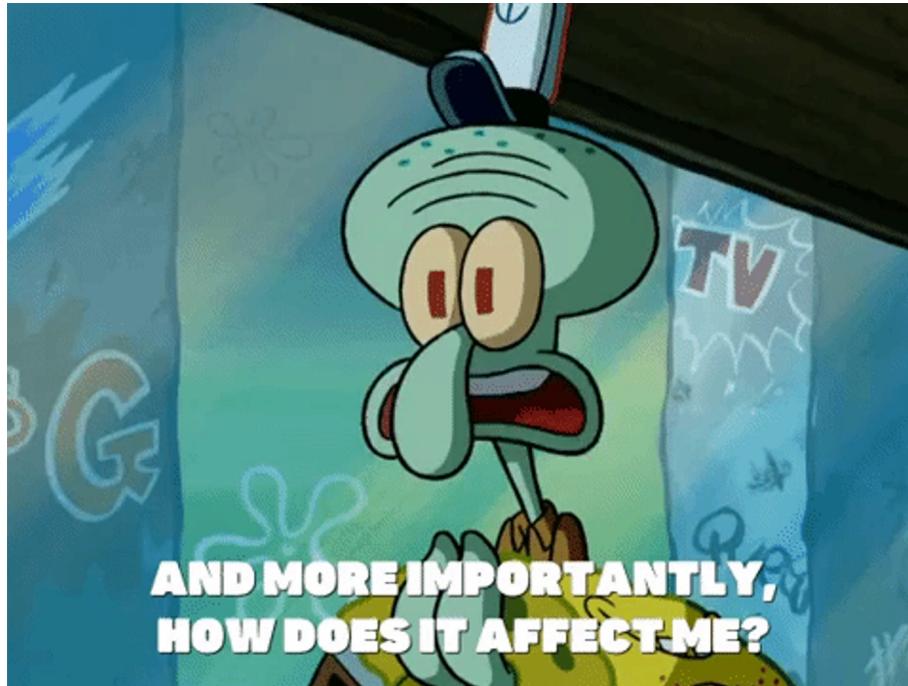


Figure 2 Evidence-based variant categorization. Somatic variants are classified into four tiers based on their level of clinical significance in cancer diagnosis, prognosis, and/or therapeutics. Variants in tier I are of strongest clinical significance, and variants in tier IV are benign or likely benign variants. FDA, Food and Drug Administration.

We are only going to focus on Therapeutic Response today...

CLINICAL ACTIONABILITY & SIGNIFICANCE!!!



AMP/ASCO/CAP

Table 4 Tier I: Variants with Strong Clinical Significance

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	Therapeutic: FDA approved or investigational with strong evidence* Diagnostic: In PG or reported evidence with consensus Prognostic: In PG or reported evidence with consensus
Mutation type	Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions
Variant frequencies	Mostly mosaic
Potential germline [†]	Mostly nonmosaic (VAF approximately 50% or 100%)
Population database: <i>ESP</i> , <i>dbSNP</i> , <i>1000Genome</i> , <i>ExAC</i>	Absent or extremely low MAF
Germline database: <i>HGMD</i> , <i>ClinVar</i>	May or may not be present
Somatic database: <i>COSMIC</i> , <i>My Cancer Genome</i> , <i>TCGA</i>	Most likely present
Predictive software: <i>SIFT</i> , <i>PolyPhen2</i> , <i>MutTaster</i> , <i>CADD</i>	Mostly damaging; information to be used for reference only
Pathway involvement	Disease-associated pathways
Publications: <i>functional study</i> , <i>population study</i> , <i>other</i>	Therapeutic: reported evidence with consensus Diagnostic: reported evidence with consensus Prognostic: reported evidence with consensus

Resources that can help you for Therapeutic Response...

- [FDA](#) - open to all
- [NCCN Guidelines](#) - open
- [WHO](#) - have to have a license
- [OncoKB](#) - available with commercial restrictions
- [CIViC](#) - open
- [Molecular Oncology Almanac](#) - open
- [VICC MetaKB](#) - open
- GOOGLE, but seriously...

Non-exhaustive list*

AMP/ASCO/CAP

Table 5 Tier II: Variants with Potential Clinical Significance

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	Therapeutic: FDA approved for different tumor type; investigational therapies with some evidence Diagnostic: not in PG but with convincing published data Prognostic: not in PG but with convincing published data
Mutation type	Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions
Variant frequencies	Mostly mosaic
Potential germline*	Mostly nonmosaic (VAF approximately 50% or 100%)
Population database: <i>ESP, dbSNP, 1000Genome, ExAC</i>	Absent or extremely low MAF
Germline database: <i>HGMD, ClinVar</i>	May or may not be present
Somatic database: <i>COSMIC, My Cancer Genome, TCGA</i>	Likely present
Predictive software: <i>SIFT, PolyPhen2, MutTaster, CADD</i>	Mostly damaging; information to be used for reference only
Pathway involvement	Involve disease-associated pathways or pathogenic pathways
Publications: <i>functional study, population study, other</i>	Therapeutic: evidence of using FDA-approved therapies for different tumor types; phase 2 or 3 clinical trials for investigational therapies Diagnostic: multiple lines of reported evidence without consensus Prognostic: multiple lines of reported evidence without consensus

AMP/ASCO/CAP

Table 6 Tier III: Variants of Unknown Clinical Significance

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	Cancer genes: none Noncancer genes (apply to cancer exome/whole genome sequencing): none
Mutation type	Functionally unknown; mostly missense, in-frame indels; less commonly, other types
Variant frequencies	Mosaic or nonmosaic
Potential germline*	Mostly nonmosaic (VAF approximately 50% or 100%)
Population database: <i>ESP, dbSNP, 1000Genome, ExAC</i>	Absent or extremely low MAF
Germline database: <i>HGMD, ClinVar</i>	Absent or downgraded from pathogenic to VUS
Somatic database: <i>COSMIC, My Cancer Genome, TCGA</i>	Absent or present without association to specific tumors (potential germline VUS); present but in very few cases
Predictive software: <i>SIFT, PolyPhen2, MutTaster, CADD</i>	Variable; information to be used for reference only
Pathway involvement	May or may not involve disease-associated pathways
Publications: <i>functional study, population study, other</i>	None or no convincing evidence to determine clinical/biological significance

AMP/ASCO/CAP

Table 7 Tier IV: Benign/Likely Benign Variants

Evidence source/type	Available evidence
FDA-approved therapies, PG, investigational therapies	None
Mutation type	Functionally benign or unknown; mostly missense; less commonly, other types
Variant frequencies	Mostly nonmosaic (VAF, approximately 50% or 100%)
Potential germline*	Mostly nonmosaic (VAF, approximately 50% or 100%)
Population database: <i>ESP</i> , <i>dbSNP</i> , <i>1000Genome</i> , <i>ExAC</i>	MAF \geq 1% in the general population; or high MAF in some ethnic populations
Germline database: <i>HGMD</i> , <i>ClinVar</i>	Absent or present but downgraded to benign/likely benign
Somatic database: <i>COSMIC</i> , <i>My Cancer Genome</i> , <i>TCGA</i>	Absent or present without association to specific tumors (potential rare germline polymorphism)
Predictive software: <i>SIFT</i> , <i>PolyPhen2</i> , <i>MutTaster</i> , <i>CADD</i>	Mostly benign; information to be used for reference only
Pathway involvement	May or may not involve disease-associated pathways
Publications: <i>functional study</i> , <i>population study</i> , <i>other</i>	Reported evidence supportive of benign/likely benign; or none

