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SPECIAL ARTICLE

Recommendations for Tumor Mutational Burden Assay Validation and Reporting

A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, and Society for Immunotherapy of Cancer

Larissa V. Furtado,[*†] Carlo Bifulco,[*‡§] Daniel Dolderer,[*¶] Susan J. Hsiao,[*‖] Benjamin R. Kipp,[*,**] Neal I. Lindeman,[*††‡‡] Lauren L. Ritterhouse,[*§§] Robyn L. Temple-Smolkin,[¶¶] Ahmet Zehir,[*‖‖] and Jonathan A. Nowak[*,***]

From the The Tumor Mutational Burden Working Group of the Association for Molecular Pathology, Clinical Practice Committee, Rockville, Maryland; the Department of Pathology,† St. Jude Children's Research Hospital, Memphis, Tennessee; the Department of Pathology,‡ Providence Portland Medical Center, Portland, Oregon; the Society for Immunotherapy of Cancer,§ Milwaukee, Wisconsin; the Department of Pathology,¶ Jupiter Medical Center, Jupiter, Florida; the Department of Pathology and Cell Biology,‖ Columbia University Medical Center, New York, New York; the Department of Laboratory Medicine and Pathology,** Mayo Clinic, Rochester, Minnesota; the Department of Pathology,†† Weill Cornell Medicine, New York, New York; the College of American Pathologists,‡‡ Northfield, Illinois; the Department of Pathology,§§ Massachusetts General Hospital, Boston, Massachusetts; the Association for Molecular Pathology,¶¶ Rockville, Maryland; the Memorial Sloan Kettering Cancer Center,‖‖ New York, New York; and the Department of Pathology,*** Brigham and Women's Hospital, Boston, Massachusetts*

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Address correspondence to Larissa V. Furtado, M.D., St. Jude Children's Research Hospital, 262 Danny Thomas Pl., MS 1160, Memphis, TN 38105.
E-mail: larissa.furtado@stjude.org.

Tumor mutational burden (TMB) has been recognized as a predictive biomarker for immunotherapy response in several tumor types. Several laboratories offer TMB testing, but there is significant variation in how TMB is calculated, reported, and interpreted among laboratories. TMB standardization efforts are underway, but no published guidance for TMB validation and reporting is currently available. Recognizing the current challenges of clinical TMB testing, the Association for Molecular Pathology convened a multidisciplinary collaborative working group with representation from the American Society of Clinical Oncology, the College of American Pathologists, and the Society for the Immunotherapy of Cancer to review the laboratory practices surrounding TMB and develop recommendations for the analytical validation and reporting of TMB testing based on survey data, literature review, and expert consensus. These recommendations encompass pre-analytical, analytical, and postanalytical factors of TMB analysis, and they emphasize the relevance of comprehensive methodological descriptions to allow comparability between assays. *(J Mol Diagn 2024, ■: 1–16; https://doi.org/10.1016/j.jmoldx.2024.05.002)*

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The Tumor Mutational Burden Working Group of the Clinical Practice Committee, Association for Molecular Pathology (AMP), with organizational representation from the American Society of Clinical Oncology (Solange Peters, M.D., Ph.D.), College of American Pathologists (N.I.L.), and Society for Immunotherapy of Cancer (C.B.). The AMP 2022 Clinical Practice Committee consisted of Jane Gibson (Chair), Steven Sperber, Diana Mandelker, Michael Kluk, Rena Xian, David Eberhard, Navid Sadri, Blake Buchan, Karissa Culbreath, Donna Wolk, Elaine Gee, Sabah Kadri, Jack Tung, and Lauren Miller.

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Immune checkpoint inhibitor (ICI) therapies targeting programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte−associated protein 4 (CTLA-4) have transformed the field of oncology by providing clinical benefit to patients with multiple cancer types.[1] Unlike chemotherapy and targeted therapies that directly target tumor cells, immunotherapy enhances the ability of a patient's immune system to fight against cancer cells. Unfortunately, only a subset (20% to 30%) of patients currently benefit from immunotherapy, with varied response rates among tumor types. Furthermore, the possibility of immune-related adverse events in patients treated with immune checkpoint blockade and the significant cost of the ICIs contribute to the importance of optimally selecting patients for ICI therapy. As a result, there continues to be significant interest in biomarkers that can identify patients more likely to benefit from immunotherapy treatments.[2] To date, the US Food and Drug Administration has approved PD-L1 protein expression, microsatellite instability/defective mismatch repair, and tumor mutational burden (TMB) as predictive biomarkers for ICIs in patients with cancer.[3−5]

TMB is defined as the total number of nonsynonymous somatic mutations per megabase (Mb) of coding DNA sequenced.[6] It is postulated that highly mutated tumors produce tumor-specific epitopes or neoantigens that have a higher chance of being recognized as nonself or foreign by the immune system and, therefore, are considered more amenable to treatment with ICIs.[1] Despite contradictory outcomes, TMB has been associated with a higher rate of ICI treatment response and longer survival in multiple cancer types.[1,6−11]

Currently, however, there exists variation in how TMB is calculated, reported, and interpreted.[1,12] Much of the variation stems from laboratory-specific assay features, including the genomic size of the territory from which it is calculated, gene content of the assay, whether somatic only or paired tumor-germline sequencing is performed, algorithmic components and settings of bioinformatic pipelines, inclusion or exclusion of specific variant types from the calculation, and other analytical methods to adjust or normalize the data.[13] Multiple pre-analytical factors may impact TMB calculation as well.[14]

Beyond laboratory aspects of calculating and reporting TMB, there is also no established standard for the interpretation of TMB values, such as what values should be considered high or actionable and whether the value or cutoff used should be dependent on tumor type, the drug or drug combination being considered, or the assay that was used. Although the US Food and Drug Administration approved pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors with TMB ≥10 mutations/Mb in 2020,[5,15] the number of clinical trials assessing TMB as part of their study design has significantly increased over the past several years (*https://clinicaltrials.gov*), and these studies may use disparate methods of calculating TMB as well as various methods of interpreting those values.

International efforts led by Friends of Cancer Research and Qualitätssicherungs-Initiative Pathologie (Quality in Pathology) are underway with the goal of developing approaches to standardize the measurement and reporting of TMB across different assays.[14,16] Standardization of TMB is expected to benefit clinical molecular diagnostic laboratories and should facilitate the implementation of this metric into routine clinical use. TMB standardization is also needed for clinical trial design, to ensure that results obtained in trials can be broadly applied and translated into clinical practice. These efforts would also benefit oncologists and treating clinicians, enabling them to know how to best interpret and use TMB for patient care. As it currently stands, it is difficult to know how TMB values translate from one laboratory to the next, which is a significant hindrance to its use and the impetus behind efforts for standardization.

In addition, validating TMB may be difficult for many laboratories in the absence of standard guidelines and reference samples that can be used to define ground truth. It can, therefore, be challenging for laboratories to assess whether their current next-generation sequencing (NGS) panels and offerings would be suitable for TMB calculations, and what the best path forward would be for validating and bringing TMB online.

Recognizing the current challenges of clinical TMB testing, the Association for Molecular Pathology (AMP) convened in 2018 a multidisciplinary working group to assess laboratory practices surrounding TMB and to develop evidence-based recommendations for the analytical validation and reporting of clinical TMB testing. The recommendations presented here are based on literature review, survey data, and subject matter expert consensus, with a focus on the technical aspects of TMB analysis.

