SPECIAL ARTICLE

Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC)

ARTICLE INFO

ABSTRACT

Purpose: Several professional societies have published guidelines for the clinical interpretation of somatic variants, which specifically address diagnostic, prognostic, and therapeutic implications. Although these guidelines for the clinical interpretation of variants include data types that may be used to determine the oncogenicity of a variant (eg, population frequency, functional, and in silico data or somatic frequency), they do not provide a direct, systematic, and comprehensive set of standards and rules to classify the oncogenicity of a somatic variant. This insufficient guidance leads to inconsistent classification of rare somatic variants in cancer, generates variability in their clinical interpretation, and, importantly, affects patient care. Therefore, it is essential to address this unmet need.
Methods: Clinical Genome Resource (ClinGen) Somatic Cancer Clinical Domain Working Group and ClinGen Germline/Somatic Variant Subcommittee, the Cancer Genomics Consortium, and the Variant Interpretation for Cancer Consortium used a consensus approach to develop a standard operating procedure (SOP) for the classification of oncogenicity of somatic variants.
Results: This comprehensive SOP has been developed to improve consistency in somatic variant classification and has been validated on 94 somatic variants in 10 common cancer-related genes.
Conclusion: The comprehensive SOP is now available for classification of oncogenicity of somatic variants.

© 2022 Published by Elsevier Inc. on behalf of American College of Medical Genetics and Genomics.

*Correspondence and requests for materials should be addressed to Peter Horak, National Center for Tumor Diseases (NCT), Im Neuenheimer Feld 460, 69120 Heidelberg, Germany. *E-mail address:* peter.horak@nct-heidelberg.de
**Debyani Chakravarty, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. *E-mail address:* chakravd@mskcc.org
***Dmitriy Sonkin, Division of Cancer Treatment and Diagnosis, National Cancer Institute, 9609 Medical Center Drive, Rockville, MD 20850. *E-mail address:* dmitriy.sonkin@nih.gov
A full list of authors and affiliations appears at the end of the paper.

Introduction

Somatic genetic variants identified in cancer are interpreted from multiple partially overlapping perspectives across the clinical oncology and molecular pathology communities. In discovery and translational research endeavors, it is important to determine whether a particular variant observed in a gene of interest is oncogenic because such knowledge provides the foundation for targeted cancer treatment research. Clinical applications are dominated by diagnostic, prognostic, or therapeutic implications, which in most cases depend on underlying variant oncogenicity determined through expert classifications.

The joint consensus of the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO), and the College of American Pathologists (CAP) somatic variant clinical interpretation and their reporting guidelines address diagnostic, prognostic, and therapeutic implications.[1] Although the AMP/ASCO/CAP guidelines describe data types that can be used to determine oncogenicity of a variant, including population frequency, functional data, computational predictions, and somatic frequency, they do not provide a clear, systematic, and comprehensive set of standards and rules to classify somatic variant oncogenicity. Multiple other levels of evidence systems published by professional societies such as the European Society for Medical Oncology (ESMO) Scale of Clinical Actionability of molecular Targets[2] and knowledge bases such as Clinical Interpretation of Variants in Cancer (CIViC),[3] Precision Oncology Knowledge Base (OncoKB),[4] and Variant Interpretation for Cancer Consortium (VICC) Meta-Knowledgebase[5] address the actionability of somatic cancer variants and provide rules for their curation but omit or differ in criteria defining variant oncogenicity. This lack of structured guidance for biological classification of rare variants and the ambiguity of biological and clinical context in which they are identified leads to inconsistency in their clinical interpretation. This further enhances the variability in reporting across clinical laboratories and institutions and has wide-reaching consequences for therapeutic decisions.

This report describes the development of standards and guidelines for the classification of oncogenicity of somatic sequence variants in cancer using a rigorous and comprehensive process. For the purpose of these guidelines, we define variant oncogenicity as the pathogenicity of the variant in the context of a neoplastic disease. We specifically categorize somatic variants for their potential to confer growth and survival advantages in tumor cells. These guidelines were inspired by the American College of Medical Genetics and Genomics (ACMG) and AMP germline pathogenicity guidelines[6] and were adapted to systematically and comprehensively categorize evidence of oncogenicity for somatic variants. Consensus was built through discussion with experts in translational cancer biology, bioinformatics, medical oncology, and molecular pathology.

On the basis of this standard operating procedure (SOP), somatic single nucleotide variants and small insertions/deletions can be assigned to 1 of the 5 categories (oncogenic, likely oncogenic, variant of uncertain significance [VUS], likely benign, and benign), aiding their further clinical interpretation. This SOP expands the classification of variants from variants with well-established oncogenicity to those that can be classified using this SOP and were previously not amenable to clinical interpretation, which improves consistency and comprehensiveness in reporting of somatic variant oncogenicity in clinical practice.

Methods

A working group consisting of individuals from multiple organizations, laboratories, institutions, and countries, including members of the ClinGen Somatic Cancer Clinical Domain Working Group, ClinGen Germline/Somatic Variant Subcommittee, Cancer Genomics Consortium (CGC), and VICC, was formed with the goal of developing this SOP. The working group evaluated the literature and recommendations from other professional societies (ACMG, AMP, ASCO, CAP, American Association for Cancer Research [AACR], and ESMO), and the SOP structure was informed by the ACMG/AMP germline pathogenicity guidelines.[1,2,6]

The evidence of oncogenicity or benign effect was categorized as Very Strong, Strong, Moderate, or Supporting. A point-based system based on the study by Tavtigian et al[7] was used for combining evidence to classify oncogenicity of somatic variants. Variants can be classified as follows: oncogenic, likely oncogenic, VUS, likely benign, or benign. Figure 1 provides a general overview of the evidence framework.