Example:

- Disease: Acute Myeloid Leukemia
- Variant: *FLT3* c.1773_1793dup (p.Glu598_Tyr599insAspValAspPheArgGluTyr)
 - ITD (internal tandem duplication)

Let's go check out the VICC MetaKB service to see what is known about *FLT3*

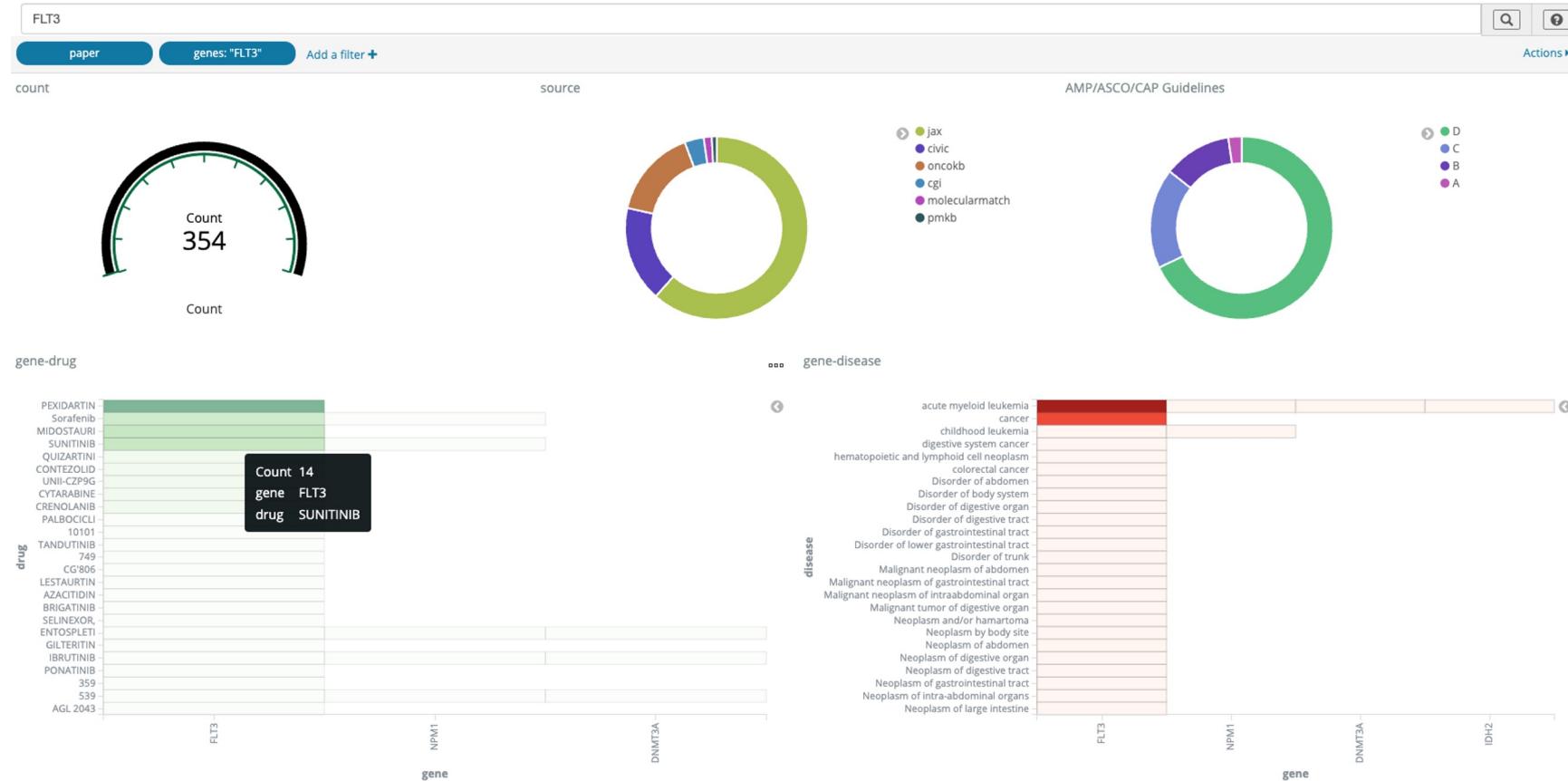
The Variant Interpretation for Cancer Consortium Meta-Knowledgebase

MetaKB

Welcome to the Variant Interpretation for Cancer Consortium (VICC) meta-knowledgebase (v1). This is a search interface for cancer variant interpretations assembled by aggregating and harmonizing across multiple cancer variant interpretation knowledgebases.

If this is your first time here, we recommend reading the [help documentation](#) and trying the example queries below. Please send any questions to help@cancervariants.org.

Also see our [paper](#) and license [FAQ](#). Other useful links: [Data Downloads](#), [API](#), and [analysis toolkit](#).



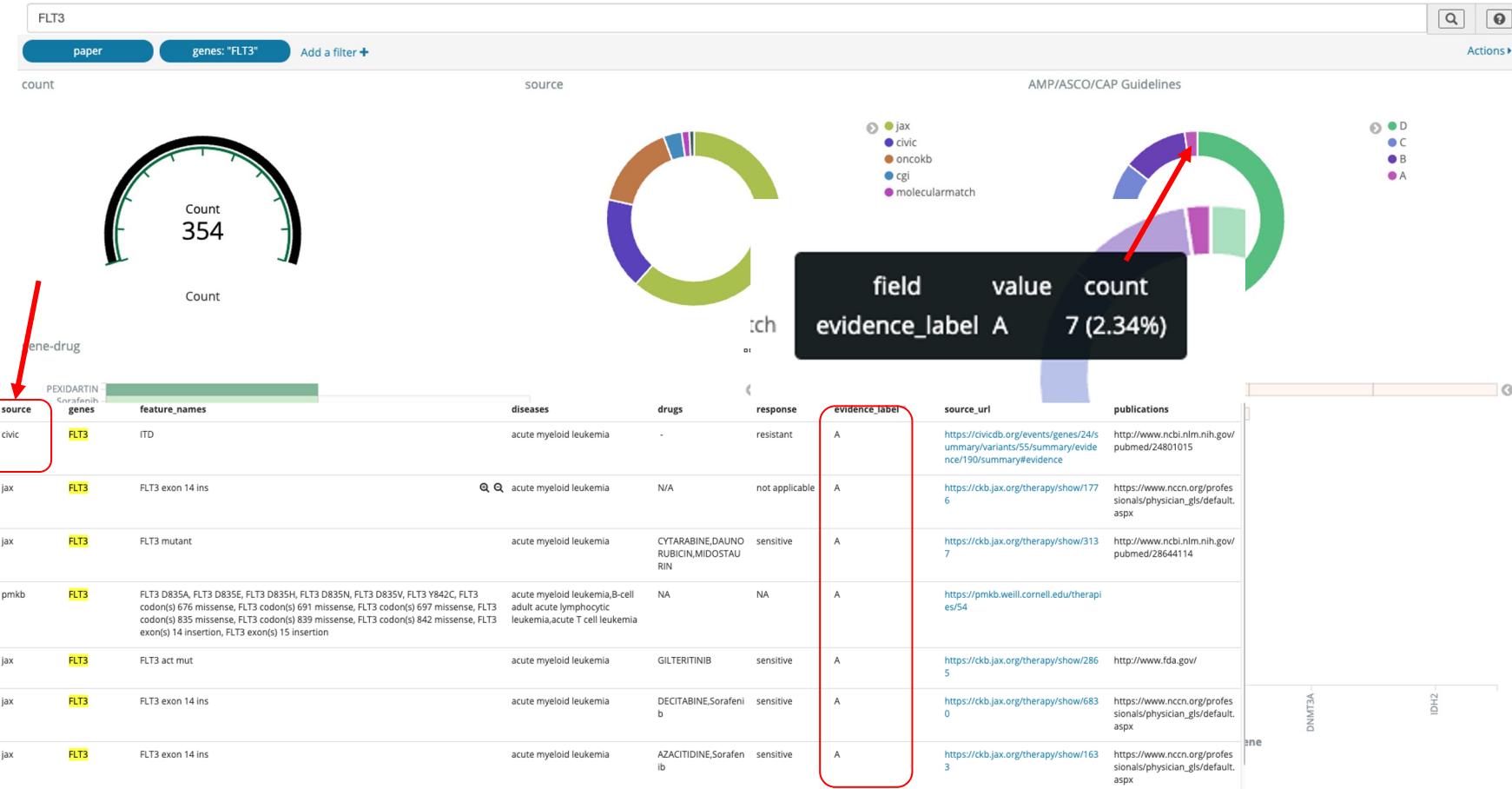
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Also see our [paper](#) and [license FAQ](#). Other useful links: [Data Downloads](#), [API](#), and [analysis toolkit](#).