Materials and Methods

Working Group Composition

AMP convened a multidisciplinary subject matter expert working group (TMB Working Group) with representation from the American Society of Clinical Oncology, the College of American Pathologists, and the Society for Immunotherapy of Cancer. The Working Group comprised 13 participants from the United States and 1 from Europe, who represented molecular pathologist, molecular geneticist, pathologist, oncologist, and bioinformatician expertise and experience in NGS testing for TMB. All TMB Working Group members complied with the AMP conflicts-of-interest policy, which required disclosure of financial or other interests that may have an actual, potential, or apparent conflict throughout the project. Funding for the administration of this project was provided exclusively by AMP; no industry funds were used in the guideline's development.

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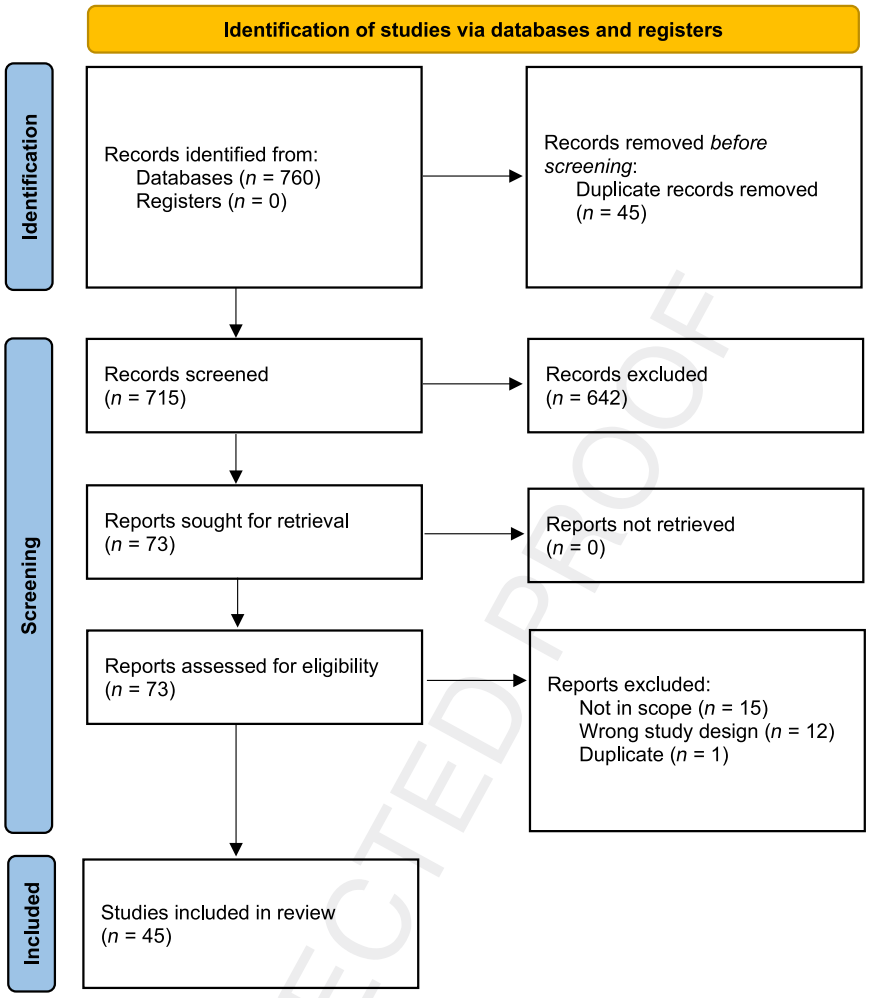

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 statement for identification of studies via databases and registers was used to report the scoping review.[20] Literature searches were performed in the PubMed* (National Library of Medicine, *https://pubmed.ncbi.nlm.nih.gov*, last accessed August 17, 2023) database using a date filter of database inception to February 9, 2020, inclusive. The filtered results were combined, deduplicated, and uploaded into Covidence software (Melbourne, VIC, Australia) to perform the literature review. Included articles were required to describe tumor mutational burden (TMB) clinical testing, calculation, pipeline, quality control metrics, algorithms, validation, paired tumor-germline or unmatched analysis, transcript annotation, clonality, reporting, mutational signatures, immunotherapy, neoantigens, hypermutability, neo-epitopes, immune blockade therapy, immune checkpoint inhibitors, or neoplasms. Articles were excluded if they were not performed on human samples; were not peer reviewed; were not published in the English language; addressed cell line, preclinical, or non–tissue-based sample testing; or were a commentary, case report, conference abstract, proceedings, or secondary source, such as a review article. Of the 28 full-text studies excluded, 15 were not in scope, 12 were of a wrong study design, and 1 was a duplicate. The literature search was repeated for articles published from February 10, 2020, through January 30, 2023 (inclusive), and title-abstract screened to identify articles that would support or refute the TMB Working Group's recommendations. When necessary, a full-text review was performed. The additional articles screened supported all recommendations; however, these articles are outside of the systematic evidence review. *PubMed search string: (tumor mutation[All Fields] OR tumor mutational[All Fields] OR tumor mutations[All Fields]) AND burden[All Fields] OR ("immunotherapy"[MeSH Terms] OR "immunotherapy"[All Fields]) AND ("high-throughput nucleotide sequencing"[MeSH Terms] OR ("high-throughput"[All Fields] AND "nucleotide"[All Fields] AND "sequencing"[All Fields]) OR "high-throughput nucleotide sequencing"[All Fields] OR ("next"[All Fields] AND "generation"[All Fields] AND "sequencing"[All Fields]) OR "next generation sequencing"[All Fields]).

All TMB Working Group members volunteered their time and were not compensated for their involvement.

Project Scope and Limitations

The aim of this project was to develop best practice recommendations for the analytical validation and reporting for TMB testing informed by literature review, survey data, and expert consensus opinion. These recommendations encompass pre-analytical, analytical, and postanalytical factors of TMB analysis that play a role in TMB calculation, analytical validation, and reporting.

This publication does not address clinical validation or clinical utility of a TMB assay. TMB measurement from circulating tumor DNA is not specifically addressed here. Specific aspects and recommendations related to NGS

assay validation and reporting are addressed elsewhere.[17–19]

TMB Laboratory Practices Survey

A 29-question survey was developed by the TMB Working Group to assess the current state of clinical TMB testing practices (Supplemental Tables S1–S4). The survey included a four-question introduction for all respondents and then directed respondents to choose one of two tracks. One track included 3 questions for those who indicated that their laboratories had not implemented TMB testing at the time the survey was completed, whereas the other track included 22 questions for respondents who answered that their laboratories were performing clinical TMB testing. Three survey questions allowed respondents to choose more than one answer. The survey was administered using the SurveyMonkey platform (Momentive Inc., San Mateo, CA) and was made available online to AMP members and nonmembers from February 4, 2019, to March 25, 2019.

Systematic Literature Review

A scoping review was performed to identify published literature describing TMB clinical testing (Figure 1).[20] The search strategy, which used a collection of keywords synonymous with the concepts of TMB clinical testing, immunotherapy, and NGS, was applied to PubMed entries with a publication date from database inception to February 9, 2020, inclusive. Articles with a title and/or abstract containing these keywords were loaded into the web-based Covidence system (Melbourne, VIC, Australia) used to manage the review process and were deduplicated.