To test the proposed SOP, we selected 9 genes covering key aspects of tumor molecular biology for initial testing. Two of these genes, *KRAS* and *BRAF,* are well-characterized oncogenes. *PIK3CA* is also a well-known oncogene that was selected as an example that is more challenging to interpret, in part due to the presence of hotspot variants in multiple domains.[8] *IDH1* was selected for its neomorphic oncogenic mechanism driven by oncometabolite 2-hydroxyglutarate.[9] The role of *EZH2* in cancer is potentially context-dependent, functioning as an oncogene or as a tumor suppressor gene (TSG). *TERT* was selected to represent noncoding oncogenic variants.[10,11] Finally, *PTEN*, *TP53*, and *RB1* were selected to represent well-characterized TSGs. Notably, *PTEN* and *TP53* have ClinGen germline expert panel guidelines available, and all variants selected for SOP evaluation in these 2 genes have been interpreted by a corresponding expert panel.[12,13] For the 9 genes, curation was completed for a set of 84 variants covering a range of classifications from benign to oncogenic. In addition, through collaboration with the ClinGen Somatic Hematologic Cancer Taskforce, oncogenicity classifications
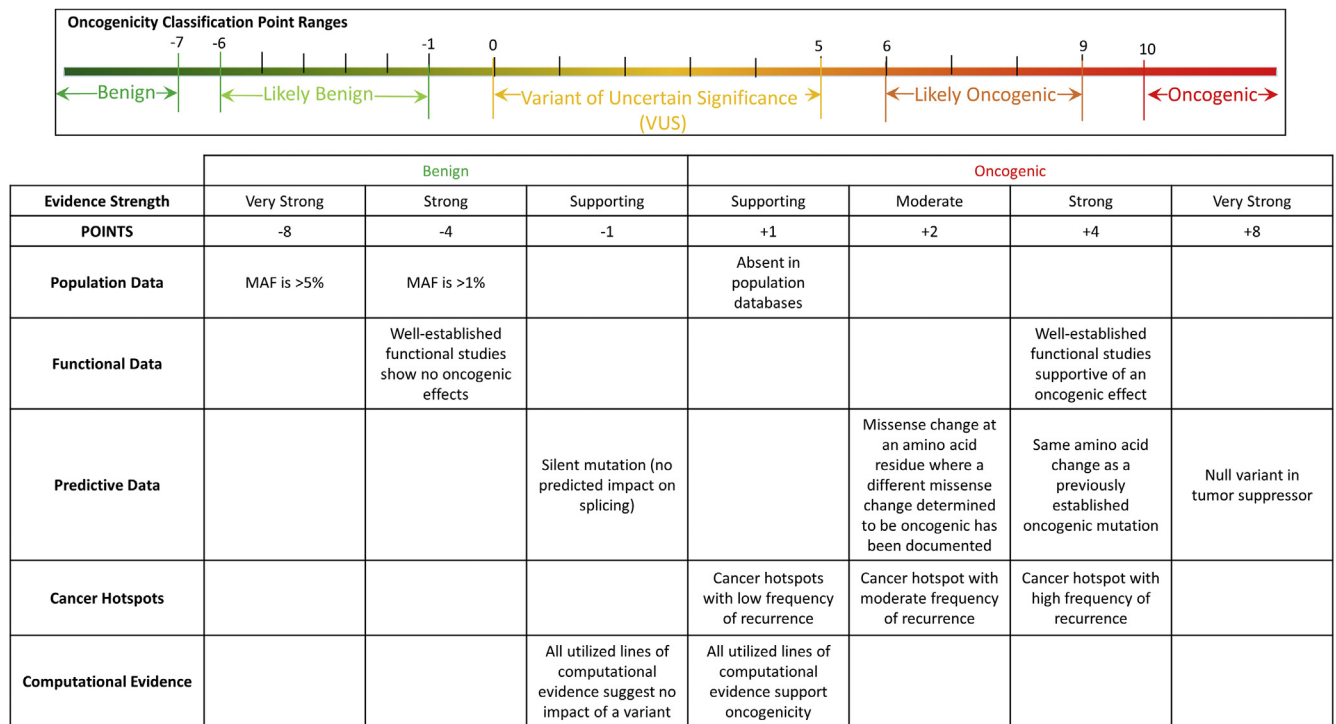
Figure 1   **Somatic standard operating procedure evidence framework overview (all criteria are not listed)**. MAF, minor allele frequency.

based on this SOP were performed for 10 *FLT3* variants involving both tyrosine kinase and non–tyrosine kinase domains. Determination of the oncogenicity for variants of *FLT3* gene is important owing to the availability of US Food and Drug Administration (FDA)-approved targeted therapeutic drugs that include highly selective tyrosine kinase inhibitors such as midostaurin and gilteritinib.[14,15] Thus, the final evaluation set consisted of 10 genes and 94 variants. All variants included in this SOP evaluation are listed in Table 1 and in Supplemental Table 1 using Human Genome Variation Society nomenclature. Each variant was evaluated independently by at least 2 curators; differences in evaluation between curators were reconciled via consensus agreement of classifications in regular monthly meetings of the working group. Classifications for all variants listed in Table 1 are provided in Supplemental Material.

General Considerations

Intended scope

This SOP is focused on the classification of oncogenicity of somatic genetic variants in tumor cells such as single nucleotide variation (SNV) and insertions/deletions, including deletions covering multiple exons.

This SOP should not be used for classification of pathogenicity of germline cancer predisposition variants.

Classification of oncogenicity of variants using this SOP should be carried out in the context of relevant tumor type(s). Detailed discussion on this topic can be found in the special considerations section.

This SOP is not intended for classification of oncogenicity of fusions, copy number variants (with exception of deletions limited to within a single gene), or other chromosomal rearrangements.

This SOP is not intended for determining the diagnostic, prognostic, or therapeutic value of variants but may be used to support these interpretations.

This SOP is intended to be used in conjunction with AMP/ASCO/CAP style somatic guidelines for clinical actionability assessment. Figure 2 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5707196/figure/fig2/) from the study conducted by Li et al[1] describes the AMP/ASCO/CAP somatic guideline tiers. Oncogenicity can be reported alongside clinical actionability assessed by these guidelines and inform the decision-making process. From a clinical perspective, we expect the main practical benefit of this SOP to be for somatic variants that do not directly align with AMP/ASCO/CAP guideline designation as tier I. For a missense somatic variant that is not directly listed in FDA approval(s) or practice guideline(s), an SOP-based oncogenicity classification may provide a rationale to assign such variant to tier III (VUS), tier IV (benign), or potentially to tier I or tier II. This assessment might further depend on the tumor type, absence of wild-type allele in tumor DNA (for TSGs), and relevant approval and/or practice guidelines. The immediate clinical relevance of independent oncogenicity assessment arises when practice guidelines/approvals omit or do not specify variants or

Table 1 Gene variants used to test somatic standard operating procedure

Gene Symbol	KRAS	BRAF	PIK3CA	IDH1	EZH2	TERT	FLT3	PTEN	TP53	RB1
Transcript ID	NM_004985.5	NM_004333.6	NM_006218.4	NM_005896.4	NM_004456.4	NM_198253.3	NM_004119.3	NM_000314.6	NM_000546.5	NM_000321.2
Variant	p.Gly12Ala	p.Pro14Arg	p.Met1?	p.Phe32Val	c.-7-2A>C	c.-124C>T	p.Asp835Tyr	c.-9C>G	p.Pro47Ser	p.Ile124ArgfsTer6
	p.Gln61Lys	p.Ala31Gly	p.Ile391Met	c.123-4C>T	p.Asp136Val	c.-146C>T	p.Asp835Glu	p.His123Arg	p.Val73Met	p.Ser443Ter
	p.Ser65Ile	p.Pro403LeufsTer8	p.Glu418Lys	p.Val71Ile	p.Asp233Gly	c.-124C>A	p.Asp835His	p.Arg130Gln	p.Cys124Ter	c.607+1G>A
	p.Arg164Gln	p.Asp445His	p.Glu453Lys	p.Arg132His	p.Val626Met	c.-332C>T	p.Asp835Val	p.Thr131Ile	p.Cys135Gly	c.2211+5G>T
		p.Asn486_Pro490del	p.Glu545Ala	p.Arg132Cys	p.Arg658Thr	c.-245T>C	p.Asp835Ile	p.Leu182Ser	p.Val173Met	c.1216-29A>G
		p.Gly469Ala	p.Lys733Arg	p.Arg132Ser	p.Ala682Gly	p.Arg230Ter	p.Asn676Lys	p.Leu193=	p.Glu180Lys	p.Gln689His
		p.Gly469Glu	p.His1047Gln	p.Val178Ile	p.Ala682Val	p.Trp581Ter	p.Tyr842Cys	p.Pro204Ala	p.Glu298Lys	p.Ser567Leu
		p.Asp594Asn	p.His1047Arg	p.Asp220Gly	p.Arg684Cys		p.Phe691Leu	c.-634+5G>C	p.Gly334Arg	p.Trp563Leu
		p.Val600Lys	p.His1065Tyr	p.Glu262Lys	p.Arg690His		p.Tyr693Cys	p.Lys322Ter	p.Ala347Asp	p.Arg661Trp
		p.Lys601Glu	p.Asn1068LysfsTer5		p.Ser695Leu		p.Tyr693Asn	p.Pro354Leu	p.His365Tyr	p.Glu554Lys
					p.Tyr731Phe					p.Tyr606Cys
					p.Tyr731His					
					p.Tyr646Phe					