Let's Check out what CIViC has on *FLT3* variants

CIViC *FLT3* Gene

FLT3 fms related receptor tyrosine kinase 3

[Revise](#) [Flag](#) [Bell](#)

Curators: None Editors: None

[Summary](#) [Comments](#) [Revisions](#) [Flags](#) [Events](#)

Description

FLT3 is an important cytokine receptor involved in normal hematopoiesis. Mutations in this gene are common in acute myeloid leukemia (AML) and screening for mutations in this gene has been recommended by the World Health Organization in patients with AML, particularly in cases of cytogenetically normal AML (CN-AML). FLT3 mutations commonly co-occur with mutations such as NPM1 that are associated with CN-AML and likely modulate prognostic impact. While FLT3-ITD mutations have been associated with poorer prognosis in AML, the prognostic impact of FLT3-TKD mutations are still up for debate.

Sources [PubMed: Stirewalt et al., 2003, Nat. Rev. Cancer](#) [PubMed: Vardiman et al., 2009, Blood](#)

Aliases CD135, FLK-2, FLK2, FLT3, STK1

Resources [DGIdb](#) [ProteinPaint](#)

MyGeneInfo

[Overview](#) [Summary](#) [Protein Domains \(10\)](#) [Pathways \(8\)](#)

Entrez Symbol: **FLT3** (ID: 2322) UniProtKB ID: P36888

Chromosome: 13 Strand: -1 Start: 28577411 Stop: 28674729

Aliases CD135, FLK-2, FLK2, STK1

Protein Domains Immunoglobulin, Immunoglobulin-like domain, Protein kinase domain, Protein kinase, ATP binding site, Protein kinase-like domain, Serine-threonine/tyrosine-protein kinase catalytic domain, Tyrosine-protein kinase, CSF-1/PDGF receptor family, Tyrosine-protein kinase, active site, Tyrosine-protein kinase, catalytic domain... [Expand](#)

Pathways Cytokine-cytokine receptor interaction - Homo sapiens (human), Hematopoietic cell lineage - Homo sapiens (human), Pathways in cancer - Homo sapiens (human), Transcriptional misregulation in cancer - Homo sapiens (human), Acute myeloid leukemia - Homo sapiens (human), Central carbon metabolism in cancer - H... [Expand](#)

Molecular Profiles **Variants**

71 Total Filter: Molecular Profile Names Show: All Molecular Profiles

[FLT3 ITD](#) [FLT3 TKD MUTATION](#) [FLT3 Mutation](#) [FLT3 D835](#) [FLT3 Y842C](#) [FLT3 D835](#) [FLT3 T227M](#) [FLT3 D835H](#) [FLT3 Overexpression](#) [FLT3 D835Y](#) [FLT3 F691L](#) [FLT3 D835I](#) [FLT3 D835H/Y](#) [FLT3 D835E](#) [FLT3 E611_F612INS25](#) [FLT3 G846S](#) [FLT3 Y591_V592INSVDFREYE](#) [FLT3 E588_Y589NSKYFYVDFRE](#) [FLT3 V592_D593NSDFREY](#) [FLT3 Amplification](#) [FLT3 FLT3](#) [FLT3 ITD N676K](#) [FLT3 G697R](#) [FLT3 N676K](#) [FLT3 Y693](#) [FLT3 ITD & TKD MUTATIONS](#) [FLT3 D839G](#) [FLT3 M664I](#) [FLT3 K663Q](#) [FLT3 D839A](#) [FLT3 D839N](#) [FLT3 D839H](#) [FLT3 N841I](#) [FLT3 ITD&F691\(I/L\)](#) [FLT3 ITD & D835\(V/Y/F/H\)](#) [FLT3 ITD & Y842C](#) [FLT3 ITD & D839G](#) [FLT3 G697S](#) [FLT3 N841K](#) [FLT3 ITD and co-mutations](#) [FLT3 ITD D651G](#) [FLT3 ITD N676D](#) [FLT3 ITD I687F](#) [FLT3 D835 & I836](#) [FLT3 I836](#) [FLT3 Y589D](#) [FLT3 Y842H](#) [FLT3 ITD & Y597F](#) [FLT3 ITD & L601F](#) [FLT3 N841T](#) [FLT3 Q575Δ](#) [FLT3 Y572Δ](#) [FLT3 E573Δ](#) [FLT3 S574Δ](#) [FLT3 I836S](#) [FLT3 I836T](#) [FLT3 ITD & N841K](#) [FLT3 N841](#) [FLT3 Q575del](#) [FLT3 ITD AND KDM6A Loss](#) [FLT3 Overexpression AND FLT3LG Expression](#) [FLT3 D835N](#) [FLT3 D835G](#) [FLT3 D593del](#) [FLT3 D385](#) [FLT3 internal tandem duplication](#) [FLT3 D835 AND FLT3 I836 AND FLT3 internal tandem duplication](#) [FLT3 ITD AND FLT3 D835 AND FLT3 I836](#) [FLT3 TKD MUTATION AND FLT3 D835Y](#) [FLT3 ITD AND FLT3 D835Y](#) [FLT3 ITD AND FLT3 D835H](#)



FLT3 MP

Description	Sources
FLT3-ITD (internal tandem duplications) frequently occur in patients with hematologic malignancies such as chronic myelogenous leukemia, acute myeloid leukemia (AML) and myelodysplastic syndrome, but particularly in cytogenetically normal AML (CN-AML). These duplication events disrupt the juxtamembrane domain of FLT3 and can be the result of a duplication of internal FLT3 sequence or other unrelated sequence resulting in an in-frame duplication event. The length of these duplications can vary widely which may have prognostic consequences, but this has not been conclusively determined. FLT3-ITD mutations overall have generally been associated with poor prognosis. Additional genes associated with CN-AML such as NPM1 may modulate the prognosis associated with this variant.	Not specified
Molecular Profile Score	Aliases
448.5	Not specified

