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SPECIAL ARTICLE

Assessments of Somatic Variant Classification Using the Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists Guidelines

A Report from the Association for Molecular Pathology

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To assess the clinical implementation of the 2017 *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*, identify content that may result in classification inconsistencies, and evaluate implementation barriers, an Association for Molecular Pathology Working Group conducted variant interpretation challenges and a guideline implementation survey. A total of 134 participants participated in the variant interpretation challenges, consisting of 11 variants in four cancer cases. Results demonstrate 86% (range, 54% to 94%) of the respondents correctly classified clinically significant variants, variants of uncertain significance, and benign/likely benign variants; however, only 59% (range, 39% to 84%) of responses agreed with the working group's consensus intended responses regarding both tiers and categories of clinical significance. In the implementation survey, 71% (157/220) of respondents have implemented the 2017 guidelines for variant classification and reporting either with or without modifications. Collectively, this study demonstrates that, although they may not yet be optimized, the 2017 guideline recommendations are being adopted for standardized somatic variant classification. The working group identified significant areas for future guideline improvement, including the

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The Variant Interpretation Testing Across Laboratories Somatic Working Group of the Clinical Practice Committee, Association for Molecular Pathology (AMP), was Chaired by M.M.L. The AMP 2021 Clinical Practice Committee consisted of Jane Gibson (chair), Fatimah Nahhas, Steven Sperber, Rashmi Goswami, Michael Kluk, Susan Hsiao, David Eberhard, Joseph Yao, Blake Buchan, Joshua Coleman, Elaine Gee, Andres Madrigal, and Jack Tung.

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need for a more granular and comprehensive classification system and education resources to meet the growing needs of both laboratory professionals and medical oncologists. *(J Mol Diagn 2023, 25: 69—86; https://doi.org/10.1016/j.jmoldx.2022.11.002)*

The technical advances associated with next-generation sequencing (NGS) and continued decreased costs affiliated with this technology have paved the way for routine molecular profiling of solid tumors and hematologic malignancies (*https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost*, last accessed May 3, 2022). Identification of acquired somatic and germline genetic variants in the setting of cancer can provide important predictive, diagnostic, and prognostic implications as well as key information for identification of therapeutic agents in patient management. Therefore, standardized reporting of complex genomic results within and between laboratories to facilitate clear and widespread understanding among health care providers is critical for proper interpretation of laboratory results and appropriate patient care.

A survey conducted among adult health care providers at a comprehensive cancer center in 2014 revealed that, although about 25% of providers were very confident in their knowledge of genomics, almost as many were "not very confident" or were "not confident at all" in this area.[1] Likewise, although about 25% noted that they were "very confident" in making treatment decisions based on genomic data, closer to 30% declared that they were "not very confident" or "not confident at all" in this regard.[1] Three years later, a survey addressing the integration of NGS into clinical practice directed toward physicians within a large pediatric oncology practice yielded similar findings, with >50% conceding that they were not confident in interpreting genomic findings and translating this information into patient care.[2] These studies highlight the complexity of NGS results and the difficulty among many health care providers in discerning the clinical relevance of a variant (eg, distinguishing a driver variant that is associated with the oncogenesis of cancer from a passenger variant that may have little clinical significance). In addition, these results illustrate the importance of developing consensus guidelines for evolving variant interpretation. In this regard, several variant classification systems have been proposed on the basis of various levels of supporting evidence for clinical significance.[3—6]

Development of "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"

In 2017, a formalized classification system for the interpretation and reporting of variants in cancer was developed by a multidisciplinary group led by the Association for Molecular Pathology (AMP), with representation from the American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP), and American College of Genetics and Genomics (hereafter referred to as the AMP/ASCO/CAP Somatic Variants Guideline).[7] The proposed standards and guidelines represented a defined framework for the classification of cancer variants and were carefully crafted on the basis of expert consensus from the working group and consideration of the previously published literature. This document was presented soon after publication of *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*, with primary applicability to mendelian disorders and an emphasis on the disease association of a variant for clinical diagnosis.[8] In the AMP/ASCO/CAP Somatic Variants Guideline, the clinical significance of a variant, detected by either NGS or non-NGS assays, is defined in a tiered system in consideration of three categories of clinical and experimental evidence: diagnostic, prognostic, and therapeutic. Levels of evidence (ranked from A to D) are defined relative to the strength of supporting evidence for the associated biomarker, with stronger evidence ranked in tier I (A/B: strong clinical significance) and lower evidence ranked in tier II (C/D: potential clinical significance). Variants lacking convincing evidence of cancer association or evidence of being benign/likely benign are designated in tier III (unknown clinical significance), with tier IV (benign/likely benign) most commonly representing rare germline variants with no known cancer association. Evidence is derived from a broad number of resources, including US Food and Drug Administration (FDA)—designated biomarkers, professional guidelines, clinical trials, function of the variants, population databases, variant databases, predictive algorithms, and published literature. Although a primary aim of cancer genomic profiling is the identification of somatic variants to aid in diagnosis, prognosis, and therapeutic decision making, careful consideration must be given to the identification of potential germline variants in genes that may predispose to hereditary cancer or hematologic disease, particularly in the setting of tumor-only analysis when no normal sample is available for comparison.[9,10]

AMP/ASCO/CAP Somatic Variants Guideline Implementation

Soon after the publication of the AMP/ASCO/CAP Somatic Variants Guideline, the Clinical Genome Resource Somatic Working Group incorporated the 2017 guideline's tiered

system into a variant curation metadata structure within the Clinical Interpretations of Variants in Cancer online resource.[11,12] The inclusion of the variant tier-based system in this widely used web-based tool promotes consensus and standardization in variant curation for laboratory professionals. In addition, >700 citations of the AMP/ASCO/CAP Somatic Variants Guideline have appeared in the literature, as of the time of this article's submission [R.L.T.-S., personal communication of Scopus search results (*http://www.scopus.com*, last accessed July 17, 2022)]. This has included the practical application of these guidelines for the purpose of variant interpretation, as well as interlaboratory and intralaboratory comparison of interpretation using the guidelines.[13–19] Furthermore, although some studies have reported easy adaptation of the guidelines, other studies have documented the need for improvement. In one multi-institutional study, the interrater concordance of categorizing 51 solid tumor variants among 20 participants was only 58%.[14] Discordance was observed for variants in tiers I, II, and III, with lack of agreement attributed to the following: i) lack of expertise with the guidelines, as only 20% of participants had incorporated the guidelines into their clinical practice; ii) the need for distinction between clinical actionability and biological relevance of the variant; and iii) the lack of clarity of the guidelines themselves. In a second multi-institutional study, participants believed that the guidelines did not incorporate the clinical significance of the effect of the variant on the gene/protein and that the relative importance of a variant for diagnosis and prognosis was limited.[15] Furthermore, most of those who completed the survey believed that the guidelines were heavily skewed toward FDA-approved therapies and National Comprehensive Cancer Network (NCCN) guidelines, which may not be as applicable to non-US health care systems.[15]

A study from the College of American Pathologists Molecular Oncology Committee reported worldwide adaptation of the 2017 AMP/ASCO/CAP Somatic Variants Guideline tier system in the fall of 2019 to be 129 of 236 (54.7%) and 64 of 131 (48.8%) for laboratories performing NGS on solid tumors and hematologic malignancies, respectively.[20] However, of those laboratories that used tiered classification systems, the 2017 AMP/ASCO/CAP Somatic Variants Guideline was used by 84.9% (129 of 152) of participants undergoing NGS on solid tumors and 73.6% (64 of 87) of participants undergoing NGS on hematologic malignancies.[20] In addition, approximately 35.2% of laboratories performing NGS on solid tumors and 39.4% of laboratories performing NGS on hematologic malignancies were planning to adopt the guideline in the future.[20] The study also reported that 74.2% of respondents undergoing NGS on solid tumors and 69.5% of respondents undergoing NGS on hematologic malignancies were "very satisfied" or "satisfied," respectively, with the 2017 guideline.[20] In addition to the publication of the AMP/ASCO/CAP Somatic Variants Guideline, the European Society for Medical Oncology has published the European Society for Medical Oncology Scale of Clinical Actionability for Molecular Targets,[21] which defines six levels of clinical evidence for molecular targets, whereas the FDA has proposed a three-tiered approach for FDA-approved NGS molecular profiling assays (FDA Fact Sheet: CDRH'S Approach to Tumor Profiling Next Generation Sequencing Tests, *https://www.fda.gov/downloads/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm584603.pdf*, last accessed May 3, 2022).