In the first review phase (phase 1), abstracts were reviewed in a double-blind manner by members of the TMB Working Group, with a third member serving as tiebreaker when needed. Included articles were required to address either TMB clinical testing, calculation, pipeline, quality control metrics, algorithms, validation, or paired tumor-germline or unmatched analysis, transcript annotation, clonality, reporting, mutational signatures, immunotherapy, neoantigens, hypermutability, neo-epitopes, immune blockade therapy, ICIs, or neoplasms. Articles were excluded if they were not reporting human sample data; were not peer reviewed; were not published in the English language; addressed cell line, preclinical, or non–tissue-based sample testing; or were a commentary, case report, conference abstract, proceedings, or secondary source, such as a review article.

Abstracts identified as potentially relevant in phase 1 were re-evaluated by a similar double-blind process in phase 2 using the full article text. Publications meeting the inclusion criteria in phase 2 advanced to phase 3, where a list of predefined data elements was extracted for each article using SurveyMonkey in a double-blind manner by Working Group members. Discrepancies in the data extracted were resolved through discussion and consensus between the individuals performing data extraction. The reviewers were divided into three groups to perform the initial review and analysis of the evidence that was compiled during the data extraction phase. Before publication, the literature search was repeated for articles published from February 10, 2020, through August 10, 2023 (inclusive), and title-abstract screened to identify articles that would support or refute the TMB Working Group's recommendations. When necessary, a full-text review was performed. The additional articles screened supported all recommendations; however, these articles are outside of the systematic evidence review.

Development of Recommendations

The TMB Working Group met periodically by conference call or virtual meeting to review published evidence and draft recommendations. An in-person public feedback session was held with attendees of a special session during the AMP 2019 Annual Meeting. As with many other professional association volunteer-driven article projects, the TMB Working Group's initial timeline was then significantly affected by the coronavirus disease 2019 (COVID-19) pandemic. On the basis of the results of the systematic evidence review, the survey, stakeholder input, and the cumulative practice experience of the members of the Working Group, the recommendation statements were developed by expert opinion consensus of the Working Group.

A public open comment period on the 13 draft recommendation statements was held from March 19, 2023, through April 2, 2023. The public comment was administered online via SurveyMonkey. The open comment period was publicized via AMP society communications across multiple outlets (eg, e-mail, member listserv announcements, and social media).

The website received 1248 comments in total (agree, agree with comment, disagree with comment, and neutral/not applicable responses, along with 27 open comments, were captured) (Supplemental Table S5). All draft recommendation statements achieved between 99.1% and 84.2% agreement (agree + agree with comment). The Working Group reviewed all comments received. Following panel discussion, the Working Group members determined whether to maintain the original draft recommendation as is, revise it with minor language change, or consider a major recommendation change. Resolution of all changes was achieved by consensus of the Working Group using a nominal group technique (rounds of e-mail, virtual meeting discussions, and multiple edited recommendations) among the group members. The final recommendation statements were approved unanimously by the group with a formal vote. The Working Group considered the risks and benefits throughout their considered judgment process. Formal cost analysis or cost-effectiveness was not performed.

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Results

TMB Laboratory Practices Survey

Fifty-seven participants from 17 countries completed the entire survey (Supplemental Tables S1–S4). Of those, 61% of the responses were from North America, 16% from Europe, 14% from Asia, 5% from Australia, and 3.5% from South America. Molecular pathology professionals (43.9%; $n = 25/57$), pathologists (29.8%; $n = 17/57$), and clinical laboratory directors (24.6%; $n = 14/57$) accounted for the professions with the most responses, although a variety of health care professionals and one patient advocate also participated in the survey. Most (59.6%) of the respondents indicated their practice setting to be university hospitals and academic medical centers.

When asked about the status of TMB testing implementation in their laboratories, 40.4% of the survey participants ($n = 23/57$) indicated that their laboratories have implemented TMB testing, and 59.7% ($n = 34/57$) answered that they were not performing testing for TMB. Of the 34 respondents who were not performing TMB testing, 33 (97%) indicated that they planned to implement TMB testing in their laboratories sometime in the future. In those laboratories, TMB testing was either handled via send out (32.4%; $n = 11/34$) or not routinely requested by oncologists (29.4%; $n = 10/34$).

The lack of standardized procedures for TMB testing and reporting was identified as the main barrier for implementation of TMB assays by 55.9% ($n = 19/34$) of the respondents who have not yet implemented TMB testing in their laboratories and by 26.1% ($n = 6/23$) of respondents who perform TMB assays in their laboratories. Lack of (or insufficient) evidence regarding the clinical utility of TMB testing (36.8%; $n = 21/57$), lack of available TMB samples for assay development and validation (31.6%; $n = 18/57$), and uncertainties regarding TMB assay reimbursement (31.6%; $n = 18/57$) were also considered to be major barriers for implementation of TMB assays by all survey respondents.

Participants indicated that they used panel-based sequencing and whole-exome sequencing for TMB testing, with the number of genes included in the assay panels ranging from 5 to 50 to >500 genes. A minority of respondents reported performing whole-genome sequencing (4.3%) for TMB assessment. Of survey respondents, 52.2% of laboratories perform tumor-only testing, and 47.8% perform tumor-germline paired testing. A variety of databases were reported to be used in filtering germline polymorphisms, including the 1000 Genomes Project (*https://www.internationalgenome.org/data*), Genome Aggregation Database (gnomAD; *https://gnomad.broadinstitute.org*), Exome Aggregation Consortium (*https://gnomad.broadinstitute.org*), and dbSNP (*https://www.ncbi.nlm.nih.gov/snp*) (all last accessed September 3, 2023), and custom or in-house databases. Reported minor allele frequency cutoffs used were 1% and 5%, although a subset of laboratories used lower cutoffs and/or ethnicity-specific cutoffs. Most respondents (34.8%) indicated the use of exonic single-nucleotide variants (SNVs) and insertion/deletion (indel) variants for TMB calculation, whereas exonic nonsynonymous SNV and indel variants, exonic nonsynonymous non-hotspot SNV and indel variants, and exonic nonsynonymous SNV variants were used for TMB calculation by 21.7%, 13%, and 4.3% of participating laboratories, respectively. All SNV and indel variants, only exonic SNV variants, and exonic SNV, indels, and splice site variants were each separately used by 8.3% of participants for calculation of TMB.

When asked about reporting approaches, most respondents (43.5%) answered that TMB was reported as the number of mutations per megabase of sequenced territory, 13% provided a TMB value and reported whether it was low or high based on a tumor-specific threshold, 13% provided a TMB value and described how TMB was distributed in that cancer type, 8.7% provided a TMB value and reported if it was low or high based on a published TMB threshold, and 8.7% provided a TMB value along with a reference percentile for the sequenced sample's TMB. Mutational signatures were also reported by 43.5% of participating laboratories.