"p." denotes the variant at the protein level, and "c." denotes the variant at the complementary DNA level. Note: the first 5 TERT variants are in promoter region and the negative number indicates the number of base pairs upstream of the ATG translation start site.

describe variant types instead (eg, *EGFR* exon 19 deletion).

Variants in genes known to be associated with hereditary cancer syndromes require special consideration. If the somatic variant is in a gene known to cause hereditary cancer, ACMG/AMP ClinGen germline gene-specific expert panel guidelines provide important gene-specific nuances, such as minor allele frequency cutoffs, functional evidence guidance, computational evidence guidance, etc. For somatic variants in hereditary cancer genes with VUS oncogenicity classification by this SOP but with evidence of pathogenicity in the germline context, we recommend using the ClinGen germline gene-specific variant curation expert panel guidelines-based curation where evidence is applicable. If such gene-specific germline expert panel guidelines do not exist for a particular gene, the generic ACMG/AMP germline pathogenicity guidelines may be considered. This recommendation can potentially improve some variant classifications by putting segregation and other gene-specific information to use. The following provides an example for such a scenario: *PTEN* NM_000314.6:c.610C>G (p.Pro204Ala) variant is classified as VUS by this SOP (Supplemental Materials); however, it is classified as likely pathogenic by ClinGen PTEN germline expert panel, in part due to putting hereditary-based information to use (https://erepo.genome.network/evrepo/ui/classification/CA000535/MONDO:0017623/003).

Recommendations for database use

Population databases
Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org) aggregates population data from multiple sources, incorporating data from several key sources (eg, Exome Aggregation Consortium and 1000 Genomes). We therefore recommend using gnomAD over these constituent resources. There are a number of important caveats to consider in regard to population data. Insertions and deletions may be underrepresented in part due to technical challenges associated with their detection. Moreover, owing to clonal hematopoiesis, some variants in gnomAD (and similar databases) may actually be somatic and not germline. Variants likely causing clonal hematopoiesis have been reported in multiple cancer relevant genes, eg, *DNMT3A*, *TET2*, *IDH2*, *TP53*, *KRAS*, and *SF3B1*. Examining read data in the gnomAD variant report page for suspiciously low variant allele frequency may help to identify such variants. Some variants are derived from normal tissue in individuals who are known to have cancer, and the noncancer subset of gnomAD excludes such individuals.

Cancer databases
Cancer hotspots (https://www.cancerhotspots.org) provides information about statistically significant recurrently mutated positions identified in approximately 25,000 tumor samples.[16,17] Determination of statistical significance takes

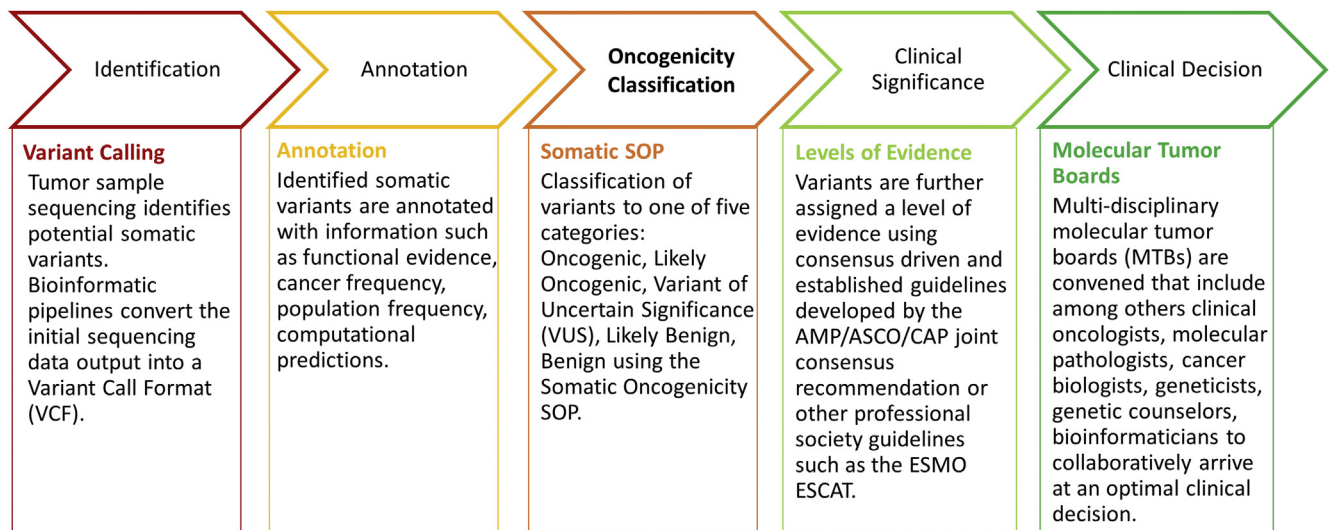| Identification	Annotation	**Oncogenicity Classification**	Clinical Significance	Clinical Decision
Variant Calling Tumor sample sequencing identifies potential somatic variants. Bioinformatic pipelines convert the initial sequencing data output into a Variant Call Format (VCF).	**Annotation** Identified somatic variants are annotated with information such as functional evidence, cancer frequency, population frequency, computational predictions.	**Somatic SOP** Classification of variants to one of five categories: Oncogenic, Likely Oncogenic, Variant of Uncertain Significance (VUS), Likely Benign, Benign using the Somatic Oncogenicity SOP.	**Levels of Evidence** Variants are further assigned a level of evidence using consensus driven and established guidelines developed by the AMP/ASCO/CAP joint consensus recommendation or other professional society guidelines such as the ESMO ESCAT.	**Molecular Tumor Boards** Multi-disciplinary molecular tumor boards (MTBs) are convened that include among others clinical oncologists, molecular pathologists, cancer biologists, geneticists, genetic counselors, bioinformaticians to collaboratively arrive at an optimal clinical decision.