AMP Variant Interpretation Testing Across Laboratories Somatic Working Group

In 2018, the AMP Variant Interpretation Testing Across Laboratories (VITAL) Somatic Working Group was formed. The purpose of this working group was to i) understand implementation and utilization of the AMP/ASCO/CAP Somatic Variants Guideline among laboratories, ii) assess concordance between laboratories in the application of the tier classification system outlined in the guidelines for a series of variants, and iii) identify content within the guidelines that may result in variant classification inconsistencies between laboratories. This project has been designed in two separate but interrelated parts: administration of somatic variant classification challenges (hereafter referred to as VITAL Somatic Challenges) and AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey. Collectively, the results obtained from these studies would be used to inform future revisions of the somatic variant classification guidelines with a goal to achieve standardization and consistency for somatic variant interpretation across the globe.

Materials and Methods

AMP VITAL Somatic Challenges

Participant Selection

VITAL Somatic Challenge volunteer participants were recruited through outreach by AMP via e-mail, membership newsletters, social media posts, and peer-to-peer communications. Registration was open for a total of 6 weeks for all community members who perform somatic variant interpretation (Supplemental Table S1). Data from variant challenges were anonymized before analysis, and participants were assigned an identification number to avoid potential bias during data analysis. Participants were given the opportunity for further acknowledgment in publications of any VITAL Somatic Challenge data.

Variant Selection

Individual members of the AMP VITAL Somatic Working Group contributed a total of 10 deidentified cases from various cancer types and indications, both solid and hematologic diseases, and variant types, including both single-nucleotide variants and copy number variants. The variants from each case were reviewed by at least five members of

the working group. Individual working group members assessed levels of evidence and determined final classifications for each variant. A consensus of variant interpretation was reached through group discussion and used as the intended classification. Further refinement based on case complexity, variant types, and classification consensus resulted in the selection of four cases encompassing a total of 11 variants to be designated for inclusion in the final variant survey (Table 1).

VITAL Somatic Challenge Data Collection

VITAL Somatic Challenge response forms were generated using Survey Monkey (Momentive Inc., San Mateo, CA). Data collection questions were reviewed and vetted by VITAL Somatic Working Group members with assistance from select community members. Registered participants were independently sent the variant interpretation challenge cases and given approximately 4 weeks for completion. Participants were encouraged to complete all variant challenges; however, completion of all cases or all variants within a case was not required for inclusion in further data analysis.

VITAL Somatic Challenge Data Analysis

Final variant tier assignment

Two algorithms were used to compare the participants' responses with the intended response: one is based on the highest tiering, and another is based on the single best answer. Each variant was considered independently and, therefore, the algorithms included from 28 to 44 participants, depending on the variant, for a total of 362 classifications. For a variant that is of strong clinical significance (tier I) or potential clinical significance (tier II), it was additionally examined whether this variant should be interpreted together as clinically significant with any of the therapeutic (T), diagnostic (D), or prognostic (P) significance type or combinations.

Algorithm 1 (highest tiering): the responses for each variant were counted based on the highest tier. Specifically, the variant's final assignment of each participant is based on the participant's highest ranking of tier I to tier IV (tier I as the highest). For example, if a participant assigned a variant as tier I in one of the T, D, and P categories, it was considered that the participant intended to assign tier I to this variant, although the participant also assigned the variant tier II in one or more of other categories.

Algorithm 2 (single best answer): If a variant with the highest tier was assigned in more than one of the T, D, and P categories, the one correspondent to the intended response was counted as the final answer. Specifically, if a variant was assigned to tier II in both T and D and the intended answer was tier II in T category, tier IIT would be counted as the final answer and tier IID would be discarded.

Summarization of final variant assessment

These data are summarized for presentation using three different methods: i) the concordance of participants' variant classification and the intended classification based on both the variant tier and the category of clinical significance (T, D, or P; method 1); ii) the concordance of participants' variant classification and the intended classification based on variant tier only (method 2); or iii) the concordance of participants' variant classification and the intended classification when clinically significant variants, tier I and tier II, were grouped together (method 3).

In addition, because of the small number of participants ($n = 19$) who completed all 11 variant classifications, but 28 to 44 participants answered questions for each variant, all the answers to each of the 11 variants were combined together to represent broader range of participants' experience.

Table 1 Summary of the 11 VITAL Somatic Challenge Variants from Four Cancer Cases

VITAL variant	Gene	Transcript[†]	Position (hg19[‡])	c.	p.	VAF	Cancer type
1A	*ESR1*	NM_001122742.1	Chr6: 152332875	c.1181G>T	p.Arg394Leu	0.32	Lung squamous cell carcinoma
1B	*TP53*	NM_000546.4	Chr17: 7577555	c.726C>G	p.Cys242Trp	0.67	
2A	*PTCH1*	NM_000264.4	Chr9: 98239039	c.1602+2T>G	p.?	0.89	Medulloblastoma
2B	*TERT*	NM_198253.2	Chr5: 1295228	c.-124C>T	NA	0.34	
2C	*PTCH1*			9q Copy number loss			
3A	*EZH2*	NM_004456.4	Chr7: 148513849	c.1432G>T	p.Glu478*	0.22	Myeloid leukemia
3B	*PHF6*	NM_001015877.1	ChrX: 133549137	c.821G>A	p.Arg274Gln	0.51	
3C	*SETBP1*	NM_015559.2	Chr18: 42531907	c.2602G>A	p.Asp868Asn	0.05	
3D	*U2AF1*	NM_001025203.1	Chr21: 44514777	c.470A>C	p.Gln157Pro	0.38	
4A	*NRAS*	NM_002524.4	Chr1:115256528	c.183A>T	p.Gln61His	0.39	Adenocarcinoma
4B	*PIK3CA*	NM_006218.2	Chr3:178927410	c.1173A>G	p.Ile391Met	0.51	

[†]Data available (National Library of Medicine GenBank Overview, https://www.ncbi.nlm.nih.gov/genbank, last accessed May 3, 2022).

[‡]Data available (National Library of Medicine GRCh37 Genome Assembly, https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13, last accessed May 3, 2022).

Chr, chromosome; NA, not applicable; VAF, variant allele fraction; VITAL, Variant Interpretation Testing Across Laboratories.

AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey

All guideline implementation survey questions were developed, reviewed, and vetted by VITAL Somatic Working Group members with assistance from select community members. Survey questions and summary data are available in Supplemental Tables S2 through S4. The survey response form was generated using Survey Monkey and utilized a combination of open- and closed-ended questions without randomization. Answers to all questions were not required. The survey was anonymous; however, the collector only permitted the survey to be completed once from any device. Skip logic was employed only on question 1 as follows:

- "Yes" answers skipped forward to question 4 and completed remainder of the survey;
- "No" answers skipped forward to answer only questions 2 and 3, then skipped to end of survey;
- "Unaware" answers were considered disqualifying to continue and skipped to end of survey.

Survey participants were recruited through outreach by AMP via member listserv (ChAMP), e-mail, member newsletters, social media posts, and peer-to-peer communications. AMP membership was not required for participation in the survey. The survey was open from September 16, 2020, to November 3, 2020, and a total of 220 responses were received. The VITAL Somatic Working Group members reviewed all survey data. Free-text responses were reviewed by the working group to identify common themes within the responses whenever similar comments were provided (data not shown).