Literature Review

The initial literature review retrieved 760 publications (Figure 1); however, a limited number of TMB validation studies were identified. Overall, the systematic review demonstrated an apparent lack of uniformity in the methods used for TMB testing and a high degree of variability pertaining to the presence and comprehensiveness of methodological descriptions of analytical aspects of TMB testing between studies. Findings from the literature review are discussed in the following sections and summarized in Table 1. [T1]

Assay Characteristics

Across publications, a wide diversity of NGS panels were used, with approximately twice as many studies choosing a laboratory-developed procedure ($n = 29$) over a commercially available panel ($n = 15$), with the most frequently chosen commercial panel being from Foundation Medicine (Cambridge, MA; $n = 9$).

Although several articles failed to identify the type of sequencer used, Illumina (Illumina, Inc., San Diego, CA; $n = 27$) was more commonly identified than Ion Torrent (Thermo Fisher Scientific, Waltham, MA; $n = 6$). Hybrid capture-based sequencing chemistry ($n = 36$) was used in most studies, with a clear predilection for larger gene panels (>300 genes). These larger panels made up 80% of those used across all publications, with a preference for panels with >500 genes ($n = 23$). Panel size, although not consistently reported, showed a wide range, with the most

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Table 1 Summary of TMB Literature Review Findings

Component	Findings summary
Assay design characteristics	• A wide diversity of assays was used for TMB testing, mostly large hybrid capture LDP panels (1–2 Mb; >300 genes). • Most laboratories performing panel-based TMB used existing assays for TMB implementation. • WES was used for TMB testing in ∼10% (6/50) of the studies. ∼35% (8/23) of survey respondents indicate the use of WES for TMB testing. • DNA extraction method was inconsistently reported across studies. • Information about paired versus unmatched sequencing and the approaches for germline variant filtering in unmatched sequencing was not consistently reported. • ∼50% of survey respondents whose laboratories perform TMB testing used paired sequencing. Approaches for germline variant filtering in unmatched sequencing varied across respondents.
TMB validation studies	• ∼60% of studies reported some orthogonal validation, which was 2:1 *in silico*/laboratory based. • ∼70% (16/23) of survey respondents reported performing orthogonal validation. • Number of validation samples used varied widely across studies. • TMB ranges and degree of concordance in validation studies were difficult to interpret. • Most studies that reported validation either performed thoracic or pan-solid tumor assays.
TMB calculation	• Most studies included only exonic mutations for TMB calculation; more studies looked only at nonsynonymous mutations versus synonymous and nonsynonymous mutations; similar findings were observed across survey responses. • Minimum tumor content and coverage data required for TMB calculation were difficult to interpret from studies. • ∼70% of survey respondents (16/23) indicated that the minimal neoplastic cellularity acceptable for TMB testing in their laboratories was 10%–20%, which is in keeping with the reported lower LoD of their assays used for TMB testing (LoD of 5%–10%). • Most studies that specify the mutation detection tool used for TMB calculation indicated the use of the original version of MuTect followed by Ion Torrent commercial software. • The transcript source used for mutation annotation and the minimum overall sequencing depth needed for TMB calculation were not reported by most studies. • Most studies used some combination of coverage, VAF, and quality score as pipeline quality control metrics. • The average sequencing depth of TMB assays varied across survey responses.
TMB reporting	• Most studies reported numerical TMB values using a qualitative interpretation (eg, TMB high or TMB low) without a percentile interpretation of the reported TMB. • Most survey participants (43.5%; 10/23) reported the number of mutations per megabase of sequenced territory without contextual or qualitative interpretation. • Approximately half of the studies did not specify whether criteria for TMB interpretation were tumor specific or encompassed all tumor types. For those that did, approximately half provided tumor-specific TMB interpretations, whereas the other half provided interpretations that included all tumor types.
Mutational signatures	• Mutational signature analysis was performed as an adjunct to TMB in approximately one-third of the studies, with microsatellite instability being the most commonly detected mutational signature. • Information on approaches for mutational signature test validation and the algorithms used for mutational signature detection was not provided in most publications.

LDP, laboratory-developed procedure (also known as laboratory-developed test); LoD, limit of detection; Mb, megabase; TMB, tumor mutational burden; VAF, variant allele fraction; WES, whole-exome sequencing.

common choices being 1 to 2 Mb ($n = 18$), followed by >10 Mb ($n = 5$). Reporting of DNA extraction method, paired versus unmatched samples, coding versus noncoding regions analyzed, and single-nucleotide polymorphism filtering databases was too inconsistent across studies to draw definite conclusions.

TMB Calculation

Most publications ($n = 29$) only counted mutations in exonic regions for TMB, whereas one publication also included splice sites and one publication included all exonic and intronic regions. However, more than a quarter of all publications ($n = 13$) did not report the genomic regions analyzed for TMB. Over three quarters of publications ($n = 34$) counted nonsynonymous mutations toward TMB, whereas synonymous mutations were also included ($n = 13$) in approximately a quarter of cases. Less than a third of studies provided more granular information about the types of variants counted, with missense mutations, in-frame insertions/deletions, frameshift mutations, and nonsense mutations included in decreasing order of

frequency. Only one study reported the reference transcript source used for variant calling.

Over three quarters of publications ($n = 35$) did not provide information about the minimum tumor content or overall sequencing depth required for TMB calculation. In the publications where this information was reported, 20% was the most common minimum tumor content ($n = 7$), while minimum required sequencing coverages were broad, ranging from 50× to 500×, likely reflecting variable use of whole-exome sequencing and targeted panels for TMB measurement. No studies explicitly addressed whether variant clonality or subclonality was considered for TMB calculation, which would require comparison with the estimated tumor content of the specimen. However, more than a quarter of studies ($n = 13$) did use allele fraction as a criterion for including variants in TMB.

Total coverage at a variant locus ($n = 13$), along with various additional quality scores and metrics generated by variant callers, such as quality by depth, median base quality, and mapping quality ($n = 9$), were used in a subset of publications. However, more than half of all publications ($n = 24$) did not report any quality control criteria for variant inclusion in TMB.

Similarly, more than half ($n = 27$) of all publications did not report the variant calling tool used for TMB calculation. For publications that reported the tool used, MuTect or Mutect2 was used in more than three quarters of cases ($n = 11$).

TMB Assay Validation Approach

One-third of publications ($n = 15$) reported validation of TMB against an orthogonal standard, and most of these publications ($n = 12$) referenced whole-exome sequencing as the orthogonal standard. When an orthogonal testing approach was specified, this approach was *in silico* only in one-third of cases ($n = 5$), wet laboratory based in another third of cases ($n = 5$), and occasionally used both methods ($n = 2$).

The number of cases included in orthogonal studies was highly variable, with nearly even numbers of studies using 1 to 50, 51 to 500, and >500 cases. Validation case sets were either focused on thoracic tumors specifically or included a broad distribution of solid tumor types.

Although some articles reported the range and distribution of TMBs that were tested in the orthogonal validation study, these results were reported in highly variable ways that made interpretation difficult and precluded meaningful comparison between studies. For example, one study reported "5.1 to 15 mutations/MB," whereas another reported "10 to 502" mutations in total, without normalization to genomic regions covered. Another study reported only average: 5.0 to 14.7 mutations, precluding unambiguous interpretation, whereas several others provided graphical depictions of TMB validation ranges but did not report discrete numbers.