MP Variants

1 ITD

Variant	ITD	Gene	FLT3
Aliases	None specified	Variant Type	Inframe Insertion
Allele Registry ID	None provided	ClinVar ID	16270
OpenCRAVAT	Allele Registry ID required.	Other Molecular Profiles	FLT3 ITD AND KDM6A... FLT3 ITD AND FLT3... + view 3 more

> Representative Variant Coordinates

Evidence 35 of 39 displayed

EID	Disease	Therapies	IT	DESC	EL	ET	ED	S	VO	R
EID190	Acute Myeloid Leukemia	N/A	N/A		A					3 ★
EID69	Acute Myeloid Leukemia	N/A	N/A		B					5 ★
EID1515	Acute Myeloid Leukemia	N/A	N/A		B					5 ★
EID7061	Acute Myeloid Leukemia	Midostaurin	N/A		B					5 ★
EID9070	Acute Myeloid Leukemia	Hematopoietic Cell... Sorafenib	...		B					5 ★
EID52	Acute Myeloid Leukemia	N/A	N/A		B					4 ★
EID128	Acute Myeloid Leukemia	N/A	N/A		B					4 ★

FLT3 ITD Assertions 1 of 1 displayed

AID	Molecular Profile	Disease	Therapies	IT	SUM	AT	AD	S	CAT	Count
AID38	FLT3 ITD	Acute Myeloid Leukemia	Gilteritinib	N/A						4



CIViC FLT3 ITD Assertion

Assertions / AID38 / Summary

Related to AID38: [FLT3 ITD](#)

AID38

[Summary](#) [Comments](#) [Revisions](#) [Flags](#) [Events](#)

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Curators: Editors:

Summary

FLT3 internal tandem duplication (ITD) mutations in relapsed / refractory acute myeloid leukemia (AML) are sensitive to Gilteritinib, a Type I FLT3 inhibitor.

Description

The constitutively activating FLT3 internal tandem duplication (ITD) mutation appears in approximately 20-30% of AML patients and are associated with high risk, high relapse rates and poor clinical outcome. The type I selective second-generation oral inhibitor, gilteritinib, received initial global approval for use to treat adults with relapsed or refractory (R/R) FLT3 ITD positive AML in Japan in September 2018. Based on interim results of the ADMIRAL trial (Perl AE, et al., 2019), the FDA approved the drug for treatment of adult R/R AML with FLT3 ITD mutations in November 2018. The FDA approved companion diagnostic, LeukoStrat CDx FLT3 Mutation assay.

Status	Submitted (Oct 30, 2020)	Accepted (Dec 2, 2020)
Accepted	by ShrutiRao	by JasonSaliba
Molecular Profile Name	FLT3 ITD	MP Expression FLT3 ITD
Disease	Acute Myeloid Leukemia	Phenotype None Specified
Therapy	Gilteritinib	Regulatory Approval
FDA Companion Test		NCCN Guideline Acute Myeloid Leukemia (2.2021)

Type	Predictive	Direction	Supports	Significance	Sensitivity / Response
Variant Origin	Somatic			AMP/ASCO/CAP Category	Tier I - Level A
ClinGen/GCG/VICC Codes	Not applicable			ACMG Codes	Not applicable

AID38 Evidence 4 of 4 displayed

EID	Molecular Profile	Disease	Therapies	IT	DESC	EL	ET	ED	S	1	R
EID7728	FLT3 Mutation	Acute Myeloid Leukemia	Gilteritinib	N/A		B					
EID7283	FLT3 Mutation	Acute Myeloid Leukemia	Gilteritinib	N/A		B					
EID8924	FLT3 ITD	Acute Myeloid Leukemia	Gilteritinib	N/A		D					
EID8923	FLT3 ITD	Acute Myeloid Leukemia	Gilteritinib	N/A		D					

1

Internal tandem
duplication (572_630ins)

Acute Myeloid Leukemia

Gilteritinib

1

FDA-recognized biomarker predictive of
response to an FDA-approved drug in this
indication

Gilteritinib is a small molecule multiple tyrosine kinase inhibitor that is FDA-approved for adult patients who have relapsed/refractory (R/R) acute myeloid leukemia (AML) with a FLT3 mutation. FDA approval was based on the results of the open-label, international, multicenter ADMIRAL trial ([NCT02421939](#)) of gilteritinib in 138 adult patients with R/R AML having a FLT3 ITD, D835, or I836 mutation, in which the rate of complete remission/complete remission with partial hematologic recovery was 21% (95% CI= 14.5, 28.8) and the median duration of response was 4.6 months ([PMID: 31665578](#), [28516360](#), [28645776](#)) ([Abstract: Levis et al. Abstract# 7000, ASCO 2019.](#)). In the final study results of the Phase I study of gilteritinib in 80 adult patients (FLT3 mutation-positive, n=44 [FLT3-ITD, n=33]) with (R/R) AML with a FLT3 mutation, complete remission rates were 79.7% overall and 90.9% among FLT3 mutation-positive patients, and overall survival was 38.6 months (21.7, NE) overall and 45.9 months (30.8, NE) among FLT3 mutation-positive patients ([Abstract: Prazt et al. Abstract# AML-256, Clinical Lymphoma, Myeloma & Leukemia 2022.](#))[01255-1/fulltext](#)). [Show less](#)

Questions?



Let's do some classifications together!



Order of Operations

1. Perform Oncogenicity Assessment
2. Perform Clinical Actionability Assessment

Variant #1

- Disease: Acute Myeloid Leukemia
- Variant: *IDH1* NC_000002.12:g.208248389G>A, p.Arg132Cys

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

What can we eliminate first?

- OVS1: This is not a null variant
- OS1 & OM4: No currently available data using this standard
- OM2: Not an in-frame indel or stop-loss variant
- OP2: Not a single gene etiology malignancy
- SBP2: Not a splice site variant

Where should we go next? What gives us the most bang for our buck?