Results

VITAL Somatic Challenge Results

Demographics of VITAL Somatic Challenge Registrants

Volunteers were asked to fill out a registration form to participate in the VITAL Somatic Challenges. Besides contact and demographic information from participants, a short series of questions describing the implementation of the AMP/ASCO/CAP Somatic Variants Guideline in their institution were administered (Supplemental Table S1). A total of 134 participants completed the registration form, among whom 69% were members of AMP. Most of the participants were from academic clinical laboratories (80%). The top two primary roles of participants included clinical laboratory directors (M.D. plus Ph.D., 45%) and variant analysts (Ph.D. plus M.S., 24%). Other participants included Molecular Genetic Pathology and American Board of Medical Genetics and Genomics trainees and residents, and medical technologists. As related to guideline implementation, most participants used a hybrid (combination of manual and automatic) approach (65%) for variant classification. Of participants, 33% applied the guidelines manually, and 2% of participants utilized automated variant classification only.

VITAL Somatic Challenges

The VITAL Somatic Challenges consisted of 11 variants across four cases, including a lung squamous cell carcinoma with 2 variants, a medulloblastoma with 3 variants, a myelodysplastic syndrome (MDS) with 4 variants, and a colorectal adenocarcinoma with 2 variants (Tables 1–5). The 11 variants were indexed across 10 unique cancer-related genes, including both loss of function ($n = 7$) and gain of function ($n = 3$).[22–33] The 11 variants included 10 single-nucleotide variants (1 nonsense, 1 splice site, 1 promoter region, and 7 missense) and 1 copy number variant. The variant allele fraction (VAF) of the variants ranged from 4.6% to 89%.

A total of 362 responses to the 11 VITAL Somatic Challenges were received and reviewed. Concordance of the participant responses to the intended responses was calculated using the three different methods, as described above (Table 6). Of the 362 reported variant classifications, 213 (59%) responses agreed with the working group's intended response both on tiers and the categories of clinical significance (method 1), 236 (65%) responses agreed with the intended tier classifications (method 2), and 312 (86%) responses agreed with the intended tier classification when combining all clinically significant variants (tier I and tier II variants) into a single group (method 3). Variants 1A (tier III), 3B (tier III), 4B (tier IV), and 4A (tier I) demonstrated the most consistent concordance rate across the three comparison methods. Variant 2A (tier I) demonstrated the lowest concordance rate (39%) across participants with comparison methods 1 and 2. When comparison was performed by combining tier I and tier II into a single category (method 3), 9 of the 11 variants demonstrated at least 80% concordance or higher, with the exception of variants 3B (tier III; 53.6%) and 4B (tier IV; 77.4%) (Figure 1).

AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey Results

The AMP/ASCO/CAP Somatic Variants Guideline implementation survey was performed to assess the current adoption of the guidelines in the clinical molecular laboratory community (Supplemental Tables S2–S4). The initial survey question was used to determine the number of respondents who had implemented the AMP/ASCO/CAP Somatic Variants Guideline (71%; 157 respondents) versus those who had not (25%; 55 respondents) or who were not familiar with the guideline (4%; 8 respondents) (Figure 2). Respondents who were not familiar with the guideline were disqualified from further survey participation. Those who had not implemented the guideline were asked two questions before being disqualified from further survey participation: perceived barriers or limitations to implementation (question 2) and changes that could be made that they

Table 2 VITAL Somatic Challenge Case 1 Variant Interpretation Summary

Case variant*	Gene	Variant NM_accession no.†	VAF	Expected classification	Evidence summary
1A	*ESR1*	NM_001122742.1: c.1181G>T: p.Arg394Leu	0.32	Tier III	*ESR1* variants like p.Arg394Leu are rarely seen outside of breast cancer and have not been reported in squamous cell or other NSCLC (COSMIC, cBioPortal, and JAX-CKB). This variant has not been reported as a germline variant (ExAC, gnomAD, and EVS). *In silico* data suggest that this variant is damaging to ESR1 protein function (CADD, MutationAssessor, MutationTaster, and GERP); however, no additional functional or clinical data on this variant result in a classification as a variant of uncertain significance.
1B	*TP53*	NM_00546.4: c.726C>G: p.Cys242Trp	0.67	Tier II, P	The p.Cys242Trp missense variant has been reported in several tumor types, including squamous cell carcinoma of the lung (COSMIC and cBioPortal) and never reported as a population variant (ExAC, gnomAD, and ClinVar). Although there are no functional data regarding the effect of this variant, this substitution targets a residue located in the DNA-binding domain and is predicted to be pathogenic.[21] Somatic variants in *TP53* are associated with unfavorable prognosis in many tumor types, including NSCLC.[22,23]

*Clinical scenario for case 1 is a 73-year—old man with a history of squamous cell carcinoma of the lung, status after chemotherapy and radiation, now presenting with brain metastases. Next-generation sequencing was performed on DNA extracted from a left cerebellum resection, consistent with metastatic involvement by the patient's squamous cell carcinoma of the lung.

†Data available (National Library of Medicine GRCh37 Genome Assembly, *https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13*, last accessed May 3, 2022).

CADD, Combined Annotation Dependent Depletion; COSMIC, Catalogue of Somatic Mutations in Cancer; EVS, Exome Variant Server (*https://evs.gs.washington.edu/EVS*, last accessed December 15, 2022); ExAC, Exome Aggregation Consortium; GERP, Genomic Evolutionary Rate Profiling; gnomAD, Genome Aggregation Database; JAX-CKB, The Jackson Laboratory Clinical Knowledgebase; NSCLC, non—small-cell lung cancer; P, prognosis; VAF, variant allele fraction; VITAL, Variant Interpretation Testing Across Laboratories.

believed would assist in implementation (question 3). The responses for barriers and limitations in the implementation of the guidelines can be broadly categorized into software-related issues (including proprietary software and existing databases) and lack of clarity for some aspects of the guidelines, either generally or for specific cancer types. Some examples included the general lack of applicability of the guidelines for variants identified in hematopoietic neoplasms, especially when establishing clonality, the lack of more objective criteria for distinguishing between tiers I and II as well as the subcategories within each tier, and the challenges in following the guidelines internationally. Potential improvements identified included clearer guidance on classifying variants of uncertain significance, how to classify types of variants, and more granular definitions within the tiers.

For the respondents indicating that they had implemented the AMP/ASCO/CAP Somatic Variants Guideline, results indicated that 71% (157/220) of the respondents implemented the guidelines for variant classification, with (46%; $n = 41$) or without (54%; $n = 48$) modification of the guidelines to meet the institutional needs. The results also showed that 48% ($n = 43$) of respondents implemented the guideline's tier-based variant classification system in their clinical reports without modification, with 43% ($n = 38$) implementing with modifications. For the remainder (9%; $n = 8$), the guidelines did not influence the structure of the report (Figure 2). The implementation experience varied from easy and very easy (30%) to difficult and very difficult (25%), with neutral experience among 44% of the respondents. The top three changes for improvement to the existing

Table 3 VITAL Somatic Challenge Case 2 Variant Interpretation Summary

Case variant[†]	Gene	Variant NM_accession no.[‡]	VAF	Expected classification	Evidence summary
2A	*PTCH1*	NM_000264.4: c.1602+2T>G: p.?	0.89	Tier I, D	The *PTCH1* p.Ser137fs*3 is a frameshift variant leading to a premature stop. It has not been reported in the general population database, such as gnomAD and ClinVar, or in somatic mutation databases, such as COSMIC. This variant has been reported in the literature in a keratocystic odontogenic bone tumor.[24] Other truncating frameshift variants downstream of this codon have been reported in the COSMIC database in various tumor types, including medulloblastoma. *PTCH1* variants are almost exclusively found in SHH-activated medulloblastomas, and most of them show loss of heterozygosity.[25–27]
2B	*TERT*	NM_198253.2: c.-124C>T	0.34	Tier II, D/P	*TERT* promotor variants, including the c.-146C>T variant (alias C250T), occur in approximately 21% of medulloblastomas. Functional studies showed that C250T generates a new transcriptional factor binding site and activates *TERT* gene.[28,29] Of those adult patients with SHH-activated medulloblastoma, 83% contained a *TERT* promoter variant and were associated with a good outcome.[25,30] The variant is not reported in the general population database, such as gnomAD and ClinVar. However, the variant is not exclusively found in SHH-activated medulloblastoma.
2C		Loss of 9q, including *PTCH1*		Tier II, D/P	Loss of 9q and 10q has been frequently reported in SHH-activated medulloblastoma.[31] The presence of a truncating *PTCH1* variant in conjunction with partial loss of 9q, including *PTCH1*, suggests biallelic loss of this gene in this tumor sample. The 9q therefore supports the diagnosis of SHH-activated medulloblastoma.