Figure 2   Stepwise process of somatic variant classification and interpretation. AMP, Association of Molecular Pathology; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; ESCAT, European Society for Medical Oncology Scale of Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; SOP, standard operating procedure.

into account nucleotide mutability and gene-specific SNV rates. It is important to keep in mind that statistical significance in this resource is calculated for amino acid (AA) position and not for particular change at that AA position. This resource has good coverage for many solid tumors; however, it has limited coverage for hematological, rare, and pediatric malignancies. For pediatric malignancies resources, PeCanPIE (https://pecan.stjude.cloud/pie) may be considered.[18]

Catalogue Of Somatic Mutations In Cancer (COSMIC, https://cancer.sanger.ac.uk/cosmic) provides information on frequency of somatic variants in cancer. It is important to keep in mind that data in COSMIC is not adjusted by nucleotide mutability and varying gene-specific SNV rates. COSMIC data are provided by methods with different degrees of genome coverage, and therefore, coverage across gene sequences may not be uniform. In some cases, the origin of the variant (germline or somatic) may not be known or available.

Medical clinical literature

When a variant cannot be found in pre-existing population or cancer databases and functional data are not available, a search of the medical literature is often performed to assess if the variant has been previously described in an oncogenic setting. This is of particular relevance for rare variants. The literature can vary in quality and scope from case reports to seminal studies of publicly available cancer genomes (eg, The Cancer Genome Atlas). Some of such literature could be of a clinical nature and by itself not applicable as evidence for oncogenicity classification; however, it might be useful to capture such information as clinical annotation. Such annotations may be helpful for use in the AMP/ASCO/CAP guidelines for clinical interpretation. Although the

clinical context in which the variant has arisen may not be relevant to the evaluation of oncogenicity, it could be described within the annotation in a relevant context, eg, *IDH1/2* variants in gliomas have a prognostic significance, whereas *IDH1/2* variants in chondrosarcomas have a diagnostic utility and those in acute myeloid leukemia have therapeutic implications. When providing information in clinical annotations for a novel variant from the medical clinical literature, it is important to report the nature of the evidence, the study type and sample size, the type of data presented (ie, genomic or transcriptomic level, tumor-only or paired tumor/normal samples), and variant relevance to the tumor type.

Computational prediction algorithms

A number of computational tools can be useful in the classification of variant oncogenicity. Such tools include conservation analysis tools such as phyloP and phastCons[19] (http://compgen.cshl.edu/phast, also available online through https://run.opencravat.org[20]); splicing effect predictors such as spliceAI[21] (https://spliceailookup.broadinstitute.org) and varSEAK (https://varseak.bio); and a number of in silico variant effect prediction algorithms for missense variants. In silico variant effect prediction algorithms performance varies depending on gene, variant type, variant frequency, and many other factors. Supplemental Table 2 lists 19 algorithms for prediction of effect of missense variants; as described later, these predictors were evaluated in context of single nucleotide missense variants classified as part of this SOP testing.

In silico variant effect prediction algorithms were evaluated for 70 single nucleotide missense variants occurring within the coding region of each gene. Supplemental Table 3 contains all variant coordinates used, oncogenicity

SOP results (predictions, points, and evidence codes), and scores for 19 such algorithms. For 15 of these 19 algorithms we also obtained variant effect interpretations (pathogenic, deleterious, damaging, high, etc.) on the basis of recommended score cutoffs. Using these scores and predictions we performed Spearman correlations, receiver operator characteristic area under the curve, positive predictive value, and negative predictive value analyses (Supplemental Methods for details) to assess their performance relative to oncogenicity SOP points and classifications (Supplemental Figure 1). In general, scores from in silico predictors were positively correlated with oncogenicity SOP points (mean Spearman correlation of 0.376). The algorithms achieved positive predictive values (mean of 0.915), negative predictive values (mean of 0.664), and receiver operator characteristic area under the curve (mean of 0.825) values when evaluated against the oncogenicity SOP classifications. The evaluation of test variants selected in this study should not be considered a comprehensive assessment of the performance of these algorithms; in this analysis, VEST-4,[22] Protein Variation Effect Analyzer,[23] and Combined Annotation Dependent Depletion (CADD)[24,25] had the highest agreement with oncogenicity SOP results. Scores from each algorithm tended to be highly correlated to the scores from other algorithms (many of which use similar assumptions and training data). Notable partial exceptions were CHASMplus General and CHASMplus Cancer Type Specific, which is interesting because these are among a small list of algorithms that actually use tumor/somatic data directly in their training. Ghosh et al[26] provide rationale for selecting potential combinations of predictors. Overall, this analysis suggests that although in silico predictions have value in assessing variant effect, they should only be evaluated in the context of other evidence types as recommended by this SOP, and multiple algorithms should not be treated as independent supporting evidence.

Proposed Criteria for Classification of Somatic Sequence Variants in Cancer

Criteria for evidence of oncogenicity of somatic variants are provided in Table 2, and criteria for evidence of benign effect of somatic variants are provided in Table 3. The first letter O in the evidence codes in Table 2 stands for oncogenic and first 2 letters SB in Table 3 stands for somatic benign.

SOP-BASED EXAMPLES OF SOMATIC VARIANT CLASSIFICATION

The following oncogenicity classifications for 2 variants provide illustrative examples of somatic variant classification.

The first example is *KRAS* p.Arg164Gln

NM_004985.5:c.491G>A, GRCh37 (hg19) chr12:g.25362805C>T, Allele Registry ID: CA135580.

An *in vitro* functional study by Smith et al[27] in NIH3T3 mouse fibroblast cells expressing mutant *KRAS* p.Arg164Gln showed foci formation similar to wild-type *KRAS*. However, messenger RNA expression profiling may have been indicative of a slight increase in MAPK activity associated with *KRAS* p.Arg164Gln relative to wild-type *KRAS*. Additionally, there was an increase in nanoscale signaling complexes on the plasma membrane; however, this is not a well-established method of variant functional characterization, and in many instances, testing was performed in cell lines with concomitant *KRAS* p.Arg164Gln and *KRAS* p.Gly12Val variants.[28] On the basis of the earlier evidence, the Somatic Benign Strong-2 (SBS2) criterion was downgraded to supporting evidence level. This is a rare variant and is not listed in cancerhotspots.org. The Functional Analysis through Hidden Markov Models (FATHMM) prediction is pathogenic (score 0.98), and CADD prediction is potentially deleterious (score 17.04); on the basis of this evidence, the Oncogenic Supporting-1 (OP1) criterion was satisfied. One supporting criterion in support of benign effect (–1 point) and 1 oncogenicity supporting criterion (1 point) were satisfied for *KRAS* p.Arg164Gln giving a total of 0 points, which leads to a VUS oncogenicity classification for this somatic variant in neoplasms.

The second example is *BRAF* p.Gly469Glu

NM_004333.6: c.1406G>A, GRCh37 (hg19) chr7:g.140481402C>T, Allele Registry ID: CA279970.

Functional studies by Smalley et al[29] and Wan et al[30] indicate low BRAF kinase activity and increased signaling through RAF1 (class 3 *BRAF* variant[31]). On the basis of this evidence, the Oncogenic Strong-2 (OS2) criterion was satisfied (note: In PMID: 15035987 the AA numbers are shifted by 1 and AA 468 corresponds to AA 469). The FATHMM prediction is pathogenic (score 0.99) and CADD prediction is deleterious (score 33), and on the basis of this evidence, the OP1 criterion was satisfied. The *BRAF* p.Gly469Glu AA change count in cancerhotspots.org is 4, and on the basis of this evidence, OP3 criterion was satisfied. This variant is absent in gnomAD (v.2.1.1), and on the basis of this evidence, the OP4 criterion was satisfied. One strong criterion in support of oncogenicity (4 points) and 3 oncogenicity supporting criteria (3 points) were satisfied for *BRAF* p.Gly469Glu giving a total of 7 points, which leads to likely oncogenic classification of this somatic variant in neoplasms.