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

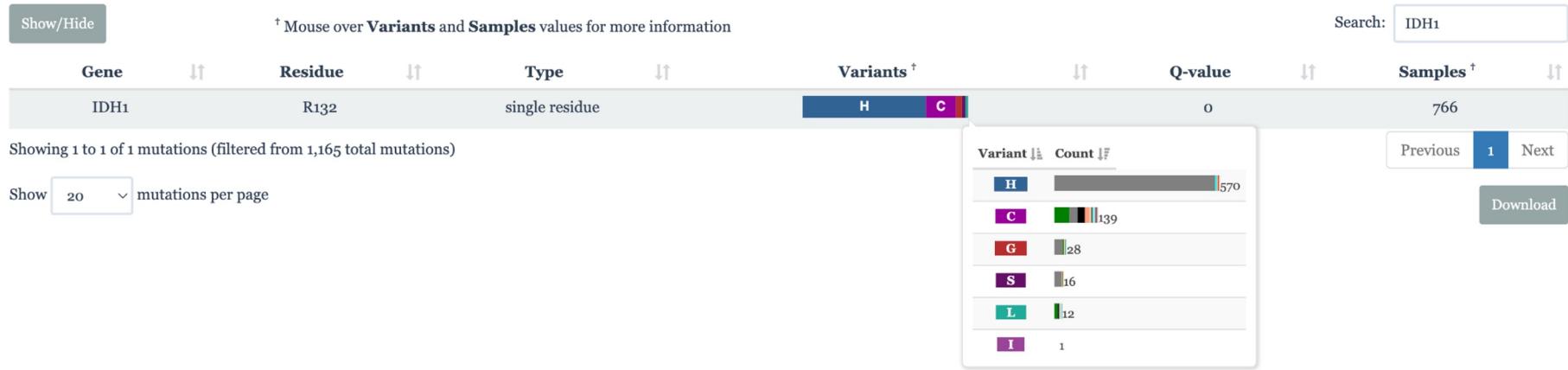
gnomAD v4

Variant ID	Source	HGVS Consequence	VEP Annotation	LoF Curation	Clinical Significance	Flags	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
2-208248376-C-T	E	p.Gly136Glu	● missense				1	833012	1.20e-6	0
2-208248378-A-G	E	p.Tyr135Tyr	● synonymous				6	1461770	4.10e-6	0
2-208248380-A-G	G	p.Tyr135His	● missense				1	151688	6.59e-6	0
2-208248385-T-G	G	p.His133Pro	● missense				1	151956	6.58e-6	0
2-208248386-G-A	E	p.His133Tyr	● missense				1	1461746	6.84e-7	0
2-208248388-C-T	E	p.Arg132His	● missense		Pathogenic/Likely p...		16	1613530	9.92e-6	0
2-208248388-CGA...	E	p.Ile128MetfsTer12	● frameshift				2	1461746	1.37e-6	0
2-208248390-A-T	E	p.Gly131Gly	● synonymous		Likely benign		32	1461844	2.19e-5	0
2-208248391-C-A	E	p.Gly131Val	● missense				2	1461838	1.37e-6	0

OP4: Absent from controls

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

Cancer Hotspots



OS3: AA position count in cancerhotspots.org is > 50 (766) and AA change count is ≥ 10 (139)

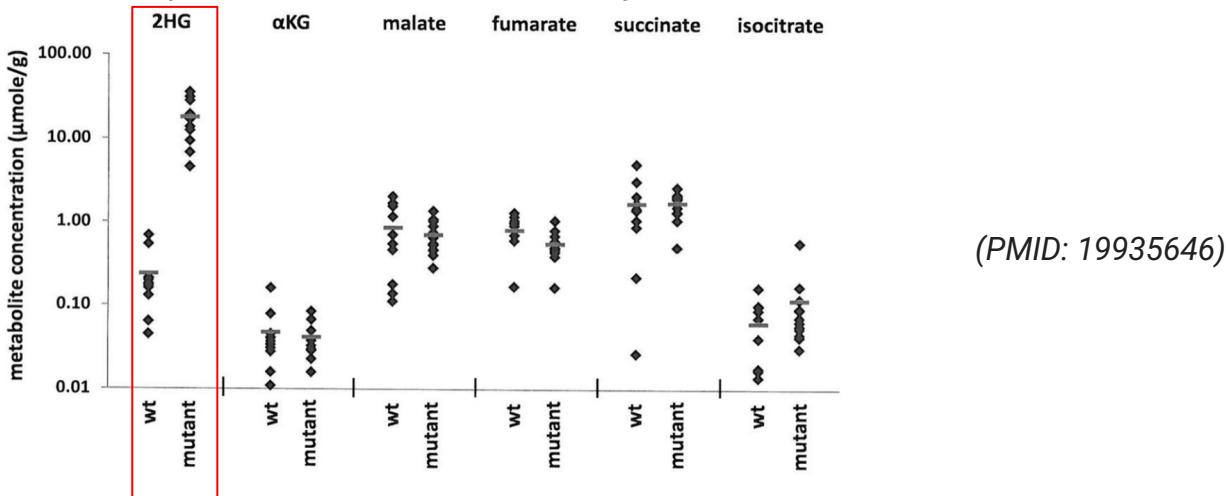
IDH1 p.Arg132Cys & Acute Myeloid Leukemia

In-silico predictor score

- REVEL: 0.9401 (Predicted Pathogenic)

OP1: Predicted Pathogenic

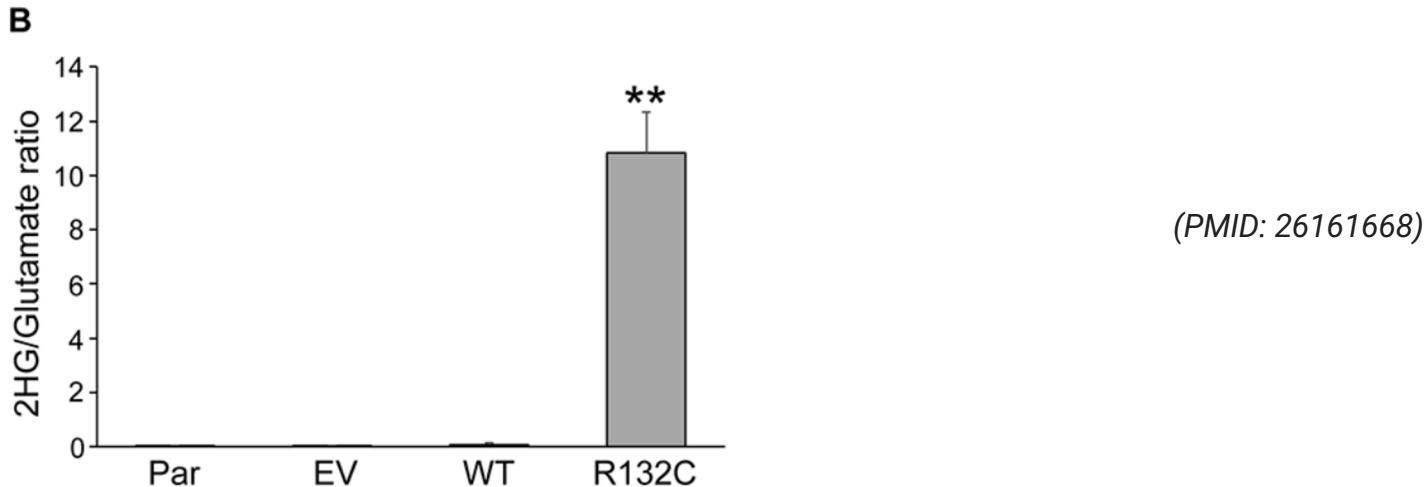
IDH1 p.Arg132Cys & Acute Myeloid Leukemia



(PMID: 19935646)

All R132 mutant IDH1 tumors examined had between 5 and 35 μmol of 2HG per gram of tumor, while tumors with wild-type IDH1 had over 100 fold less 2HG. This increase in 2HG in R132 mutant tumors was statistically significant ($p<0.0001$). We confirmed that (R)-2HG was the isomer present in tumor samples (data not shown). Together these data establish that the novel enzymatic activity associated with R132 mutations in IDH1 results in the production of 2HG in human brain tumors that harbor these mutations.

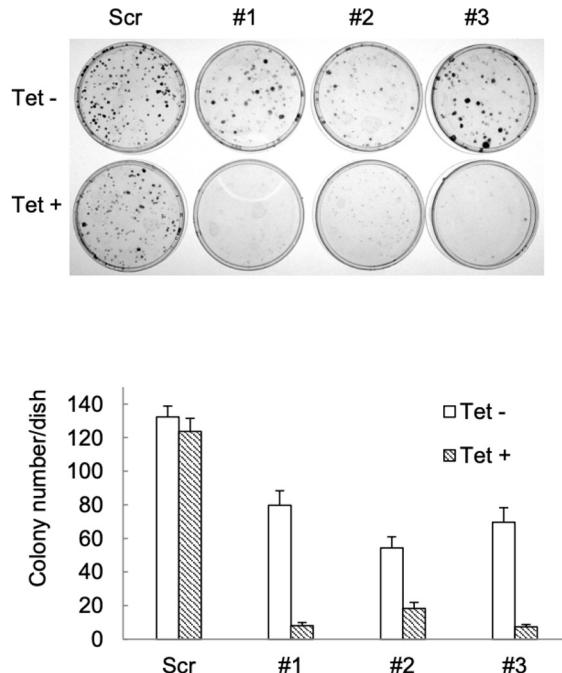
IDH1 p.Arg132Cys & Acute Myeloid Leukemia



Similar results were obtained in the other two hMSCs (data not shown), and hMSCs expressing *IDH1* R132C produced markedly larger amounts of 2-HG than cells expressing the exogenous wild-type *IDH1* gene, in which the relative amount of 2-HG was equal to that in parental or cells infected with the empty vector ([Fig 1B](#)).