[†]Clinical scenario for case 2 is a 29-year–old man with a poorly differentiated tumor, most likely of neural origin. The tumor is a malignant round blue cell tumor of the posterior fossa, World Health Organization grade IV. The pathologist suspected medulloblastoma of the posterior fossa.

[‡]Data available (National Library of Medicine GRCh37 Genome Assembly, *https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13*, last accessed May 3, 2022).

COSMIC, Catalogue of Somatic Mutations in Cancer; D, diagnostic; gnomAD, Genome Aggregation Database; P, prognostic; SHH, Sonic hedgehog; VAF, variant allele fraction; VITAL, Variant Interpretation Testing Across Laboratories.

guidelines included clearer guidance on variant classification, clearer guidance on interpretation of variants of uncertain significance, and more granular definitions within tiers. A common theme noted from the respondents was to incorporate more granular, and possibly numerical, scoring systems to provide objectivity to the variant classification process. The survey revealed an interesting observation related to the satisfaction of using the guidelines; the satisfaction was higher in the context of use of the guidelines for self-learning and in molecular pathology perspective in contrast to use by nonpathology professionals (eg, oncologists and genetic counselors).

Table 4 VITAL Somatic Challenge Case 3 Variant Interpretation Summary

Case variant[†]	Gene	Variant NM_accession no.[‡]	VAF	Expected classification	Evidence summary
3A	*EZH2*	NM_004456.4: c.1432G>T: p.Glu478*	0.218	Tier I, P	*EZH2* variants are seen in myeloid disorders, including MDS, AML, and MPN. Specifically, c.1432G>T represents a nonsense variant in exon 12 of the *EZH2* gene, whereby glutamic acid at codon 478 is replaced by a stop codon and leads to premature truncation of the encoded protein (p.Glu478*). The *EZH2* gene (Drosophila) is located on chromosome 7q36.1. The gene encodes a histone methyltransferase, a component of the polycomb repressive complex 2 involved in epigenetic regulation of cell fate decisions. Somatic variants in *EZH2* have been reported in various tumor types, including myeloid and lymphoid malignancies. Variants usually observed are missense, frameshift, and nonsense variants that cluster in the D2 and the catalytic SET domains of the protein. The predicted truncated protein described herein lacks the conserved C-terminal SET catalytic domain, which is required for histone methyltransferase activity, thereby leading to loss of function of the EZH2 protein. *EZH2* variants are independently associated with a poor prognosis in MDS [NCCN Guidelines Myelodysplastic Syndromes, *https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf* (registration required), last accessed February 16, 2022].
3B	*PHF6*	NM_001015877.1: c.821G>A: p.Arg274Gln	0.510	Tier III	*PHF6* variants occur in <5% of myeloid malignancies, including MDS and AML. Evidence for the clinical significance of *PHF6* variants in myeloid neoplasms is still emerging and remains under investigation. The alteration c.821G>A represents a missense variant in *PHF6*, whereby arginine is replaced by glutamine in the encoded protein (p.Arg274Gln). This variant is located within the zinc finger domain of the protein, and it has been reported as a somatic event in a few hematologic malignancies, including MDS. This variant has not been reported as a germline polymorphism (gnomAD and ClinVar). The *PHF6* is located on chromosome Xq26.2 and encodes a protein with two PHD-like zinc finger domains. The PHF6 protein is involved in transcriptional regulation via suppression of rRNA transcription, and it has been reported to function as a tumor suppressor. Nonsense and frameshift variants are most commonly observed. NCCN guidelines (NCCN Guidelines Myelodysplastic Syndromes, *https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf*, last accessed February 16, 2022) state nonsense/frameshift or splice site variants are more frequent in cases with excess blasts, but there is no association with decreased survival.
3C	*SETBP1*	NM_15559.2: c.2602G>A: p.Asp868Asn	0.046	Tier I, P	The variant c.2602G>A represents a missense variant in *SETBP1*, whereby aspartic acid is replaced by asparagine at codon 868 of the encoded protein (p.Asp868Asn). Amino acid 868 is a highly conserved residue and is a recurrent hot spot variant within the SKI-homologous domain of SETBP1. The *SETBP1* is located on chromosome 18q12.3. SETBP1 interacts with the protein encoded by the SET oncogene involved in DNA replication and cell cycle regulation. Functional studies indicate that p.Asp868Asn leads to gain of function with higher proliferation rate, leukemic progression, and adverse outcomes (NCCN Guidelines Myelodysplastic Syndromes, *https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf*, last accessed February 16, 2022).

(*table continues*)

Table 4 (*continued*)

Case variant[†]	Gene	Variant NM_accession no.[‡]	VAF	Expected classification	Evidence summary
3D	*U2AF1*	NM_001025203.1: c.470A>C: p.Gln157Pro	0.382	Tier I, P	The variant c.470A>C represents a missense variant in *U2AF1*, whereby glutamine is replaced by proline in the encoded protein (p.Gln157Pro). This base change alters a highly conserved amino acid within the N-terminal zinc finger motif flanking the U2AF homology motif and likely functions as an activating alteration. The *U2AF1* gene (alias U2AF35) is located on chromosome 21q22.3. The gene encodes a member of the SR family of splicing factors and is required for accurate 3′-splice site selection in mRNA splicing. Hot spot substitutions at two amino acid positions in the N- and C-terminal zinc finger domains (S34 in exon 2 and Q157 in exon 6, respectively) account for most of the reported variants in *U2AF1*. These gain-of-function variants are associated with decreased overall survival and increased risk of leukemic transformation (NCCN Guidelines Myelodysplastic Syndromes, *https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf*, last accessed February 16, 2022).

[†]Clinical scenario for case 3 is a 68-year—old man with a history of thrombotic thrombocytic purpura with ongoing transfusion-dependent anemia, intermittent neutropenia, and persistent thrombocytopenia. A bone marrow biopsy result shows involvement by MDS, with 7.6% blasts and a normal male karyotype. Next-generation sequencing was performed on DNA extracted from the bone marrow using a myeloid 37 gene panel.

[‡]Data available (National Library of Medicine GRCh37 Genome Assembly, *https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13*, last accessed May 3, 2022).

AML, acute myeloid leukemia; EZH2, enhance of zeste homolog 2; gnomAD, Genome Aggregation Database; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NCCN, National Comprehensive Cancer Network; P, prognostic; PHD, plant homeodomain; PHF6, PHD finger protein 6; SETBP1, SET-binding protein 1; SKI, SKI proto-oncogene; SR, serine-arginine-rich protein; U2AF, U2 small nuclear RNA auxiliary factor; VAF, variant allele fraction; VITAL, Variant Interpretation Testing Across Laboratories.

International participation in the survey was noted among respondents who has implemented the AMP/ASCO/CAP Somatic Variants Guideline, including the United States (72%), Canada (6%), Australia (5%), China (5%), India (3%), Iran (2%), and the United Kingdom (2%). Algeria, Singapore, Norway, Republic of Korea, and Spain were all represented at 1% each. Both germline and somatic testing was performed in 60% of these laboratories, whereas somatic only testing was performed in the remainder (40%).