Similarly, the degree of concordance between the TMB assay being validated and the orthogonal standard was difficult to assess because of variation in how agreement was reported. However, most studies reported a correlation coefficient, which was most likely a Pearson r value, although this was not always specified. These values ranged from 0.62 to 0.99, with most >0.85. No studies commented on potential sources of bias that led to imperfect correlation with orthogonal standards.

TMB Reporting

In most publications ($n = 35$), numerical TMB values were reported using a qualitative interpretation (eg, TMB high or TMB low), but a percentile interpretation of the reported TMB was not provided by most articles ($n = 40$). Approximately half of the publications ($n = 24$) did not mention if their criteria for TMB interpretation were tumor specific or encompassed all tumor types. For those that did, approximately half ($n = 11$) provided tumor-specific TMB interpretation, and the other half ($n = 10$) provided interpretations that included all tumor types.

Mutational signature analysis was performed as an adjunct to the TMB assay in approximately one-third of the studies ($n = 16$), with microsatellite instability being the most commonly detected mutation signature ($n = 11$). Information on approaches for mutational signature assay validation, as well as the algorithms used for mutational signature detection, was not provided in most publications.

Recommendations for TMB Assay Validation, Reporting, and Publications

The TMB Working Group reviewed the available published literature on TMB assays to establish an evidence base for a set of best practice recommendations. Findings from this analysis emphasized the fact that conclusions about TMB calculation, validation, and reporting are difficult to draw from the literature because of incomplete or absent methodological descriptions. The limited number of publications addressing analytical performance characteristics of TMB testing and the scarcity of detailed information regarding technical aspects of TMB assay validation available in the published studies support the need for guidance in analytical performance assessment and reporting approaches for TMB testing. Despite these limitations, the TMB Working Group generated 13 subject matter expert consensus recommendations that address laboratory-related validation, reporting, and publication considerations for clinical TMB testing (Table 2 and Figure 2). [T2] [F2]

TMB Validation Recommendations

Laboratories implementing clinical TMB testing must follow the regulatory and accreditation requirements

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Table 2 Summary of Recommendations for Clinical TMB Validation, Reporting, and Publications

Recommendation no.	Related area	Recommendation
1	Testing	Laboratories should validate and report the enrichment method used in the TMB assay.
2	Testing	Laboratories should validate and report the size and describe the genomic regions (ie, exons, introns, and intergenic regions) used for TMB calculation.
3	Testing	Laboratories should validate TMB measurement against an orthogonal assay, and the method of TMB calculation used by the orthogonal comparison assay should be documented.
4	Testing	Laboratories should include validation samples that reflect the intended use of the TMB assay with respect to both specimen type and representative tumor types.
5	Testing	Laboratories may use reference materials to supplement but not supplant clinical samples for TMB assay validation.
6	Testing	Laboratories may use *in silico* validation studies to supplement but not supplant a TMB assay wet laboratory validation.
7	Testing	Laboratories should specify the sequencing mode (tumor-germline paired or somatic only) used by the TMB assay during TMB assay validation. If somatic-only sequencing is performed, filter settings used to remove common population variants should also be documented.
8	Testing	Laboratories should establish the performance parameters of bioinformatic pipelines used for TMB calculation during validation.
9	Reporting	Laboratories should report the assay name, version, and sequencing platform used for clinical TMB assays.
10	Reporting	Laboratories should report the name, version, properties, and/or settings of bioinformatic pipeline software components used for TMB calculation.
11	Reporting	Laboratories should report the specific types and/or categories of variants included in and omitted from the TMB calculation.
12	Reporting	Laboratories should report the sequencing mode (tumor-germline paired or somatic only) used by the TMB assay. If somatic-only sequencing is performed, filter settings used to remove common population variants should be provided or made available on request.
13	Publication	Publications describing TMB assays intended for clinical applications, including description of clinical validation, should include performance characteristics that would facilitate methodological assessment.

See text for key details regarding each of these recommendations.
TMB, tumor mutational burden.

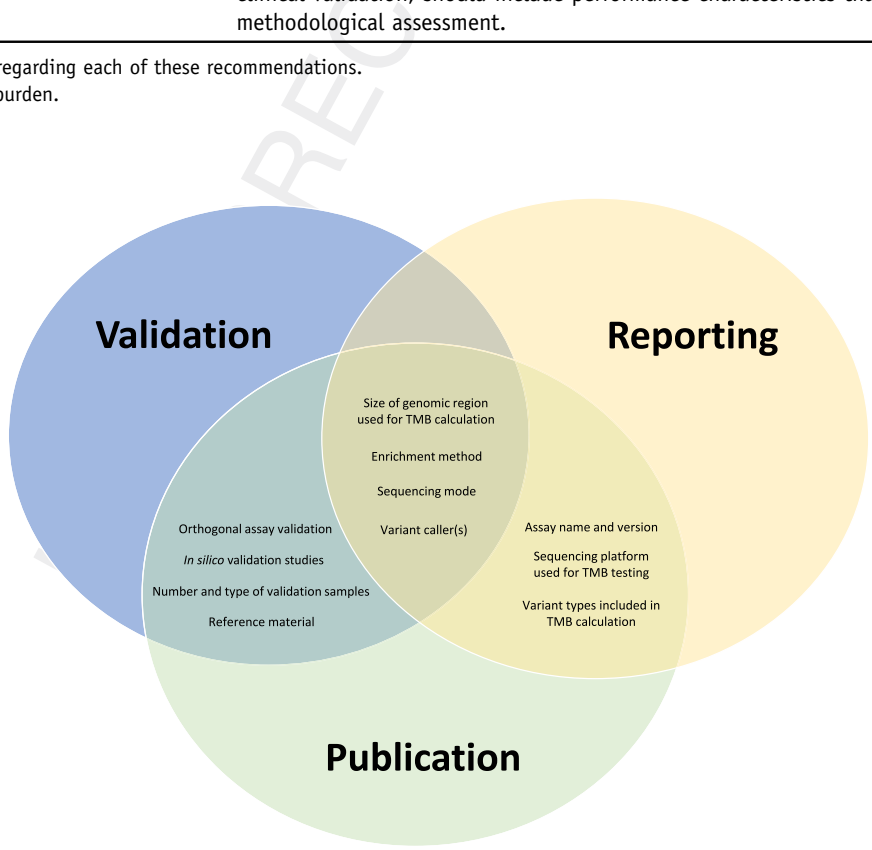

Figure 2 Recommended elements for inclusion in tumor mutational burden (TMB) validation studies and clinical reports.

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relevant for their practice setting (eg, Clinical Laboratory Improvement Amendments, College of American Pathologists, The Joint Commission, and/or European Medicines Agency) (*https://www.govinfo.gov/content/pkg/USCODE-2011-title42/pdf/USCODE-2011-title42-chap6A-subchapII-partF-subpart2-sec263a.pdf*; *https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493*; *https://www.cap.org/laboratory-improvement/accreditation*; *https://www.jointcommission.org/what-we-offer/accreditation*; *https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R0746*, all last accessed September 7, 2023). For this article, the recommendations that follow will assume that a robust clinical validation for the TMB assay is being performed by the testing laboratory.[17,18] This process includes, but is not limited to, the following.

- Defining and describing clinical TMB assay performance characteristics (sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and concordance) appropriate for the medical indication for the test;
- Defining and describing the analytical sensitivity (limit of detection) of the TMB assay;
- Defining and describing potential sources of TMB assay interference;
- Evaluating and addressing potential sources of result interpretation error.