Criteria Clarifications

To keep this SOP reasonably compact, criteria descriptions are concise and do not provide extensive clarifications. To

Table 2 Criteria for evidence of oncogenicity of somatic variants

Category	Evidence	Criteria	Comments/Caveats
Very Strong (8 points)	OVS1	Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single-exon or multiexon deletion) in a bona fide tumor suppressor gene.	• Use caution interpreting pLOF variants at the extreme 3′ end of a gene after the nonsense mediated decay site. • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact (in frame events). • Use caution if splice variant leads to expression of a well-known alternative iso-form that preserves tumor suppressor functionality. • Use caution in the presence of multiple transcripts.
Strong (4 points)	OS1	Same amino acid change as a previously established oncogenic variant (using this standard) regardless of nucleotide change. Example: Val→Leu caused by either G>C or G>T in the same codon.	• Beware of changes that impact splicing rather than the changes at the amino acid/protein level.
	OS2	Well-established in vitro or in vivo functional studies, supportive of an oncogenic effect of the variant.	• Functional studies that have been shown to be reproducible and robust are considered the most well established. • If OS1 is applicable, this rule can be used only if functional studies are based on the particular nucleotide change of the variant.
	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.	• Use caution with hotspots driven by truncating somatic variants. • If the somatic variant is in a tumor type not well covered by cancerhotspots.org, resources such as COSMIC or a tumor type–specific study could be used. • This rule cannot be used if OS1 is applicable, unless it is possible to observe hotspots on the basis of the particular nucleotide change.
Moderate (2 points)	OM1	Located in a critical and well-established part of a functional domain (eg, active site of an enzyme).	• This rule cannot be used if OS1 or OS3 is applicable.
	OM2	Protein length changes as a result of in-frame deletions/insertions in a known oncogene or tumor suppressor gene or stop-loss variants in a known tumor suppressor gene.	• This rule cannot be used if OVS1 is applicable.
	OM4	Missense variant at an amino acid residue where a different missense variant determined to be oncogenic (using this standard) has been documented. Amino acid difference from reference amino acid should be greater or at least approximately the same as for missense change determined to be oncogenic.	• Example: p.Arg156His is oncogenic; now you observe p.Arg156Cys. This rule cannot be used if OS1 or OS3 or OM1 is applicable. • Beware of changes that impact splicing rather than the changes at the amino acid/protein level.
	OM3	Located in one of the hotspots in cancerhotspots.org with <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.	• This rule cannot be used if OM1 or OM4 is applicable. • Use caution with hotspots driven by truncating somatic variants. • If the somatic variant is in a tumor type that is not covered well by cancerhotspots.org, resources such as COSMIC or a tumor type–specific study could be used.

(continued)

Table 2 Continued

Category	Evidence	Criteria	Comments/Caveats
Supporting (1 point)	OP1	All used lines of computational evidence support an oncogenic effect of a variant (conservation/evolutionary, splicing effect, etc.).	• Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. • Can be used only once in any evaluation of a variant.
	OP2	Somatic variant in a gene in a malignancy with a single genetic etiology. Example: retinoblastoma is caused by bi-allelic *RB1* inactivation.	• A small fraction of cases may be caused by an alternative mechanism; histological similarities may cause misdiagnosis.
	OP3	Located in one of the hotspots in cancerhotspots.org and the particular amino acid change count in cancerhotspots.org is below 10.	• Use caution with hotspots driven by truncating somatic variants. • If somatic variant is in a tumor type that is not covered well by cancerhotspots.org, resources such as COSMIC or a tumor type–specific study could be used.
	OP4	Absent from controls (or at an extremely low frequency) in gnomAD.	• Population data for insertions/deletions may be poorly called by next-generation sequencing. Population data may contain somatic variants associated with clonal hematopoiesis.

COSMIC, Catalogue Of Somatic Mutations In Cancer; *gnomAD*, Genome Aggregation Database; *OM1*, Oncogenic Moderate-1; *OM2*, Oncogenic Moderate-2; *OM3*, Oncogenic Moderate-3; *OM4*, Oncogenic Moderate-4; *OP1*, Oncogenic Supporting-1; *OP2*, Oncogenic Supporting-1; *OP3*, Oncogenic Supporting-3; *OP4*, Oncogenic Supporting-4; *OS1*, Oncogenic Strong-1; *OS2*, Oncogenic Strong-2; *OS3*, Oncogenic Strong-3; *OVS1*, Oncogenic Very Strong-1; *pLOF*, predicted loss-of-function.

address this, the following section provides criteria clarifications.

The oncogenic moderate-4 (OM4) criterion is intended to be used for a missense variant at an AA residue where a different missense variant, determined to be oncogenic (using this standard), has been documented. One of the notes for this rule stipulates that the AA difference from reference AA should be greater or at least approximately the same as for the missense change determined to be oncogenic. This note is intended to prevent potential misuse of this criterion in cases of AA substitutions with very similar physicochemical properties. We recommend using one of the well-known AA difference metrics such as Grantham's distance, Epstein's coefficient of difference, or Miyata's distance. In a generic example provided in the SOP, p.Arg156His is known to be oncogenic and p.Arg156Cys is observed, the Grantham's distance of 180 from Arg to Cys is greater than Grantham's distance of 29 from Arg to His; therefore, this would be an appropriate use of this criterion.

Some criteria have a potential overlap that may lead to double counting. To decrease this possibility, a number of criteria have explicit exclusion(s) specified. For example, OM1 is based on variants being located in a critical and well-established part of a functional domain (eg, active site of an enzyme). It is noted that this criterion cannot be used if OS3 is applicable because OS3 is based on a variant in a known cancer hotspot, and such hotspots are frequently located in critical functional domains.

During evaluation of Oncogenic Very Strong-1 (OVS1) criteria, we recommend considering ClinGen guidance on the interpretation of loss-of-function variants.[32] OVS1 can also be used for splicing variants up to –16 position at the splice acceptor site and +5 position at the splice donor site if there is experimental evidence indicating splicing defects.[33] A recently developed resource MutSpliceDB (https://brb.nci.nih.gov/splicing) provides a place for the genomic community to catalog RNA-sequencing based evidence for splice site variants effects on splicing.[34]

During evaluation of OS2 and SBS2 criteria we recommend considering ClinGen guidance on interpretation of functional evidence.[35]

Population-based criteria Somatic Benign Very Strong-1 (SBVS1) and Somatic Benign Strong-1 (SBS1) adopt most of the relevant ClinGen guidance.[36] General continental gnomAD populations: African, East Asian, European (non-Finnish), Latino, and South Asian have well over 2000 observed alleles. Finnish European population and Ashkenazi Jewish population may be used only if founder effects have been ruled out for the gene in question.