Discussion

The objectives of this project were to i) understand the implementation and utilization of the AMP/ASCO/CAP Somatic Variants Guideline recommendations, ii) assess concordance between laboratories in the application of the tier classification system outlined in the guidelines for a series of variants, and iii) identify content within the guidelines that may result in classification inconsistencies between participating laboratories.[7]

The VITAL Somatic Challenges represented adult and childhood clinical scenarios, with three solid tumor cases and one hematologic case (Table 1). Genes and associated variants spanned a wide range of VAFs, with five variants in tier I that showed strong clinical significance; three variants in tier II associated with potential clinical significance; two variants in tier III of unknown clinical significance; and one variant in tier IV that is considered likely benign or benign. The type of variants included, and concepts observed, are those recognized during routine NGS testing. Multiple missense variants as well as a promoter, splice site, and a nonsense variant were included, as was a benign polymorphism with a reported allele frequency of >5%, in multiple population databases. In addition, loss of heterozygosity (*TP53* and *PTCH1*), common hot spot variants (*NRAS* and *U2AF1*), a variant uncommon for a specific tumor type (*ESR1*), and a copy number variant were included for analysis.

Somewhat expectedly, VITAL Somatic Challenge responses showed that the primary role of most of the participants was clinical laboratory director or variant analyst. In addition, most participants were members of the AMP community (2:1), most worked at an academic medical center (2.5:1), and most hold a doctoral degree (Supplemental Table S1). Most participants reported spending 15 to 30 minutes per variant classification, with the exception of hot spot variants in *U2AF1* and *NRAS* and the benign *PIK3CA* variant, in which most completed in <15 minutes. Although not all participants may work in a laboratory that performs NGS testing for all the specific tumor types or associated genes described in the variant challenge survey, the working group believed that a thorough understanding of the principles outlined in the AMP/

ASCO/CAP Somatic Variants Guideline would enable participants to determine the intended classification of each variant.

Classification Concordance within the VITAL Somatic Challenges

Of the 362 responses summarized, only 213 (59%) agreed with the VITAL Somatic Working Group's intended responses for both the variant tiers and the categories of clinical significance (method 1). The low concordance can be attributed partially to participants' understanding of the correspondence of the levels of clinical evidence to the tier classification and partially to potential for ambiguity and/or user interpretation of survey instructions. For example, some participants listed level C or level D evidence for a specific variant, but classified the variant as tier I, or cited level B evidence for a tier II variant. These results reflect participants' familiarity of the guidelines. A recent study evaluating interrater agreement of variant classification based on the AMP/ASCO/CAP Somatic Variants Guideline showed a significant increase in agreement (from $\kappa = 0.35$ to $\kappa = 0.7$) after sharing the summary of variant classifications and additional information about each variant with the participants.[14]

The VITAL Somatic Challenge instructions could have been clarified in some areas. For instance, the therapeutic significance was intended for variant-associated targeted therapies only. Some participants included the therapeutic significance, based on prognostic biomarkers, or treatment decisions, based on risk stratification. The intention of the VITAL Somatic Challenges was to have participants fill in all evidence-based clinical significance for a given variant. Because the instructions required an answer for each category of clinical significance (D, P, or T) when applicable, some participants filled in the same tier for all three (D, P, or T) categories of clinical significance, although they may really mean the significance in one category based on the evidence provided. For example, variant 4A *NRAS* p.Gln61His is a tier I variant based on level A evidence that it predicts resistance to treatment with epidermal growth factor receptor monoclonal antibodies in colon cancer. Therefore, there is virtually no benefit to treat patients with colon cancer with known *KRAS* and *NRAS* variants with cetuximab or panitumumab, either alone or in combination with other anticancer agents, and the exposure to toxicity and the expense of the treatment cannot be justified [NCCN, *https://www.nccn.org/professionals/physician_gls/pdf/colon_basic.pdf* (registration required), last accessed May 3, 2022].[34,35] Three participants classified the variant as tier I with level A evidence for therapeutics, diagnosis, and prognosis; one participant reported it as a tier I variant with level B evidence for therapeutics, diagnosis, and prognosis; and two participants reported it as a tier II variant with level B evidence for therapeutics, diagnosis, and prognosis. When using method 2, the overall concordance of participants' variant classification and the intended classification based on tiers only, the agreement increased to 65% (236/362). This method eliminated the potential misunderstanding of what constitutes the therapeutic significance as well as the possible confusion caused by the VITAL Somatic Challenge design.

The largest improvement in the agreement of the participants' responses and intended responses is observed when combining all clinically significant variants (ie, all tier I and tier II variants) into one group, method 3. The agreement for method 3 is 86% (312/362), indicating that most of the participants can correctly identify clinically significant variants based on the AMP/ASCO/CAP Somatic Variants Guideline. However, the data also show that some participants were unfamiliar with the relationship between different levels of evidence and the tiers of variant classification. In a few cases, participants classified variants with the level B evidence as tier II variants, such as the example listed above that two participants classified the variant 4A *NRAS* p.Gln61His in colon cancer as a tier II variant but supported their classification by the level B evidence (data not provided). There were also challenges in differentiating level A/B evidence from level C/D evidence, especially for variants with diagnostic and/or prognostic significance. This is exemplified in the responses to variant 3D *U2AF1* p.Gln157Pro in a patient with MDS. The intended answer was a tier I variant with level A prognostic significance [NCCN, *https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf* (registration required), last accessed May 3, 2022].[36] Although all participants considered the variant clinically significant, 13 of the 31 participants classified the variant as tier II. The results indicate that not all participants are equally familiar with the tiered classification system. This evaluation suggests that more education about the AMP/ASCO/CAP Somatic Variants Guideline and how to integrate the guidelines into clinical practice is needed. The results also indicate the need for additional clarification and more granular criteria to define different levels of evidence, especially the evidence of diagnostic and prognostic significance, to facilitate more consistent variant classification.

The variants with high and low concordance were further analyzed to identify possible factors that may affect classification concordance. Variants 1A and 4A presented the highest concordance (82% and 84%, respectively) using method 1. Variant 2A exhibited the lowest concordance (39%) using method 1 and the highest increase in concordance (to 91%) when method 3 was used. Variant 3B showed the same low concordance (54%) regardless of what method was used. Variant 1A *ESR1* p.Arg394Leu was seen in a 73-year−old man with a history of squamous cell carcinoma of the lung. Variants in the *ESR1* gene are rarely seen outside of breast cancer. The *ESR1* p.Arg394Leu has not been reported in population databases, such as Genome Aggregation Database, or somatic or germline variant databases, such as Catalogue of Somatic Mutations in Cancer, cBioPortal, Human Gene Mutation Database, and ClinVar. Although *in*

silico predicting tools suggest that this variant is damaging to the estrogen receptor 1 (ESR1) protein function [eg, Combined Annotation Dependent Depletion (CADD), *https://cadd.gs.washington.edu*; MutationAssessor, *http://mutationassessor.org*; MutationTaster, *https://www.mutationtaster.org*; and Genomic Evolutionary Rate Profiling (GERP), *https://genome.ucsc.edu/cgi-bin/hgTrackUi?db=hg19&g=allHg19RS_BW*; all last accessed December 15, 2022], no additional functional or clinical data suggesting oncogenicity of this variant result in a classification of a variant of uncertain significance (tier III). Variant 4A, *NRAS* p.Gln61His, was reported in a 56-year−old man with colon cancer. This variant is a well-documented gain of function, and hot spot variant in colorectal cancer, and is associated with resistance to epidermal growth factor receptor monoclonal antibody therapies. Classification of these two variants is relatively easy: one is an extremely rare variant with no available evidence of clinical significance, and one is a well-published hot spot variant documented in multiple professional guidelines [NCCN, *https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf* (registration required), last accessed May 3, 2022].[37] The variant 2A, *PTCH1* c.1602+2T>G, is a loss-of-function mutation found in a patient suspected of having a medulloblastoma. Although most of the participants considered the variant clinically significant, only 13 of the 33 participants classified the variant as a tier I variant for diagnosis.[38] Eight participants classified it as tier II for diagnosis, and 9 classified it as tier I or II for therapy or prognosis. This variant may not be as easy to classify for individuals who mostly encounter adult cancers as the disease exists mostly in pediatric patients. Because the *PTCH1* gene is a known tumor suppressor, the loss of function itself (canonical splicing site variant) may have led to the high concordance (91%) using method 3, although more participants classified it as a tier II than a tier I variant (data not provided). Variant 3B, *PHF6* p.Arg274Gln, is another variant difficult to classify. Alterations in *PHF6* have been reported in different hematologic malignancies, most commonly in T-cell acute lymphoblastic leukemia and occasionally in MDS. The *PHF6* gene encodes a tumor suppressor, and most disease-associated variants cause a loss of function (nonsense, frameshift, or splice-site variant). The significance of the missense variant *PHF6* p.Arg274Gln in MDS is uncertain (NCCN, *https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf*, last accessed May 3, 2022).[39,40] The intended classification for this variant is tier III. However, the *PHF6* p.Arg274Gln variant is in a variant hot spot, and there is emerging evidence suggesting potential prognostic significance in MDS.[41] Not surprisingly, the variant showed the same low concordance using all three methods of analysis. Detailed analysis of each response to the VITAL Somatic Challenges concludes that the concordance between participants' responses and the intended answers is largely dependent on the variants themselves: common variants with well-established clinical evidence are associated with higher concordances, and rare variants with limited clinical evidence or variants with emerging evidence manifest lower concordances.