Existing recommendations for clinical NGS assay validation, interpretation, and reporting also apply to TMB assays.[17–19] The following recommendations address specific considerations for validation of TMB clinical testing that were considered by the Working Group as in need of providing additional guidance.

Recommendation 1: Laboratories Should Validate and Report the Enrichment Method Used in the TMB Assay

The scoping review and survey results demonstrated a lack of uniformity in the panel-based methods adopted by clinical laboratories for TMB assessment. The most popular targeted enrichment approach for panel TMB measurement is hybridization capture, although amplicon-based sequencing is also being used.

Several analytical differences between targeted enrichment technologies may influence TMB calculation, such as different sample input and processing specifications, chemistries, library construction methods, sequencing platforms, and bioinformatic analysis pipelines.[21–24] For instance, amplicon-based panel testing and whole-exome sequencing assays have demonstrated differences in analytical performance related to on-target alignment rates, coverage uniformity, and variant calling in comparison with hybridization capture methods.[13,21,25,26]

According to the survey results, most laboratories implement TMB testing using an existing NGS assay. For this reason, it is expected that in most clinical laboratories, the beginning-to-end performance of the assay used for TMB testing should have been previously validated according to regulatory requirements, accreditation criteria, and professional guidelines,[17,18] and that quality control metrics and acceptability criteria for samples and sequencing data have been established. However, the validation of an NGS assay must reflect its intended clinical use. Laboratories should assess how existing bioinformatics parameters, sequencing artifacts, and assay performance metrics may affect TMB calculation. Reporting the type of enrichment method used in the TMB assay in clinical reports is relevant to allow comparability across assays.

Recommendation 2: Laboratories Should Validate and Report the Size and Describe the Genomic Regions (ie, Exons, Introns, and Intergenic Regions) Used for TMB Calculation

The published literature contained insufficient evidence to enable a specific recommendation for the ideal extent of genomic sequence to interrogate to optimize the TMB estimation. In general, the accuracy of TMB estimation correlates directly with the extent of genomic sequencing, with small panels causing the most error, and with minimal error associated with either whole-exome or whole-genome sequencing.[16,27–31] Not all publications reviewed specified the extent of genomic sequence analyzed, but among those that did, there was a broad range from <0.5 to >10 Mb, but a clear modal size of 1 to 2 Mb (approximately 60% of methods). The reasons for the high frequency of this size of panel were not explained, but it is unlikely to have been driven solely by TMB calculation considerations, rather than a combination of other reasons.

Given that assay size influences TMB calculation accuracy, it is important to validate and report the extent of genomic territory that is included in the TMB analysis. However, this might not be the same as the total genomic landscape of the assay. For example, assays that include intron baits to enable structural variant detection or assays that bait thousands of single-nucleotide polymorphism regions across the genome to normalize copy number assessment should not include the genomic regions associated with these baits, unless those laboratories are also analyzing SNVs in these regions and including them in the TMB calculation.

Recommendation 3: Laboratories Should Validate TMB Measurement Against an Orthogonal Assay, and the Method of TMB Calculation Used by the Orthogonal Comparison Assay Should Be Documented

Given the myriad of laboratory and bioinformatic components of a sequencing assay that can each affect TMB calculation, it is not feasible to judge the analytic performance of a TMB assay only with external reference standards (reference materials). For this reason, the Working Group recommends that all TMB assays be subject to orthogonal validation studies (eg, whole-exome sequencing or large targeted panel) that measure their performance relative to high-quality controls. In many situations, such

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controls would represent tumors analyzed by whole-exome sequencing that are matched for the specimen types, tumor types, and tumor content of specimens that will be analyzed using the assay under validation. Although specimens could be sequenced using whole-exome sequencing for the purposes of validation, it is expected that reference materials will offer the advantage of enabling statistical calibration between laboratories to harmonize TMB measurements.[28] Given the variation in design between TMB assays, it is not expected that TMB scores from different assays will match perfectly,[32] and it will be incumbent on each laboratory to ensure that the degree of concordance with orthogonal TMB measures is high enough to ensure that patients are appropriately classified for clinical decision-making.

Recommendation 4: Laboratories Should Include Validation Samples that Reflect the Intended use of the TMB Assay with Respect to Both Specimen Type and Representative Tumor Types

The choice of samples (eg, DNA from formalin-fixed, paraffin-embedded tissue and/or circulating tumor DNA) to be included in the validation of TMB assays should take into account the type of assay being developed, as different assays have different characteristics and sample requirements. For instance, NGS panel assays are generally designed to work with low input amounts of DNA obtained from formalin-fixed, paraffin-embedded tissue samples, whereas whole-exome and whole-genome sequencing usually requires higher input amounts of DNA. Targeted panels also typically obtain deeper sequencing coverage than whole-exome or whole-genome sequencing, which provides panel-based assays higher analytical sensitivity for detection of subclonal variants or variants with low allele fractions, which is particularly relevant if subclonal variants are included in TMB calculation.

Given the impact of tumor-cell content and DNA input on TMB measurement,[14,17] enough samples with variable tumor purity and DNA concentration should be included in the validation studies to assess the impact of various pre-analytic conditions on assay performance. Estimation of tumor cell percentage is relevant for interpreting variant allele fractions, as low/subclonal variant allele fractions can occur because of biological reasons: intratumor genetic heterogeneity, proportion of cancer cells versus nonneoplastic tissue in a tumor sample, cancer-clone evolution, or from pre-analytical and analytical technical artifacts. Each laboratory should establish its own specimen acceptability criteria based on assay coverage depth and limit of detection studies performed during assay validation. When possible, it is recommended to test a mix of samples at different purity levels and DNA input amounts. Samples with variant allele fractions near the assay lower level of detection should be included. In addition, quality control metrics should be established for each step of the sequencing analytical procedure to assist with interpreting sequencing findings and to prevent the inclusion of artifactual variants in TMB calculation, which could reduce accuracy.

Because specimen processing and fixation conditions can influence the quality and integrity of nucleic acids obtained from a sample, laboratories should include in their TMB assay validation an adequate number and representative distribution of specimen types expected to be tested by the assay (eg, DNA from formalin-fixed, paraffin-embedded tissue, fresh/frozen samples, and/or circulating tumor DNA).