During evaluation of the criteria OVS1, OS2, OM1, OM2, OM4, and SBS2, underlying evidence may be insufficient to fully satisfy a criterion. In such cases, curators have the option to downgrade the criterion to a lower evidence level. For example, evidence of functional effect for SBS2 may be limited or partially conflicting. In such cases, curators can downgrade evidence level to moderate or supporting.

Table 3 Criteria for evidence of benign effect of somatic variants

Category	Evidence	Criteria	Comments/Caveats
Very Strong (−8 points)	SBVS1	Minor allele frequency is >5% in gnomAD in any 5 general continental populations: African, East Asian, European (non-Finnish), Latino, and South Asian.	If the somatic variant is in a gene known to cause predisposition to hereditary cancer, ACMG/AMP ClinGen germline expert panel gene-specific guidelines (if they exist) must be consulted to establish a cutoff that takes disease prevalence into account.
Strong (−4 points)	SBS1	Minor allele frequency is >1% in gnomAD in any 5 general continental populations: African, East Asian, European (non-Finnish), Latino, and South Asian.	If the somatic variant is in a gene known to cause predisposition to hereditary cancer, ACMG/AMP ClinGen germline expert panel gene-specific guidelines (if they exist) must be consulted to establish a cutoff that takes disease prevalence into account.
	SBS2	Well-established in vitro or in vivo functional studies show no oncogenic effects.	NA
Supporting (−1 point)	SBP1	All used lines of computational evidence suggest no effect of a variant (conservation/evolutionary, splicing effect, etc.).	Because many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. Can be used only once in any evaluation of a variant.
	SBP2	A synonymous (silent) variant for which splicing prediction algorithms predict no effect on the splice consensus sequence nor the creation of a new splice site and the nucleotide is not highly conserved.	NA

ACMG/AMP, American College of Medical Genetics and Genomics/Association of Molecular Pathology; *ClinGen*, Clinical Genome Resource; *gnomAD*, Genome Aggregation Database; *NA*, not applicable; *SBP1*, Somatic Benign Supporting-1; *SBP2*, Somatic Benign Supporting-2; *SBS1*, Somatic Benign Strong-1; *SBS2*, Somatic Benign Strong-2; *SBVS1*, Somatic Benign Very Strong-1.

Guidelines for Reporting Results

We endorse a stepwise and structured approach to classifying variants, in which oncogenicity classification by this guideline precedes assessment of clinical actionability, as shown in Figure 2. Oncogenicity classifications should be provided for all variants included in a clinical report. From the perspective of storing classifications of oncogenicity of somatic variants on the basis of this SOP in knowledge bases, there are a few possible approaches. Ideally, each met criteria code should be captured separately in addition to the overall classification, relevant tumor type(s), and comments. However, such an approach might not always be possible, especially in the short term. As an alternative, criteria codes satisfied could be captured in the comments section or overall classification statement depending on knowledge base structure. The ClinGen Evidence Repository, which provides access to variant level evidence used and applied by ClinGen Variant Curation Expert Panels in the classification of germline variants, can be used as an example of a fully structured resource. On the contrary, ClinVar provides an example of an alternative approach. The 2 links that provide examples from ClinGen Evidence Repository and ClinVar for a germline *TP53* variant NM_000546.5:c.537T>A (p.His179Gln) are https://erepo.genome.network/evrepo/ui/classification/CA16615708/MONDO:0018875/009 and https://www.ncbi.nlm.nih.gov/clinvar/variation/406578, respectively.

Somatic variant nomenclature should follow similar guidance as outlined for germline variants in the study by Richards et al.[6] A key part of this guidance is based on Human Genome Variation Society (HGVS) (https://varnomen.hgvs.org) nomenclature. We also strongly recommend providing a ClinGen Allele Registry ID[37] for each variant (http://reg.clinicalgenome.org) to decrease the chance of incorrect variant mapping between different genome builds and transcripts. Laboratories should adopt the Matched Annotation from NCBI and EMBL-EBI (MANE) Select transcripts as the default reporting transcript and additionally report variants in MANE Plus Clinical transcripts for comprehensive coverage. The MANE project (https://www.ncbi.nlm.nih.gov/refseq/MANE) provides a set of matching Reference Sequence and Ensembl transcripts, with the MANE Select version being well-supported by experimental data and representing the biology of the gene. Use of MANE transcripts allows harmonization across the community and with major genomic resources and also decreases the chance of variant misinterpretation due to disparate transcript use.

Discussion and Special Considerations

Curation and interpretation of oncogenic variants is a process involving several distinct steps. As indicated in the intended scope, this SOP is primarily intended to be used in

conjunction with AMP/ASCO/CAP style somatic guidelines for assessing the therapeutic, diagnostic, or prognostic relevance of variants. This SOP can also be integrated with other guidelines for assessing the clinical significance of cancer variants, such as ESMO Scale of Clinical Actionability of molecular Targets.[2] A more precise classification of rare and less well-characterized variants may overcome some challenges in the clinical interpretation process. In particular, this SOP might facilitate assignment of therapeutic, diagnostic, or prognostic significance to variants classified as likely oncogenic or oncogenic, which might result in reassigning variants from AMP/ASCO/CAP tier III to tier I/II.

Functional effects of individual variants might be highly dependent on the histologic subtype and the molecular context of the tumor in which they occur. This SOP suggests that the classification of the oncogenicity of variants should be performed in context of the relevant tumor type. We recommend specifying a tumor type at the most generic level possible, eg, for *KRAS* variants we specify neoplasms as the tumor type because *KRAS* variants have been observed in most of the solid and hematological malignancies. For *FLT3* variants, hematopoietic neoplasms is specified as the tumor type because *FLT3* variants are generally limited to hematological malignancies. Some genes may have oncogenic effects and tumor suppressive effects that depend on tumor type, *EZH2* is potentially one such gene. For activating *EZH2* variants, neoplasms in tissues with *EZH2* expression is specified as a tumor type. For loss-of-function *EZH2* variants, myeloid malignancies is specified as a tumor type because on the basis of the current understanding of the molecular biology of myeloid malignancies, *EZH2* plays a tumor suppressive role in this context. *EZH2* serves as an example of cancer relevant genes with a limited understanding of the underlying molecular biology, indicating the need to perform periodic review of variant oncogenicity curations, due in part to the potential emergence of new information on the molecular biology of a gene in question.

In essentially all cases, the underlying molecular mechanism of the same variant in germline and somatic context is the same. However, the implications of the same variant in germline and somatic context could be different. For example, although the *BRAF* p.Val600Glu variant has oncogenic effects and therapeutic implications in several malignancies, its presence in the germline results in embryonic lethality.[38] Germline pathogenic variants in hereditary cancer genes have lifelong implications for affected individuals and also have potential implications to multiple family members. In sporadic malignancies, oncogenic *TERT* alterations are mostly copy number gains and promoter variants leading to an increase in *TERT* expression. In the germline context, pathogenic *TERT* alterations are loss-of-function events, and on the basis of the current body of evidence, such alterations fall under the VUS umbrella in the somatic context.

Some variants in hereditary cancer genes may be present as germline, but not as somatic and vice versa. There are a few possible explanations for such observations. Variant

genomic location and nucleotide composition around such locations may be susceptible to a particular type of mutational process, and that process might only be relevant in embryonic or postnatal context. In addition, some variants in TSGs may particularly have strong dominant negative effects and potentially result in embryonic lethality.