Variant Classification Resources and Utilization

Resources proposed in the AMP/ASCO/CAP Somatic Variants Guideline for variant classification include FDA-approved therapy data, professional guidelines, population databases, and germline and somatic variant databases. Predictive software and publications on variant impact/function, population studies, or clinical trial information are additionally referenced.

Within the VITAL Somatic Challenges, participants were asked to report resource use in variant classification. Responses consisted of yes, no, or not applicable as related to the resources described above. Among certain variants within the survey, the use of the appropriate resource was critical for interpretation. This finding was true for variants 3A (*EZH2* c.1432G>T), 3C (*SETBP1* c.2602G>A), and 3D (*U2AF1* c.470A>C) as related to prognosis in the setting of MDS. The NCCN guidelines (NCCN, *https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1446*, last accessed May 3, 2022) provide specific information as related to disease progression or poor prognosis for these variant types among the three genes. In addition, the *WHO Classification of Tumours of Haematopoeitic and Lymphoid Tissues*[42] lists *EZH2* and *U2AF1* as commonly altered genes in MDS, with adverse prognostic impact.[42] Given that inclusion in professional guidelines serves as tier I, level A evidence, those participants who answered yes in relation to "indicated in a clinical practice guideline" were highly likely to classify these variants correctly. For example, for variant 3A (*EZH2*), among the 15 participants of 32 who indicated that the variant type was addressed in clinical guidelines, 14 of 15 (93%) correctly indicated tier I prognostic status, with 13 of 15 (87%) indicating level A evidence. By comparison, 17 of 32 participants answered no or not applicable in relation to clinical practice guidelines, with only a single participant among this group (6%) ranking the *EZH2* variant as prognostic within tier I (level B). Similar trends were observed for variants 3D and 3C.

Barriers to use of professional clinical guidelines may include access, cost, ability to search the resource for relevant information, and knowledge of resource existence. For example, NCCN guidelines require a registration to view guideline information, as well as knowledge of cancer site/type for use of the appropriate guideline. In addition, these guidelines list variant types (eg, missense or frameshift) or location of critical variants by amino acid residue numbers or ranges of amino acids, and often have information embedded within flowcharts, tables, and footnotes, making search functionality limited. As another example, the *WHO Classification of Tumours of Haematopoeitic and Lymphoid Tissues* can be challenging to search as genes or genetic

Table 5 VITAL Somatic Challenge Case 4 Variant Interpretation Summary

Case variant*	Gene	Variant NM_accession no.†	VAF	Expected classification	Evidence summary
4A	*NRAS*	NM_002524.4: c.183A>T: p.Gln61His	0.39	Tier I, T	This variant, c.183A>T, represents a missense variant in the *NRAS* gene, which is expected to replace glutamine by histidine in the encoded protein (p.Gln61His). The glutamine residue at codon 61 is highly conserved, and this missense change has been well documented to result in the gain of function of the *NRAS* gene that results in constitutive up-regulation of the Ras-Raf oncogenic pathway. This variant is a hot spot mutation, documented in colorectal and other cancer types in somatic variant databases, including COSMIC, OncoKB, CIVIC, and JAX-CKB. This variant is not documented in the general population databases, such as gnomAD. Professional society guidelines for colorectal cancer molecular testing state that *NRAS* variants in codon 61, among other hot spot variants in *KRAS* and *NRAS* genes, are associated with resistance to treatment with EGFR monoclonal antibodies.[32] This variant is also documented in the NCCN guidelines for colorectal cancers [NCCN Guidelines Colon Cancer, *https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf* (registration required), last accessed February 16, 2022].
4B	*PIK3CA*	NM_006218.2: c.1173A>G: p.Ile391Met	0.51	Tier IV	This variant is documented in the general population frequency databases at a minor allele frequency of >1% (1000 Genomes: 8.79%; ExAC: 6.49%; and gnomAD: 6.18%). The variant allele fraction of the variant is 51% in this case. All *in silico* predictions are unanimously benign/tolerated (Polyphen2, SIFT, and CADD).

*Clinical scenario for case 4 is a 56-year–old man diagnosed with a T2N0M0 invasive adenocarcinoma involving the transverse colon. Targeted next-generation sequencing assay identified variants.

†Data available (National Library of Medicine GRCh37 Genome Assembly, *https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13*, last accessed May 3, 2022).

CADD, Combined Annotation Dependent Depletion; CIVIC, Clinical Interpretations of Variants in Cancer; COSMIC, Catalogue of Somatic Mutations in Cancer; EGFR, epidermal growth factor receptor; ExAC, Exome Aggregation Consortium; gnomAD, Genome Aggregation Database; JAX-CKB, The Jackson Laboratory Clinical Knowledgebase; NCCN, National Comprehensive Cancer Network; Raf, rapidly accelerated fibrosarcoma; Ras, rat sarcoma virus; SIFT, sorting intolerant from tolerant; T, therapeutic; VAF, variant allele fraction; VITAL, Variant Interpretation Testing Across Laboratories.

alterations may be described broadly in relation to diagnostic, prognostic, or therapeutic relevance, necessitating a user to seek additional information from the cited publications within, and access to the resource incurs a cost. Thus, user knowledge, access, and experience with these resources is anticipated to reflect success in correct variant classification.

The breadth of utilized resources varied across VITAL Somatic Challenges. However, as expected, somatic variant

Table 6 Concordance Rate with Three Different Methods

	Method 1 comparison		Method 2 comparison		Method 3 comparison	
VITAL variant	Correct responses	% Correct	Correct responses	% Correct	Correct responses	% Correct
1A	36	81.8	37	84.1	37	84.1
1B	22	56.4	30	76.9	33	84.6
2A	13	39.4	13	39.4	30	90.9
2B	17	53.1	20	62.5	27	84.4
2C	14	48.3	18	62.1	26	89.7
3A	15	46.9	16	50.0	30	93.8
3B	15	53.6	15	53.6	15	53.6
3C	15	46.9	17	53.1	28	87.5
3D	16	51.6	17	54.8	31	100.0
4A	26	83.9	29	93.5	31	100.0
4B	24	77.4	24	77.4	24	77.4
Total	213	58.8	236	65.2	312	86.2

Please refer to *Materials and Methods* for full descriptions of each method.
VITAL, Variant Interpretation Testing Across Laboratories.

databases were used at high frequency, except for variant 2C, representing a copy number alteration. Clear preferences in respondent use of cancer databases were observed: Catalogue of Somatic Mutations in Cancer[43] (*https://cancer.sanger.ac.uk/cosmic*, last accessed May 3, 2022) (81%) > Clinical Interpretations of Variants in Cancer[12] (*https://civicdb.org/home*, last accessed May 3, 2022) (49%) > cBioPortal[44,45] (*https://www.cbioportal.org*, last accessed May 3, 2022) (46%) > The Cancer Genome Atlas[46] (*https://portal.gdc.cancer.gov*, last accessed May 3, 2022) (23%) > American Association for Cancer Research Genie[47] (*https://www.aacr.org/professionals/research/aacr-project-genie/aacr-project-genie-data*, last accessed May 3, 2022) (8%). The percentages represent the average across challenges, with the trend of frequency of database use consistent throughout. Clinical Interpretations of Variants in Cancer and cBioPortal demonstrated similarity in reported use, with Catalogue of Somatic Mutations in Cancer clearly represented as the most frequently consulted database. Participants also had the opportunity to supply open-ended responses about resources additionally used in the classification. For example, the TP53 International Agency for Research on Cancer database[48] was used in consultation for variant classification by 38.5% (15/39) of participants for variant 1B, *TP53* c.726C>G. Among those reporting use of the database, 66.7% (10/15) listed this variant as prognostically relevant (tier I/II), consistent with the intended response. Of those who did not specifically list consultation of the TP53 International Agency for Research on Cancer database, 15 of 24 participants (63%) also classified the variant as prognostically relevant (tier I/II). The concordance in classification may reflect participant's familiarity with *TP53*, use of other resources to support classification, lack of critical data in the TP53 International Agency for Research on Cancer database to influence this variant's classification, or potential use of the TP53 database without specific designation in the survey.