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

B



As shown in Figure 1, treatment with 200ng/ml tetracycline for 96 hours efficiently knocked down both wild type and mutant IDH1 expression in three different IDH1 inducible knockdown HT1080 clones by the shRNA. Decreased IDH1 expression led to a ten-fold decrease in levels of D-2HG (Figure 1B).

(PMID: 22885298)

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

Oncogenicity Assessment

- OP4: Absent from controls, gnomAD v4
- OS3: hotspot in cancerhotspots.org, AA position count in cancerhotspots.org is > 50 (766) and AA change count is ≥ 10 (139).
- OP1: REVEL prediction pathogenic (score 0.9541)
- OS2: gain of alternative enzymatic activity (producing 2HG) (PMID: 19935646, 26161668, 22885298)

Final Oncogenicity Classification: **Oncogenic (10 points)**

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

▼ Biomarker-Directed Therapies

[View Therapies for IDH1 R132C](#)

IDH1 R132C is a predictive biomarker for use of ivosidenib in patients.

Of the therapies with IDH1 R132C as a predictive biomarker, 1 is FDA-approved and 1 has NCCN guidelines in at least one clinical setting.

Acute myeloid leukemia, cholangiocarcinoma, and chondrosarcoma have the most therapies targeted against IDH1 R132C or its related pathways [5].

Ivosidenib	-
Acute Myeloid Leukemia	-
Biomarker Criteria: Sample must match one or more of the following: IDH1 R132C, IDH1 R132G, IDH1 R132H, IDH1 R132L, IDH1 R132S	<u>Predicted Response:</u> Primary Sensitivity <u>Clinical Setting(s):</u> First Line of Therapy (FDA), Refractory (FDA, NCCN), Relapse (FDA, NCCN)
	<u>Note:</u> Approved for adult patients with newly diagnosed or relapsed/refractory acute myeloid leukemia (AML) with a susceptible IDH1 mutation.

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

1

R132C

Acute Myeloid Leukemia

Ivosidenib

Ivosidenib is a small molecule inhibitor of mutant IDH1 that is FDA-approved for adult patients with relapsed or refractory acute myeloid leukemia (AML) with a susceptible IDH1 mutation. FDA approval was based on the results from a Phase I dose escalation and expansion study of ivosidenib in IDH1-mutant AML in which 27 and eleven of 125 patients who received ivosidenib achieved complete remission and complete remission with partial hematologic recovery, respectfully, yielding a complete response rate of 30.4% ([PMID: 29860938](#)). In the multicenter, double-blind, randomized, placebo-controlled Phase III AGILE study of combination treatment of ivosidenib and azacitidine in 146 patients with newly diagnosed, untreated IDH1-mutant AML (ivosidenib plus azacitidine treatment, n=72; placebo and azacitidine treatment, n=72), 46.2% maintained red blood cell transfusion independence in the ivosidenib plus azacitidine group and 17.5% in the placebo plus azacitidine group ([Abstract: Dohner et al. Abstract# 7042, ASCO 2022.](#)). Preclinical studies in glioma and acute myeloid leukemia (AML) models demonstrated that ivosidenib promotes cellular differentiation by inhibiting the production of the mutant IDH1 "oncometabolite," 2-hydroxyglutarate (2-HG), which can inhibit cell growth *in vitro* ([PMID: 23558169, 23393090](#)) ([Abstract: Hansen et al. Abstract# 3734, ASH 2014.](#)). Show less

IDH1 p.Arg132Cys & Acute Myeloid Leukemia

1	R132C	Acute Myeloid Leukemia	Olutasidenib	Olutasidenib is a small-molecule inhibitor of mutant IDH1 that is FDA-approved in patients with acute myeloid leukemia (AML) with a susceptible IDH1 mutation. FDA approval was based on the results of the Phase II cohort of the phase I/II trial (Study 2102-HEM-101) of olutasidenib in 147 evaluable IDH1 inhibitor-naïve patients with relapsed/refractory IDH1 R132-mutant AML in which the complete remission (CR) plus complete remission with partial hematologic recovery (CRh) rate was 35% (n=51; 95%CI= 27, 43), with a median duration of CR + CRh of 25.9 months (95% CI= 13.5, NR). The median duration of CR alone was 28.1 months (95% CI= 13.8, NR), and the observed duration of CRh (n=4) was 1.8, 5.6, 13.5 and 28.5+ months, respectively (Abstract: Cortes et al. Abstract #6193, ASH 2022.). Show less
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IDH1 p.Arg132Cys & Acute Myeloid Leukemia

AMP/ASCO/CAP Classification

Final Assessment: Tier I Level A, strong clinical significance with Ivosidenib and Olutasidenib

Variant #2

- Disease: Follicular Lymphoma
- Variant: *EZH2* NC_000007.14:g.148811635T>A, p.Tyr646Phe

EZH2 p.Tyr646Phe & Follicular Lymphoma

What can we eliminate?

- OVS1: This is not a null variant
- OS1 & OM4: No currently available data using this standard
- OM2: Not an in-frame indel or stop-loss variant
- OP2: Not a single gene etiology malignancy
- SBP2: Not a splice site variant

EZH2 p.Tyr646Phe & Follicular Lymphoma

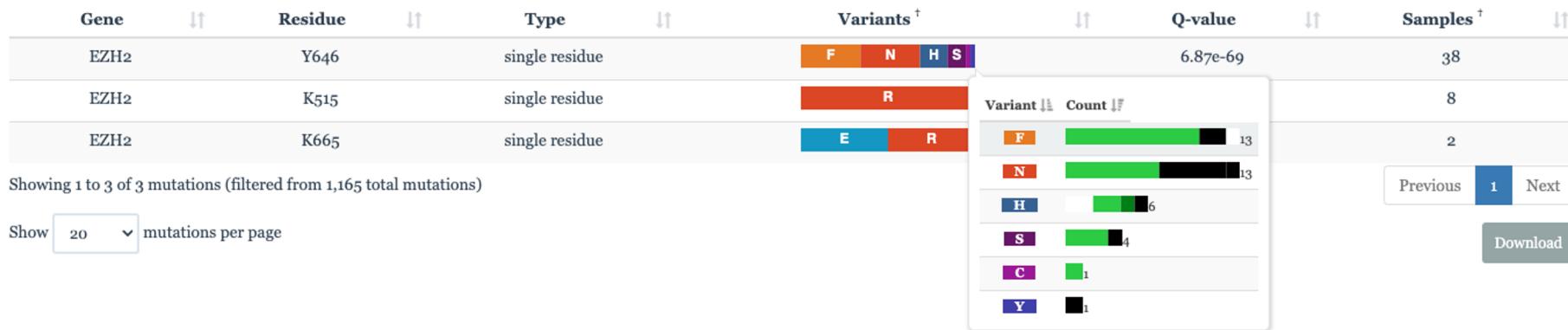
gnomAD v4

7-148811625-C-T			p.Glu649Glu		Uncertain significance...	106	1612492	6.57e-5	0
7-148811646-G-T			p.Phe642Leu			1	628370	1.59e-6	0
7-148811649-T-C			p.Glu641Glu			1	833096	1.20e-6	0
7-148811650-TC-T			p.Glu641AsnfsTer34			1	628408	1.59e-6	0
7-148811651-C-G			p.Glu641Gln			9	985274	9.13e-6	0
7-148811652-A-C			p.Asn640Lys			1	1461496	6.84e-7	0