Some somatic variants may not be oncogenic in a classical sense because they do not provide growth and/or survival advantage by themselves; however, they may provide tumor cells with the ability to tolerate potential increases in metabolic or other stress caused by other classical oncogenic variants. In addition, some somatic variants may provide only limited growth, and/or survival advantage, but may potentially play an important role during the initial stages of neoplasm development. We consider a somatic variant's involvement in oncogenesis as described by the Hallmarks of Cancer[39] as relevant for the scope of this guideline. However, because many of these variants are part of an active and unfolding area of research, they might not be well covered by these guidelines.

Clinical considerations

The current practice of precision oncology relies on individualized assessments of somatic variants and might have immediate clinical consequences for patients with cancer. To harmonize reporting across institutions, we present this SOP for the classification of oncogenicity to be used as an element of clinical variant assessment. We are aware that many of the detected variants may fall into the VUS category. Routine clinical decisions should not be made on the basis of VUS. The functional relevance of these variants should be further explored in a preclinical setting or in some instances inform therapeutic interventions in well-documented interventional or observational trials, preferably in high-volume academic centers.[40] The underlying rationale for inclusion of VUS in clinical trials should be based on the combination of a careful review of the respective variant, knowledge about the particular tumor type and its molecular drivers, potential therapeutic alternatives, patient medical history, performance status, and additional clinical parameters. In such cases it is especially critical to provide all relevant information in a way that allows patients to make informed decisions on potential participation in clinical trials. There are a number of significant pitfalls in allowing participation in clinical trials on the basis of VUS variants, and such trials should warrant additional vigilance by clinical trial monitoring authorities.

Clinically relevant variants detected during tumor-only sequencing can be of somatic or germline origin. In a number of instances, there are significant differences in clinical implications depending on the somatic or germline variant origin. In such cases, follow-up germline testing should be considered to confirm the germline vs somatic origin.[41]

The ACMG/AMP germline pathogenicity guidelines provide a framework to systematically and comprehensively

categorize evidence of pathogenicity or the lack thereof. However, it is a generic framework, and evaluation criteria may vary owing to gene-specific nuances. To address such nuances, a number of ClinGen Variant Curation Expert Panels have been formed and gene-specific guidelines have been developed through modifications and adjustments to the underlying ACMG/AMP germline pathogenicity guidelines following a comprehensive and rigorous ClinGen process. Classifications by ClinGen Variant Curation Expert Panels are recognized by the FDA (https://clinicalgenome. org/about/fda-recognition). We envision a similar approach in the somatic space with development of gene-specific somatic oncogenicity guidelines on the basis of this SOP.

In summary, we provide a standardized and universally applicable framework and operating procedure for classification of oncogenicity of somatic variants that is an integral part of clinical variant interpretation, harmonizes reporting of variant oncogenicity, and supports clinical decision-making within and outside the clinical trials.

Data Availability

All data used in this article are publicly available and also listed in supplemental Data.

Author Information

Conflict of Interest

Additional Information

The online version of this article (https://doi.org/10.1016/j. gim.2022.01.001) contains supplementary material, which is available to authorized users.

Authors

Peter Horak[1,*], Malachi Griffith[2], Arpad M. Danos[2], Beth A. Pitel[3], Subha Madhavan[4], Xuelu Liu[5], Cynthia Chow[6], Heather Williams[7], Leigh Carmody[8], Lisa Barrow-Laing[9], Damian Rieke[10], Simon Kreutzfeldt[1], Albrecht Stenzinger[11], David Tamborero[12], Manuela Benary[10], Padma Sheila Rajagopal[13], Cristiane M. Ida[3], Harry Lesmana[14], Laveniya Satgunaseelan[15], Jason D. Merker[16], Michael Y. Tolstorukov[5], Paulo Vidal Campregher[17], Jeremy L. Warner[18], Shruti Rao[4], Maya Natesan[2], Haolin Shen[2], Jeffrey Venstrom[19], Somak Roy[20], Kayoko Tao[21], Rashmi Kanagal-Shamanna[22], Xinjie Xu[3], Deborah I. Ritter[23], Kym Pagel[24], Kilannin Krysiak[2], Adrian Dubuc[25], Yassmine M. Akkari[26], Xuan Shirley Li[27], Jennifer Lee[28], Ian King[29], Gordana Raca[30], Alex H. Wagner[31,32], Marylin M. Li[33], Sharon E. Plon[23], Shashikant Kulkarni[23], Obi L. Griffith[2], Debyani Chakravarty[34,**], Dmitriy Sonkin[35,***]

Affiliations

[1]National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Washington University School of Medicine in St. Louis, St. Louis, MO; [3]Mayo Clinic, Rochester, MN; [4]Georgetown University Medical Center, Washington, DC; [5]Dana-Farber Cancer Institute, Boston, MA; [6]BC Cancer Agency, Vancouver, British Columbia, Canada; [7]Columbia University, New York, NY; [8]The Jackson Laboratory for Genomic Medicine, Farmington, CT; [9]QIAGEN Inc, Redwood City, CA; [10]Charité-Universitätsmedizin Berlin, Berlin, Germany; [11]Institute of Pathology, University of Heidelberg, Heidelberg, Germany; [12]Karolinska Institute, Stockholm, Sweden; [13]Cancer Data Science Laboratory, Center for Cancer Research, National Cancer Institute, Bethesda, MD; [14]Genomic Medicine Institute, Cleveland Clinic Lerner Research Institute, Cleveland, OH; [15]Royal Prince Alfred Hospital, Sydney, New South Wales, Australia; [16]UNC School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC; [17]Hospital Israelita Albert Einstein, São Paulo, São Paulo, Brazil; [18]Vanderbilt University, Nashville, TN; [19]Foundation Medicine, Inc, Cambridge, MA; [20]Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [21]National Cancer Center Hospital, Tokyo, Japan; [22]The University of Texas MD Anderson Cancer Center, Houston, TX; [23]Baylor College of Medicine, Houston, TX; [24]Johns Hopkins University, Baltimore, MD; [25]Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [26]Legacy Health, Portland, OR; [27]Congenica Ltd, Cambridge, United Kingdom; [28]Frederick National Laboratory for Cancer Research, National Cancer Institute, Rockville, MD; [29]University Health Network, Toronto, Ontario, Canada; [30]University of Southern California, Los

Angeles, CA; [31]Nationwide Children's Hospital, Columbus, OH; [32]The Ohio State University College of Medicine, Columbus, OH; [33]Children's Hospital of Philadelphia, Philadelphia, PA; [34]Memorial Sloan Kettering Cancer Center, New York, NY; [35]National Cancer Institute, Rockville, MD

References

1. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19(1):4–23. http://doi.org/10.1016/j.jmoldx.2016.10.002.

2. Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann Oncol*. 2018;29(9):1895–1902. http://doi.org/10.1093/annonc/mdy263.

3. Griffith M, Spies NC, Krysiak K, et al. CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nat Genet*. 2017;49(2):170–174. http://doi.org/10.1038/ng.3774.

4. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. *JCO Precis Oncol*. 2017;2017:PO.17.00011. http://doi.org/10.1200/PO.17.00011.