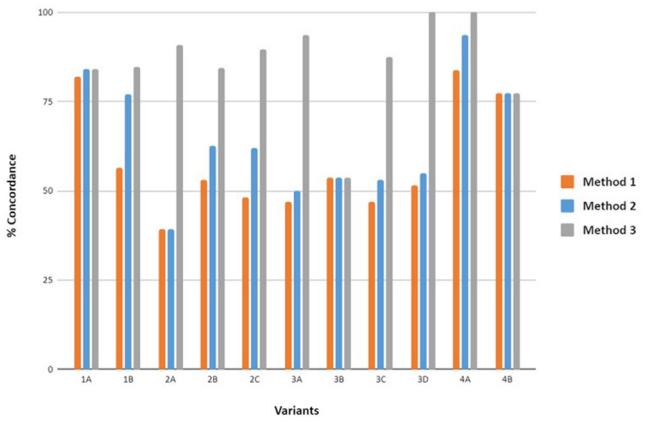

Figure 1 Concordance rate of each variant of the Association for Molecular Pathology Variant Interpretation Testing Across Laboratories Somatic Challenges using three different methods. Method 1, the concordance of participants' variant classification and the intended classification based on both the variant tier (tiers I through IV) and the category of clinical significance (therapeutics, diagnosis, or prognosis); method 2, the concordance of participants' variant classification and the intended classification based on variant tier only; and method 3, the concordance of participants' variant classification and the intended classification when tier I and tier II (ie, clinically significant variants) are combined as one group.

In the open-ended responses provided by participants, examples of other reported data sources used in classification included the following: MSK-Impact (*https://www.mskcc.org/msk-impact#a-focus-on-data-sharing*, last accessed May 3, 2022), OncoKB (*https://www.oncokb.org*, last accessed May 3, 2022), The Jackson Laboratory Clinical Knowledgebase (JAX-CKB; *https://ckb.jax.org*, last accessed December 15, 2022), My Cancer Genome (*https://www.mycancergenome.org*, last accessed May 3, 2022), St. Jude PeCan (*https://pecan.stjude.cloud*, last accessed May 3, 2022), NCCN (*http://www.nccn.org*, last

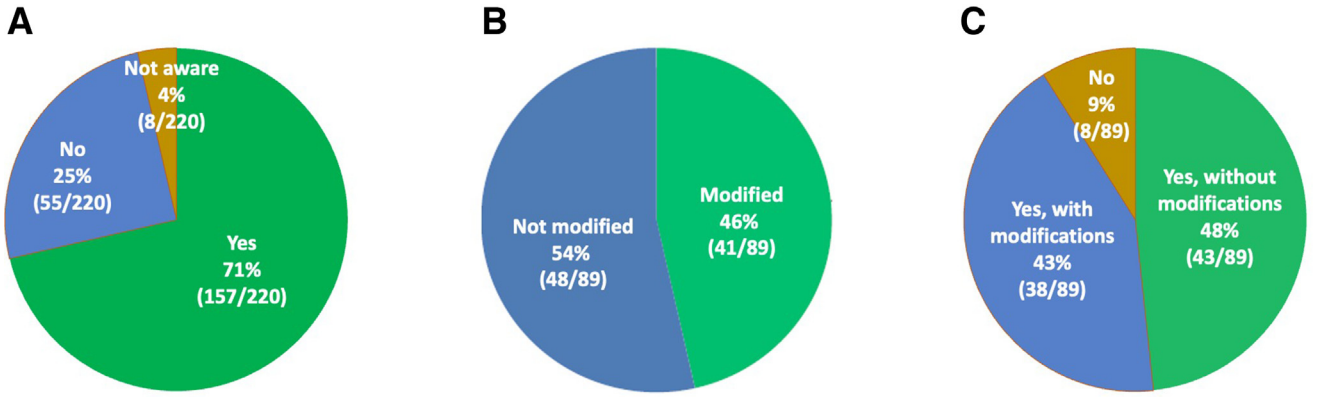

Figure 2 Association for Molecular Pathology (AMP)/American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) Somatic Variants Guideline Implementation Survey: selected results. **A:** Question (Q) 1: Have you implemented the AMP/ASCO/CAP somatic variant classification guidelines in your laboratory/institution? Answer choices: yes, no, and was not aware of these guidelines before survey. **B:** Q4: Have you modified the AMP/ASCO/CAP somatic variant classification guidelines to meet your institutional needs? **C:** Q6: Do you structure your reports based on the tiered system recommended by the AMP/ASCO/CAP guidelines? Additional AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey summary results provided in Supplemental Tables S2–S4.

accessed May 3, 2022), World Health Organization (*https://whobluebooks.iarc.fr*, last accessed May 3, 2022), Atlas of Genetics and Cytogenetics in Oncology and Haematology (*http://atlasgeneticsoncology.org*, last accessed May 3, 2022), Alamut (SOPHiA GENETICS, Inc., Boston, MA), VarSome (*https://varsome.com*, last accessed May 3, 2022), and proprietary sources.

Identification of Germline Variants within the VITAL Somatic Challenges

The use of appropriate resources for variant classification also shaped VITAL Somatic Challenge participants' identification of germline variants of relevance. Cancer genomic profiling has the capability of identifying variants of both germline and somatic origin. Discerning variant origin has important implications for determining variant context and meaning, particularly in the setting of a tumor-only analysis lacking a comparator germline sample for study. Limited knowledge of the patient's personal and family history of cancer can further confound analysis. Prior study suggests that the frequency of pathogenic germline variation may be in the range of 4% to 12% in the setting of cancer genomic profiling.[49] Within the AMP/ASCO/CAP Somatic Variants Guideline, evidence to discern variant origin relates primarily to VAF of the observed variant, while also recognizing the importance of literature and database review to discern any prior association with cancer predisposition. Where possible, review of available patient demographic or clinical history can also inform analysis. Furthermore, inherent complexities in the interpretation of VAF exist, including the influence of tumor content, clonal diversity, altered copy number, or loss of heterozygosity. For this reason, it is critical for those engaged in variant classification, reporting, and interpretation to consider a variant and its related attributes in context. Each case provided in the VITAL Somatic Challenges included a patient vignette with age, tumor, or disease type and a limited clinical history, along with VAF. The AMP/ASCO/CAP Somatic Variants Guideline suggests that apparently nonmosaic VAFs (at approximately 0.5 and 1) may represent potential evidence of germline origin. VAFs included in the challenge ranged from 0.046 to 0.89, with four variants demonstrating a VAF >0.50 (1B, 2A, 3B, and 4B). Enough information was provided in the challenge to also allow for review of population minor allele frequency to eliminate benign germline variants. Variant 4B (*PIK3CA* c.1173A>G) served to test participants' knowledge in this setting with an aggregate global minor allele frequency of >6%, indicating the benign nature of this germline variant, a concept recognized by 87% of participants (27/31) who deemed the variant to be germline in origin and 77% (24/31) classifying it as tier IV, consistent with the intended response.