Considering that mutation burden varies across different tumor types,[31,33] laboratories should include commonly tested tumors in the analytical performance assessment of their TMB assays. Including representative samples with a broad spectrum of TMBs, particularly samples that are at or near the clinical decision threshold, is also important for establishing the analytical measurement range and accuracy of the assay. Using well-characterized validation samples representing the variant types included in the TMB calculation (eg, nonsynonymous/missense/synonymous SNVs, indels, splice sites, or other intronic variants) is also recommended, and laboratories should attempt to include variants distributed across different genomic regions that are targeted by the assay to broadly evaluate the performance of TMB measurement. Each laboratory should determine an appropriate number of samples required to validate the performance characteristics of their TMB assays, in particular the linear/reportable range at the clinical decision threshold. In the systematic literature review, information about the number of samples included in TMB validation studies was found to be highly variable, ranging from 37 to 2908.[14,16,34–38]

Recommendation 5: Laboratories May Use Reference Materials to Supplement but Not Supplant Clinical Samples for TMB Assay Validation

According to the practice survey results, lack of available samples with defined TMBs for assay development and validation is a major hindrance to TMB assay implementation. Validation specimens can be obtained through interlaboratory exchange of previously characterized samples, from ongoing drug trials, or from tissue repositories. Obtaining an adequate number of well-characterized samples that span variant counts across the entire analytical measurement range of the assay, particularly its lower limit of clinical decision threshold, is not straightforward. Laboratories may include reference materials in their TMB assay validation studies, as a supplementary approach to standard validation samples, to aid in the assessment of performance characteristics of TMB assays. Multiple reference sample sources (eg, commercially available control materials, cell lines,[39,40] and known microsatellite instability positive or *POLE* hypermutated) are some of the sample types that can be used to evaluate the accuracy and precision of TMB and to assess the analytical measurement range and lower limits of detection of TMB assays. In addition, laboratories could routinely include these reference materials as run-level controls, and they could also assist with the harmonization

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and standardization of TMB measurements by different NGS assays.

Recommendation 6: Laboratories May Use *in Silico* Validation Studies to Supplement but Not Supplant a TMB Assay Wet Laboratory Validation

Performing *in silico* orthogonal validation experiments offers numerous advantages, including significantly decreased time and cost for validation, the opportunity to include more specimens than is often feasible with a full wet laboratory–based evaluation, and the ability to validate the bioinformatic portion of the assay in an isolated manner. Because *in silico* testing typically uses aligned or unaligned raw sequencing data as a starting input, it is possible to analyze the exact same sequencing reads that have been previously used to calculate TMB, eliminating the effects of stochastic differences than can arise when a single DNA aliquot is split between two different assays. *In silico* approaches for NGS bioinformatics pipeline validation and performance assessment have demonstrated utility and viability for clinical testing applications.[41,42]

For *in silico* validation of panel-based TMB calculation using pre-existing whole-exome sequencing as a starting point, it is straightforward to restrict the whole-exome sequencing data to only regions that overlap with the panel-based assay for TMB calculation for comparison. For all these reasons, it may often be desirable to perform *in silico* validation of TMB calculation. However, because *in silico* methods test only a portion of the total TMB assay, they should be viewed as an adjunct to a validation that includes both wet bench and bioinformatics performance assessment, and not a replacement.[18,43] In the systematic literature review, five studies relied on purely *in silico* validation, whereas only two studies used a combination of *in silico* and full wet laboratory–based validation, suggesting that additional attention is needed to ensure that all parts of a TMB assay are included in a validation study.

Recommendation 7: Laboratories Should Specify the Sequencing Mode (Tumor-Germline Paired or Somatic Only) Used by the TMB Assay during TMB Assay Validation; If Somatic-Only Sequencing Is Performed, Filter Settings Used to Remove Common Population Variants Should also Be Documented

From the survey results, approximately half of laboratories were performing paired tumor-germline sequencing, and half were performing somatic-only analysis. However, in the literature review, we found this information to be inconsistently reported. In addition, some laboratories may assay both paired tumor-germline samples and somatic-only samples.

Because only somatic variants have the capacity to generate tumor neoantigens, it is important to remove germline variants before TMB quantification. Thus, if somatic-only sequencing is performed, filters and data sources used to remove known population variants should be evaluated during assay validation and described. Different filtering approaches and databases, as well as different cutoffs, some of which were ethnicity based, were reported by survey respondents but were not well described in published reports. Given that a substantial proportion of variants identified by somatic-only sequencing are germline in origin, seemingly minor differences in allele frequency filtration can have outsized impacts on final reported variants, especially for small gene panels[30] and samples with lower TMB values. A recent study demonstrated significant discordance in TMB calculations between germline variant subtraction, population filtering, and algorithmic approaches applied to somatic-only sequencing data of different tumor types obtained from a 595-gene panel assay.[44] Somatic-only filtering methods tend to overestimate TMB because of the inability of this approach to exclude completely all germline variants,[45] which can lead to tumor miscategorization and suboptimal patient management.[30,44] This is especially true for patients with ethnic ancestries that are underrepresented in major germline variant databases. Even in gnomAD, which contains data from >140,000 individuals, representation across ethnicities is unbalanced, with >64,000 individuals of non-Finnish European ancestry, whereas <18,000 individuals are Latino or African/African American, and <10,000 individuals are East Asian.[46] In somatic-only testing, African ancestry has been associated with higher TMB in patients across multiple studies and tumor types, highlighting the real-world potential for variation in TMB measurement based on ancestry.[28,47,48] For these reasons, although routine paired tumor-germline sequencing for TMB assessment may not be feasible in many laboratories, the specific method used for germline variant filtering as well as its limitations should be properly validated.

Recommendation 8: Laboratories Should Establish the Performance Parameters of Bioinformatic Pipelines Used for TMB Calculation during Validation

Algorithmic strategies for variant calling vary between NGS assays and some laboratories and may use multiple callers and take the union of variant calls for TMB analysis. Given the impact that variant callers have on variant detection,[40,49–54] it is important to determine how specific variant caller(s) and their settings may affect TMB calculation. For this reason and because TMB assay validation should assess all individual components of the bioinformatics pipeline used in the analysis, the performance of the pipeline caller(s) on variants included in the TMB measurement should be carefully assessed at various allele fractions, tumor purities, DNA quality levels and concentrations, and sequencing quality conditions, and the settings and filtering strategies for the variant callers used should be documented and reported. For instance, it is not a customary practice for laboratories to analyze and report synonymous variants, but for TMB assays that include this variant type, a thorough assessment of the pipeline's ability to detect these variants reliably and accurately is recommended.

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TMB Reporting Recommendations

The 2017 AMP/American Society of Clinical Oncology/College of American Pathologists variant interpretation and reporting guideline does not provide specific guidance on TMB interpretation and reporting,[19] as clinical TMB analysis was just beginning to enter routine practice at the time of publication, but the general principles of the guideline apply. At the time of this article's publication, the AMP/American Society of Clinical Oncology/College of American Pathologists guideline is being updated to reflect advances in both technology and scientific knowledge, including mutational signatures and TMB (R. Temple-Smolkin, personal communication). Laboratory reporting for clinical assays, including TMB, should clearly describe any pre-analytical, analytical, and interpretive variables pertinent to molecular laboratory professional, pathologist, and provider understanding of reported results. The recommendations in the upcoming sections identify specific elements essential to optimal reporting of clinical TMB assays.

Recommendation 9: Laboratories Should Report the Assay Name, Version, and Sequencing Platform Used for Clinical TMB Assays

Reference to the specific assay used for TMB testing is important, as an assay's genomic coverage and analytical methods may vary between versions. Laboratories may offer multiple NGS assays, not all of which may report TMB. With the pace of development and identification of genes and genomic alterations important in cancer diagnostics, laboratory tests and assay design may undergo updates, or assays may be discontinued and replaced. TMB values are not necessarily comparable between assays even within the same laboratory; therefore, information about the specific TMB assay used for testing may assist with interpretation of TMB values and enable comparison between assays.