5. Wagner AH, Walsh B, Mayfield G, et al. A harmonized meta-knowledgebase of clinical interpretations of somatic genomic variants in cancer. *Nat Genet*. 2020;52(4):448–457. http://doi.org/10.1038/s41588-020-0603-8.

6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–424. http://doi.org/10.1038/gim.2015.30.

7. Tavtigian SV, Harrison SM, Boucher KM, Biesecker LG. Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines. *Hum Mutat*. 2020;41(10):1734–1737. http://doi.org/10.1002/humu.24088.

8. Ligresti G, Militello L, Steelman LS, et al. PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches. *Cell Cycle*. 2009;8(9):1352–1358. http://doi.org/10.4161/cc.8.9.8255.

9. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2010;465(7300):966. http://doi.org/10.1038/nature09132.

10. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959–961. http://doi.org/10.1126/science.1230062.

11. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. *Science*. 2013;339(6122):957–959. http://doi.org/10.1126/science.1229259.

12. Mester JL, Ghosh R, Pesaran T, et al. Gene-specific criteria for PTEN variant curation: recommendations from the ClinGen PTEN Expert Panel. *Hum Mutat*. 2018;39(11):1581–1592. http://doi.org/10.1002/humu.23636.

13. Fortuno C, Lee K, Olivier M, et al. Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. *Hum Mutat*. 2021;42(3):223–236. http://doi.org/10.1002/humu.24152.

14. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. *N Engl J Med*. 2017;377(5):454–464. http://doi.org/10.1056/NEJMoa1614359.

15. Perl AE, Martinelli G, Cortes JE, et al. Gilteritinib or chemotherapy for relapsed or refractory *FLT3*-mutated AML. *N Engl J Med*. 2019;381(18):1728–1740. http://doi.org/10.1056/NEJMoa1902688.

16. Chang MT, Asthana S, Gao SP, et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol*. 2016;34(2):155–163. http://doi.org/10.1038/nbt.3391.

17. Chang MT, Bhattarai TS, Schram AM, et al. Accelerating discovery of functional mutant alleles in cancer. *Cancer Discov*. 2018;8(2):174–183. http://doi.org/10.1158/2159-8290.CD-17-0321.

18. McLeod C, Gout AM, Zhou X, et al. St. Jude Cloud: a pediatric cancer genomic data-sharing ecosystem. *Cancer Discov*. 2021;11(5):1082–1099. http://doi.org/10.1158/2159-8290.CD-20-1230.

19. Hubisz MJ, Pollard KS, Siepel A. PHAST and RPHAST: phylogenetic analysis with space/time models. *Brief Bioinform*. 2011;12(1):41–51. http://doi.org/10.1093/bib/bbq072.

20. Pagel KA, Kim R, Moad K, et al. Integrated informatics analysis of cancer-related variants. *JCO Clin Cancer Inform*. 2020;4:310–317. http://doi.org/10.1200/CCI.19.00132.

21. Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, et al. Predicting splicing from primary sequence with deep learning. *Cell*. 2019;176(3):535–548.e24. http://doi.org/10.1016/j.cell.2018.12.015.

22. Douville C, Masica DL, Stenson PD, et al. Assessing the pathogenicity of insertion and deletion variants with the variant effect scoring tool (VEST-indel). *Hum Mutat*. 2016;37(1):28–35. http://doi.org/10.1002/humu.22911.

23. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015;31(16):2745–2747. http://doi.org/10.1093/bioinformatics/btv195.

24. Rentzsch P, Schubach M, Shendure J, Kircher M. CADD-splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med*. 2021;13(1):31. http://doi.org/10.1186/s13073-021-00835-9.

25. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 2019;47(D1):D886–D894. http://doi.org/10.1093/nar/gky1016.

26. Ghosh R, Oak N, Plon SE. Evaluation of in silico algorithms for use with ACMG/AMP clinical variant interpretation guidelines. *Genome Biol*. 2017;18(1):225. http://doi.org/10.1186/s13059-017-1353-5.

27. Smith G, Bounds R, Wolf H, Steele RJC, Carey FA, Wolf CR. Activating K-Ras mutations outwith "hotspot" codons in sporadic colorectal tumours - implications for personalised cancer medicine. *Br J Cancer*. 2010;102(4):693–703. http://doi.org/10.1038/sj.bjc.6605534.

28. Šolman M, Ligabue A, Blaževitš O, et al. Specific cancer-associated mutations in the switch III region of Ras increase tumorigenicity by nanocluster augmentation. *Elife*. 2015;4:e08905. http://doi.org/10.7554/eLife.08905.

29. Smalley KSM, Xiao M, Villanueva J, et al. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. *Oncogene*. 2009;28(1):85–94. http://doi.org/10.1038/onc.2008.362.

30. Wan PTC, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell*. 2004;116(6):855–867. http://doi.org/10.1016/s0092-8674(04)00215-6.

31. Yao Z, Yaeger R, Rodrik-Outmezguine VS, et al. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature*. 2017;548(7666):234–238. http://doi.org/10.1038/nature23291.

32. Abou Tayoun AN, Pesaran T, DiStefano MT, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat*. 2018;39(11):1517–1524. http://doi.org/10.1002/humu.23626.

33. Rehder C, Bean LJH, Bick D, et al. Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(8):1399–1415. http://doi.org/10.1038/s41436-021-01139-4.

34. Palmisano A, Vural S, Zhao Y, Sonkin D. MutSpliceDB: a database of splice sites variants with RNA-seq based evidence on effects on splicing. *Hum Mutat*. 2021;42(4):342–345. http://doi.org/10.1002/humu.24185.

35. Brnich SE, Abou Tayoun AN, Couch FJ, et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the

ACMG/AMP sequence variant interpretation framework. *Genome Med*. 2019;12(1):3. http://doi.org/10.1186/s13073-019-0690-2.

36. Ghosh R, Harrison SM, Rehm HL, Plon SE, Biesecker LG. ClinGen Sequence Variant Interpretation Working Group. Updated recommendation for the benign stand-alone ACMG/AMP criterion. *Hum Mutat*. 2018;39(11):1525–1530. http://doi.org/10.1002/humu.23642.

37. Pawliczek P, Patel RY, Ashmore LR, et al. ClinGen Allele Registry links information about genetic variants. *Hum Mutat*. 2018;39(11):1690–1701. http://doi.org/10.1002/humu.23637.

38. Mercer K, Giblett S, Green S, et al. Expression of endogenous oncogenic V600EB-raf induces proliferation and developmental defects in mice and transformation of primary fibroblasts. *Cancer Res*. 2005;65(24):11493–11500. http://doi.org/10.1158/0008-5472.CAN-05-2211.

39. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–674. http://doi.org/10.1016/j.cell.2011.02.013.

40. Dickson D, Johnson J, Bergan R, Owens R, Subbiah V, Kurzrock R. The master observational trial: a new class of master protocol to advance precision medicine. *Cell*. 2020;180(1):9–14. http://doi.org/10.1016/j.cell.2019.12.009.

41. Li MM, Chao E, Esplin ED, et al. Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2020;22(7):1142–1148. http://doi.org/10.1038/s41436-020-0783-8.