Most participants (73%) also reported that variant 2A (*PTCH1* c.1602+2T>G; VAF of 0.89) was suspected to be germline in origin, consistent with the intended response. Patient age (29 years, per vignette), along with disease type (medulloblastoma), provided additional evidence as related to the potential for germline cancer predisposition. This tumor also displayed a loss of 9q (encompassing *PTCH1*), lending further data in support of the high VAF. Among those participants who indicated potential for this variant to be germline in origin (24/33), 37.5% further described an explicit concern for basal cell nevus syndrome (alias Gorlin syndrome; Online Mendelian Inheritance in Man, *https://www.omim.org/clinicalSynopsis/109400*, last accessed May 3, 2022), a disease known to be associated with SHH-activated medulloblastoma due to *PTCH1*-inactivating variation. Twenty-seven percent (9/33) of participants indicated that the variant was not suspected to be germline in nature. Cited reasons amid the open-ended responses included the variant's absence from germline

databases and high VAF, suggesting further education is necessary for appropriate interpretation. Overall concordance in variant classification was high for this variant, with 91% (30/33) of respondents ranking it as tier I/II.

Among the 11 variants in the VITAL Somatic Challenges, most participants (>70%) were in agreement in judging the potential for germline etiology on a per-variant basis (Supplemental Figure S1). Seven of the 11 variants had a concordance of >90% among respondents. The variant with greatest discordance was a *PFH6* variant on chromosome X (c.821G>A) at VAF of 0.51, with 29% of participants indicating potential for germline etiology. This variant was detected in a male patient with a history of thrombotic thrombocytic purpura and a new diagnosis of MDS with normal karyotype. The VAF was likely to be a confounding attribute for participants given that it was close to 50%; however, a germline variant in a male on chromosome X would display a hemizygous frequency of 1.

The impact in identifying a germline cancer or hematologic disease predisposition variant includes potential alteration in patient management, increased surveillance, cascade testing for at-risk family members, and reproductive counseling. Therefore, it is crucial that those individuals participating in variant classification have deep knowledge and understanding of germline and somatic disease, inheritance patterns, the influence of concurrent genomic alterations on VAF, and familiarity with resources to allow for a full analysis of variant attributes.

VITAL Somatic Challenge Study Limitations

Although the VITAL Somatic Challenge participant specific questions were carefully written and designed to define the participants and allow for comparison between individual distinct details and the committee's intended response for each variant, unfortunately only 14% (19/134) of participants completed all 11 variant challenges. This finding emphasizes the importance of questions with required answers instead of optional responses in future similarly based challenges when correlation studies are important. Furthermore, the impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic on participation in the VITAL Somatic Challenges cannot be determined but was a potential factor in the low completion rates observed.

Key Findings to Inform AMP/ASCO/CAP Somatic Variants Guideline Updates

The AMP/ASCO/CAP Somatic Variants Guideline recommendations were developed to provide standardization in reporting of somatic variants among laboratories and allow for uniformity in communication of complex NGS results to our clinical colleagues to improve patient care and management. The relatively low consensus for all variants, based on VITAL Somatic Challenges, suggests lack of granularity, insufficient instructions, and need for additional guidance on using the current guidelines.

Understanding the current limitations and barriers to the AMP/ASCO/CAP Somatic Variants Guideline was ascertained with the AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey. Most respondents (55%) requested clearer guidance on the classification of variants of uncertain significance, whereas an equally high number communicated the need for clearer guidance on types of variants (50%) specifically, potential germline variants, structural variants, copy number variants, and fusions. A similar number of individuals asked for more granular definitions within specific tier categories (45%), or some suggested altering the number or tiers of tier group assignments. Furthermore, a significant number of respondents (38%) believe more educational resources should be available for clarification and understanding of the guidelines (33%) as well as the availability of educational resources to share with their clinical colleagues (33%). Some respondents voiced the requirement for clearer guidance on reclassification of variants as new evidence regarding their clinical significance emerges. Respondents also reported system-based processes as barriers for implementation of the AMP/ASCO/CAP Somatic Variants Guideline within their facility. Seventy-one percent (157/220) of respondents implemented the guidelines for variant classification. Among respondents not currently using the guidelines, most (40%) cited limitations of software systems, either proprietary (21%) or commercial (19%), as an impediment for conversion to the tier classification, whereas 29% reported converting an existing database as a barrier. Only 26% of respondents reported no barriers to implementation. Overall, the implementation survey revealed that most of our molecular professional colleagues are either satisfied or somewhat satisfied (73%) with the AMP/ASCO/CAP Somatic Variants Guideline, 11% are neutral, and 15% expressed some level of dissatisfaction (Supplemental Table S3).

Importance of Standardization and Guideline Updating

Taken together, the VITAL Somatic Challenges and AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey demonstrate that, although not yet perfected, the AMP/ASCO/CAP Somatic Variants Guideline initiated standardization and conversation regarding somatic variant classification among laboratories. Of the AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey respondents, 71% have implemented the tiered system for somatic variant interpretation recommended by the AMP/ASCO/CAP Somatic Variants Guideline. This implementation rate is higher than the results published by CAP,[20] which could be attributable to several factors (eg, participants from both CAP- and non—CAP-accredited

Clinical Laboratory Improvement Amendments laboratories and international laboratories). Now 5 years later, with molecular profiling becoming the standard of care for most solid tumors and hematopoietic malignancies, the need for universal adaptation of a clear, comprehensive, and standardized method for somatic classification is even greater. During the development of the AMP/ASCO/CAP Somatic Variants Guideline, it was fully recognized that the initial classification, interpretation, and reporting structure and recommendations may require several revisions and improvements. Ultimately, its usefulness would depend on the implementation, input, and feedback from many in the community, but it has served as an effective initial starting point. To ensure that the tiered system evolves to incorporate real-world experience and addresses problematic areas that have been identified, AMP has convened a multistakeholder working group to conduct an update of the 2017 AMP/ASCO/CAP Somatic Variants Guideline in 2022. The AMP/ASCO/CAP Somatic Variants Guideline classification system is intended to codify laboratory somatic variant interpretation and reporting, facilitate the most appropriate and effective treatment for patients, and enable advancements in understanding of both the clinical significance and the oncogenicity of variants in these complex disease processes.

Conclusion

The VITAL Somatic Challenges, based on the *AMP/ASCO/CAP Standards and Guidelines for Somatic Variant Interpretation and Reporting*, demonstrated that 86% of the participants correctly differentiated clinically significant variants from variants of uncertain significance and benign/likely benign variants, and most participants (>70%) agreed in judging the potential for germline variants. Furthermore, the AMP/ASCO/CAP Somatic Variants Guideline Implementation Survey showed that 71% of respondents implemented the guidelines for variant classification and >90% of them utilized the recommended tier-based reporting system. However, significant gaps remain to be addressed. The most desired changes to close the gaps were identified to be the following: i) a more granular, comprehensive, and standardized system for somatic variant classification, especially clearer definition of clinical actionability in each of D/P/T categories; ii) more detailed guidelines on interpretation and reporting of germline variants/suspected germline variants identified through tumor sequencing with or without a paired normal sample; and iii) additional educational programs tailored to fulfill the needs of clinical laboratory professionals and medical oncologists. The AMP will continue collaborating with relevant community stakeholders to modify and improve the current AMP/ASCO/CAP Somatic Variants Guideline based on the results from the VITAL Somatic Working Group and cancer genomics community feedback.

Disclaimers

The Association for Molecular Pathology (AMP) Clinical Practice Guidelines and Reports are developed to be of assistance to laboratory and other health care professionals by providing guidance and recommendations for particular areas of practice. The Guidelines or Reports should not be considered inclusive of all proper approaches or methods, or exclusive of others. The Guidelines or Reports cannot guarantee any specific outcome, nor do they establish a standard of care. The Guidelines or Reports are not intended to dictate the treatment of a particular patient. Treatment decisions must be made on the basis of the independent judgment of health care providers and each patient's individual circumstances. The AMP makes no warranty, express or implied, regarding the Guidelines or Reports and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The AMP shall not be liable for direct, indirect, special, incidental, or consequential damages related to the use of the information contained herein.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2022.11.002*.

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