OP4: Absent from controls

EZH2 p.Tyr646Phe & Follicular Lymphoma

Cancer Hotspots



OM3: AA position count in cancerhotspots.org is < 50 (38) and AA change count is ≥ 10 (13)

EZH2 p.Tyr646Phe & Follicular Lymphoma

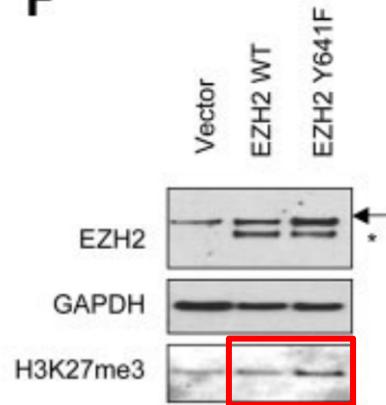
In-silico Predictor

- REVEL: 0.9547 (Predicted Pathogenic)

OP1: Predicted Pathogenic

EZH2 p.Tyr646Phe & Follicular Lymphoma

F

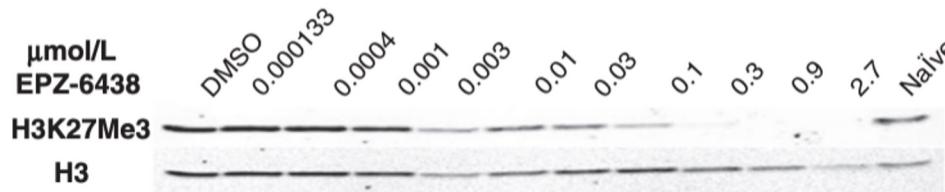


B-cell lineages in the mouse. Primary murine primary bone marrow cells were transduced with retroviruses expressing GFP and either wild-type EZH2 or EZH2^{Y641F} and differentiated into the B-cell lineage in the presence of IL7 and FLT3-L. Measurement of total H3K27me3 again showed increased H3K27me3 (Figure 2F) in cells bearing EZH2^{Y641F}, whereas EZH2^{WT} constructs did not confer this phenotype (Figure 2F).

(PMID: 21190999)

EZH2 p.Tyr646Phe & Follicular Lymphoma

B



(PMID: 24563539)

Similar to its effect in rhabdoid tumor cells, EPZ-6438 treatment of the *EZH2* Y646F-mutant lymphoma cell line WSU-DLCL2 for 4 days induced a concentration-dependent reduction in global H3K27Me3 levels with an IC₅₀ value of 9 nmol/L (H3K27Me3 levels determined by immunoblot; Fig. 1B and Table 1).

EZH2 p.Tyr646Phe & Follicular Lymphoma

Oncogenicity Assessment:

- OS2: gain of function demonstrated by altered substrate specificity and increased catalytic efficiency for H3K27 trimethylation (PMID: 21190999, 24563539, 30692681)
- OM3: AA position count in cancerhotspots.org is < 50 (38) and AA change count is ≥ 10 (13)
- OP4: absent from controls (gnomAD v4)
- OP1: REVEL prediction pathogenic (score 0.9547)

Final Oncogenicity Classification: **Likely Oncogenic (8 points)**

EZH2 p.Tyr646Phe & Follicular Lymphoma

Level	Alterations	Level-associated cancer types <small> ⓘ</small>	Drugs	Description
1	Y646F, Y646N, A682G, A692V, Y646H, Y646S, Y646C	Follicular Lymphoma	Tazemetostat	Tazemetostat is a small molecule inhibitor of EZH2 that is FDA-approved for adult patients with relapsed or refractory follicular lymphoma (FL) whose tumors are positive for an EZH2 mutation as detected by an FDA-approved test and who have received at least two prior systemic therapies, or who have no satisfactory alternative treatment options. Approval was based on the open-label, multicenter, phase II trial of tazemetostat in 99 patients with mutant (n=45) or wildtype (n=54) EZH2 relapsed or refractory FL in which the objective response rate was 77% (95% CI= 61.4-88.2) in the EZH2 mutant group versus 34% (95% CI=21.5-48.3) in the EZH2 wildtype group, with median progression-free survival of 11.1 months (95% CI=8.4-15.7) versus 5.7 (95% CI=3.5-11.1) months, respectively (PMID: 33035457). <small>Show less</small>

EZH2 p.Tyr646Phe & Follicular Lymphoma

AMP/ASCP/CAP Classification

Final Classification: Tier I Level A, strong clinical significance with Tazemetostat

Variant #3

- Disease: Non-Small Cell Lung Carcinoma
- Variant: *PIK3CA* NC_000003.12:g.179209622A>G, p.Ile391Met

PIK3CA p.Ile391Met & Non-Small Cell Lung Carcinoma

What can we eliminate?

- OVS1: This is not a null variant
- OS1 & OM4: No currently available data using this standard
- OM2: Not an in-frame indel or stop-loss variant
- OP2: Not a single gene etiology malignancy
- SBP2: Not a splice site variant

PIK3CA p.Ile391Met & Non-Small Cell Lung Carcinoma

gnomAD v4

SNV: 3-179209622-A-G(GRCh38)

[Copy variant ID](#)

	Exomes	Genomes	Total
Filters	Pass	Pass	
<u>Allele Count</u>	91077	15283	106360
<u>Allele Number</u>	1457418	152100	1609518
<u>Allele Frequency</u>	0.06249	0.1005	0.06608
<u>Grpmax Filtering AF</u> ⓘ <u>(95% confidence)</u>	0.2076	0.2076	0.2087
<u>Number of homozygotes</u>	3729	1192	4921
<u>Fraction of individuals with >20x coverage</u>	0.9	0.9	

SBVS1(-8)

PIK3CA p.Ile391Met & Non-Small Cell Lung Carcinoma

Oncogenicity Assessment

- SBVS1: Allele frequency of 0.06608 (6.6%, 106360/1609518 alleles) in gnomAD with a Grpmax of 0.2087.

Final Oncogenicity Classification: **Benign (-8 points)**

PIK3CA p.Ile391Met & Non-Small Cell Lung Carcinoma

AMP/ASCO/CAP Classification

Final Classification: Tier IV, Likely Benign or Benign variant

Why? Too common in the general population

Variant #4

- Disease: Neuroblastoma
- Variant: *BRAF* NC_000007.14:g.140781602C>G, p.Gly469Ala

BRAF p.Gly469Ala & Neuroblastoma

What can we eliminate?