Recommendation 10: Laboratories Should Report the Name, Version, Properties, and/or Settings of Bioinformatic Pipeline Software Components Used for TMB Calculation

Algorithmic strategies for variant calling vary between NGS assays, and some laboratories may use multiple callers and take the union of variant calls for TMB analysis. Given the impact that variant callers have on variant detection,[46,49–54] it is important to report the settings and filtering strategies for the variant callers used in TMB calculation.

Recommendation 11: Laboratories Should Report the Specific Types and/or Categories of Variants Included in and Omitted from the TMB Calculation

The published literature contained insufficient evidence to enable a specific recommendation for the variant types to include in the TMB calculation. Most (29/32) published methods tested only exons, whereas there was a more balanced distribution between laboratories that did, and did not, include synonymous variants in their calculations (12/45 versus 34/45). A relatively small proportion of studies indicated which classes of nonsynonymous variants were included.

Enabling comparison between TMB assays, in the absence of technical standardization, requires communication of this information as part of the report. Reports should clearly indicate the types of variants that are included and excluded in TMB calculation [ie, synonymous/nonsynonymous variants, types of nonsynonymous variants (missense, nonsense)], variant types other than SNVs (eg, indels), and whether pathogenic (hotspot) variants in canonical oncogenes and tumor suppressor genes are included or excluded. The variant description should be more detailed than synonymous or nonsynonymous variants. The criteria for inclusion and exclusion of variants on TMB calculation (eg, allele fraction, sequencing coverage and other variant-level quality metrics, population allele frequency filters, and exclusion of specific variants interpreted as oncogenic drivers) should also be documented.

Given the variation in genomic territory interrogated across assays, the Working Group recommends reporting TMB as a mutation per megabase value and not as a total number of mutations identified across the NGS assay to facilitate interpretation of TMB reports and to enable comparison between assay results.

Recommendation 12: Laboratories Should Report the Sequencing Mode (Tumor-Germline Paired or Somatic Only) Used by the TMB Assay; If Somatic-Only Sequencing Is Performed, Filter Settings Used to Remove Common Population Variants Should Be Provided or Made Available on Request

Survey data showed that laboratories were approximately split between performing paired tumor-germline sequencing and somatic-only analysis, although this information was inconsistently reported in the reviewed literature. Laboratories may additionally offer both paired tumor-germline and somatic-only testing. As discussed in Recommendation 7, minor differences in allele frequency filtration can have a significant impact on variants identified by somatic-only sequencing, particularly for small gene panels[30] and samples with lower TMB values. As this approach typically overestimates TMB, especially in underrepresented patient populations, appropriate tumor categorization and subsequent clinical interpretation and management may be affected.[30,44] For this reason, the sample and sequencing mode should be clearly delineated in TMB reports. Providing filter settings used to remove common population variants from somatic-only sequencing on the report or making them available on request can help inform users about assay specifics that may impact TMB results, assist with interpretation of TMB values, and allow meaningful comparison between assays.

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The Working Group also recommends including a reference to the validation study used to implement the assays' TMB calculation algorithm if one exists. An example of what this reporting might look like is provided in the next paragraph:

TMB was assessed using CancerPanel version 1.2.3, a somatic-only hybrid capture–based NGS assay that interrogates 1.5 Mb of exonic DNA across 550 genes. All missense, frameshift, nonsense, and splice-site variants identified using Mutect2 are filtered to remove variants present at >0.1% allele frequency in any subpopulation in gnomAD and must have an allele fraction of at least 5% and coverage of at least 50× for inclusion in the TMB calculation.

Optional Elements for TMB Reporting

Some data elements (eg, reference genome, transcript source, and mutational signatures) may have a more modest impact on TMB calculation and interpretation but provide additional information about specific pipeline settings that could boost reproducibility and harmonization across studies and may provide greater context for how TMB is clinically interpreted. Specification of a reference genome and transcript source is fundamental to the process of calling variants. Currently, laboratories generally used either Genome Reference Consortium Human Build 37 or 38, whereas numerous reference transcript sources are in use, most commonly the National Center for Biotechnology Information Reference Sequence Database and Ensembl (*https://www.ncbi.nlm.nih.gov/refseq*, *https://ensemblgenomes.org*, both last accessed September 1, 2023). In a comparison of the Reference Sequence Database and Ensembl as reference transcript sources in the WGS500 project, 83% concordance was achieved across all exonic variants using the ANNOVAR annotation tool, whereas concordance was <50% when analysis was restricted to loss-of-function variants.[55] These results suggest that the reference transcript source can have an impact on variants detected, which may, in turn, affect TMB calculation.

An increasing number of laboratories perform mutational signature detection in addition to TMB measurement, with mismatch repair deficiency being the most frequently assessed signature. Although mutational signature detection is formally a separate process from TMB calculation, they both operate using the same underlying data and can be mutually informative when interpreted in a clinical context.

TMB Publication Recommendation

Recommendation 13: Publications Describing TMB Assays Intended for Clinical Applications, including a Description of Clinical Validation, Should Include Performance Characteristics that Would Facilitate Methodological Assessment

Improving reporting in TMB publications has enormous potential to standardize clinical TMB assay development, identify variables that affect assay performance, and facilitate assay implementation. This literature review and other publications have demonstrated inconsistent reporting of important clinical diagnostic assay parameters.[56] Recognized deficits in the literature have resulted in consensus recommendations and/or checklists for reporting diagnostic assays being developed by multiple groups.[20,57–59] Education of stakeholders, funders, institutions, and journals regarding the importance of requiring authors and reviewers to evaluate minimum reporting requirement compliance when clinical TMB assay studies are published is needed.[59,60]

Conclusion

TMB has emerged as a potential predictive biomarker for ICI therapy. Despite the enthusiasm surrounding this biomarker, the variety of approaches for calculating and reporting TMB and the lack of comprehensive methodological descriptions regarding assay validation pose challenges to clinical adoption. This document summarizes the existing knowledge and challenges related to TMB testing and provides consensus recommendations on validation and reporting for TMB assays in the clinical setting. The TMB Working Group recommendations reflect the published evidence reviewed and available at the time of writing. It is anticipated that these recommendations will need to be reviewed and updated as technological and scientific advances change. The AMP Clinical Practice Committee is responsible for reviewing the article within 3 years post-publication to determine if updates are needed.

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Disclosure Statement

To provide active management of potential perceived and/or actual conflicts of interest (COIs), a Working Group chair without relevant conflicts was appointed, and COI disclosures were requested from and/or provided by all authors throughout all phases of the consensus manuscript development process. A.Z. participated in the Working Group only while employed at Memorial Sloan Kettering Cancer Center; subsequent employment at AstraZeneca (New York, NY) constituted an unmanageable COI, and he recused himself from the Working Group on hire. L.L.R. participated in the Working Group only while employed at Massachusetts General Hospital; subsequent employment at Foundation Medicine Inc. (Cambridge, MA) constituted an unmanageable COI, and she recused herself from the Working Group on hire. L.L.R. received honoraria for participation in a Bristol Myers Squibb scientific advisory board meeting. C.B. owns stock in and serves on the scientific advisory boards of PrimeVax and Bio-AI Health; received honoraria for participation in a Lunaphore scientific advisory board meeting; has an ongoing advisory relationship with Sanofi and Agilent; and received institutional research support from Illumina, Inc. S.J.H. received honoraria for participation in an AstraZeneca scientific advisory board meeting.

Supplemental Data

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