- OVS1: This is not a null variant
- OS1 & OM4: No currently available data using this standard
- OM2: Not an in-frame indel or stop-loss variant
- OP2: Not a single gene etiology malignancy
- SBP2: Not a splice site variant

BRAF p.Gly469Ala & Neuroblastoma

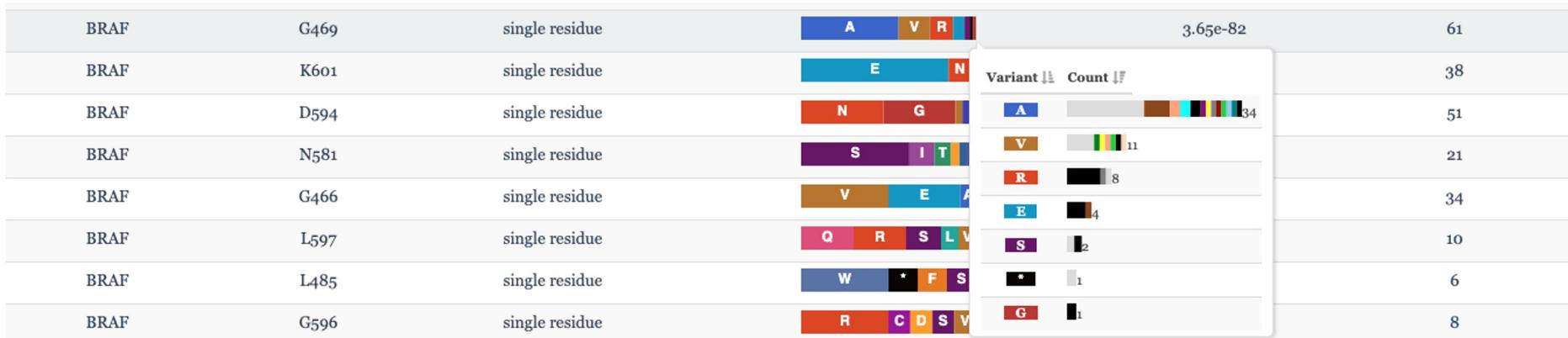
gnomAD v4

7-140783042-G-A	E	p.Ser471Ser	● synonymous	Likely benign	17	1461610	1.16e-5	0
7-140783043-G-A	E	p.Ser471Phe	● missense		1	628494	1.59e-6	0
7-140783044-A-T	E	p.Ser471Thr	● missense		1	833102	1.20e-6	0
7-140783060-T-C	E	p.Glu465Glu	● synonymous		1	833108	1.20e-6	0
7-140783064-C-T	E	G	p.Arg464Gln	● missense	21	1613876	1.30e-5	0
7-140783065-G-A	E	p.Arg464Ter	● stop gained		1	628656	1.59e-6	0
7-140783071-G-A	E	p.Pro462Ser	● missense		1	628688	1.59e-6	0
7-140783073-C-G	E	p.Gly461Ala	● missense	Uncertain significance	1	628690	1.59e-6	0
7-140783084-C-G	E	p.Gln457His	● missense		1	628696	1.59e-6	0

OP4: Absent in controls

BRAF p.Gly469Ala & Neuroblastoma

Cancer Hotspots



OS3: AA 469 change count in cancerhotspots.org is ≥ 50 (61), p.Gly469Ala count is 34

BRAF p.Gly469Ala & Neuroblastoma

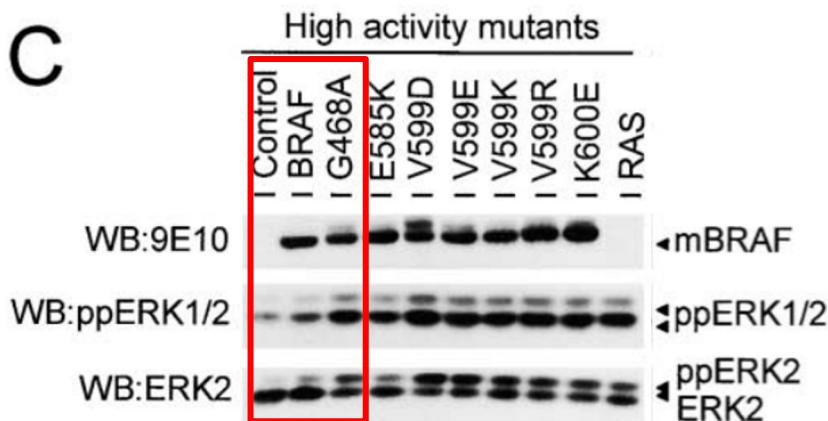
In-silico Predictor

- REVEL: 0.9721(Predicted Pathogenic)

OP1: Predicted Pathogenic

BRAF p.Gly469Ala & Neuroblastoma

C

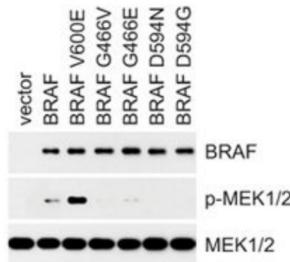


Next, we examined the ability of these mutants to activate endogenous ERK in COS cells. Western blotting with an antibody that specifically recognizes the dually phosphorylated and active forms of ERK1 and ERK2 indicated that all eighteen mutants stimulate endogenous ERK in COS cells (Figures 1C and Supplemental Table S1 online). The high activity mutants stimulated ERK phosphorylation to a similar level as that stimulated by $G^{12}V$ RAS, whereas the intermediate activity mutants were less efficient at stimulating ERK phosphorylation (Figure 1C). We used an immunoprecipitation kinase

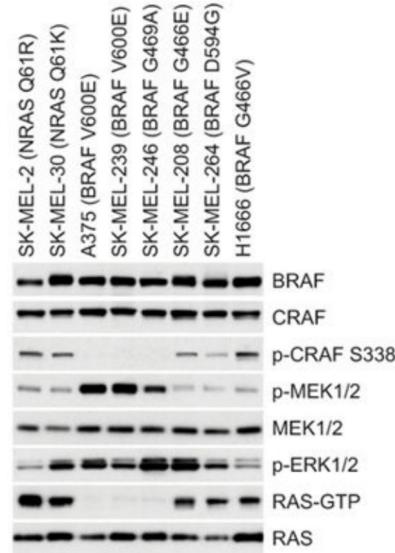
(PMID: 15035987)

BRAF p.Gly469Ala & Neuroblastoma

a



b



(PMID: 28783719)

Expression of these mutants increases the levels of phosphorylated MEK (p-MEK) and cyclin D1, but to a much lesser extent than do activating *BRAF* mutants (V600E, K601E or G469A) (Fig. 1a). Moreover, whereas activating mutants

BRAF p.Gly469Ala & Neuroblastoma

Oncogenicity Assessment

- OS2: functional evidence PMID: 15035987, 28783719; class 2 *BRAF* variant
- OS3: AA 469 change count in cancerhotspots.org is ≥ 50 (61), p.Gly469Ala count is 34
- OP4: Absent in controls (gnomAD v4)
- OP1: REVEL prediction pathogenic (score 0.9721)

Final Oncogenicity Classification: **Oncogenic (10 points)**

BRAF p.Gly469Ala & Neuroblastoma

AMP/ASCO/CAP Classification

- Previously reported in an individual diagnosed with neuroblastoma (PMID: 35652680)
- Tumors harboring Class II *BRAF* alterations are resistant to treatment with Vemurafenib (PMIDs: 26343582, 28783719)
- Preclinical trials demonstrated sensitivity to a few experimental inhibitors in tumors harboring the Gly469Ala; stable disease was observed in some individuals treated with trametinib (PMIDs: 35652680, 36049549, 32981611)

Final Classification: Tier II Level C, potential clinical significance for vemurafenib and trametinib