



Tumor Mutational Burden as a Predictive Biomarker for Response to Immune Checkpoint Inhibitors: A Review of Current Evidence

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ABSTRACT

Treatment with immune checkpoint inhibitors (ICPIs) extends survival in a proportion of patients across multiple cancers. Tumor mutational burden (TMB)—the number of somatic mutations per DNA megabase (Mb)—has emerged as a proxy for neoantigen burden that is an independent biomarker associated with ICPI outcomes. Based on findings from recent studies, TMB can be reliably estimated using validated algorithms from next-generation sequencing assays that interrogate a sufficiently large subset of the exome as an alternative to whole-exome sequencing. Biological processes contributing to elevated TMB can result from exposure to cigarette smoke and ultraviolet radiation, from deleterious mutations in mismatch repair leading to microsatellite instability, or from mutations in the DNA repair machinery. A variety of clinical studies have shown that patients

with higher TMB experience longer survival and greater response rates following treatment with ICPIs compared with those who have lower TMB levels; this includes a prospective randomized clinical trial that found a TMB threshold of ≥ 10 mutations per Mb to be predictive of longer progression-free survival in patients with non-small cell lung cancer. Multiple trials are underway to validate the predictive values of TMB across cancer types and in patients treated with other immunotherapies. Here we review the rationale, algorithm development methodology, and existing clinical data supporting the use of TMB as a predictive biomarker for treatment with ICPIs. We discuss emerging roles for TMB and its potential future value for stratifying patients according to their likelihood of ICPI treatment response. *The Oncologist* 2020;25:e147–e159

Implications for Practice: Tumor mutational burden (TMB) is a newly established independent predictor of immune checkpoint inhibitor (ICPI) treatment outcome across multiple tumor types. Certain next-generation sequencing-based techniques allow TMB to be reliably estimated from a subset of the exome without the use of whole-exome sequencing, thus facilitating the adoption of TMB assessment in community oncology settings. Analyses of multiple clinical trials across several cancer types have demonstrated that TMB stratifies patients who are receiving ICPIs by response rate and survival. TMB, alongside other genomic biomarkers, may provide complementary information in selecting patients for ICPI-based therapies.

BACKGROUND

The development and therapeutic potential of immune checkpoint inhibitors (ICPIs) has fundamentally changed cancer treatment paradigms across multiple tumor types. However, only a subset of patients treated with ICPIs experience durable clinical responses [1, 2]. Ongoing optimization of ICPI treatment requires additional predictive biomarkers that further establish which patients are most likely to benefit from such therapies. Expression of programmed death-ligand 1 (PD-L1)

assessed by immunohistochemistry (IHC) has been established as one biomarker that is predictive of ICPI response in many cancer types including non-small cell lung cancer (NSCLC), urothelial carcinoma, and most recently triple-negative breast cancer [3–6]. However, stratification by PD-L1 IHC alone is insufficient to identify the patient population most likely to respond, and approved companion and complementary diagnostics for PD-L1 expression are variable with respect to

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performance and reporting thresholds [7–9]. Therefore, additional independent biomarkers that predict ICPI response are needed.

Broad genomic sequencing approaches that have an established role in identifying oncogenic alterations—whole-exome sequencing (WES) and whole genome sequencing—have been applied to samples from ICPI clinical trials. These initial studies clearly suggested that patients with a higher number of somatic tumor mutations derived more benefit from ICPI therapy compared with patients who had fewer mutations [10]. This has paved the way for the use of targeted next-generation sequencing (NGS) panels to derive the same information while sequencing fewer DNA base pairs. When properly designed and sufficient in size, these assays can assess tumor mutational burden (TMB; the number of somatic mutations per megabase [Mb] of sequenced DNA), which is a novel biomarker and a newly established independent predictor of ICPI treatment outcome [11, 12]. Targeted yet comprehensive NGS panels can accurately recapitulate the TMB assessment from WES, allowing for broader clinical use of this biomarker [12, 13].

In this review, we summarize the development of currently available methods for assessing TMB, the rationale for TMB to be used as an independent biomarker for ICPI treatment outcome across tumor types, the association between TMB and other aspects of the tumor microenvironment, efforts to prospectively evaluate TMB as a predictive biomarker, and the potential implications for utilizing TMB as a selection tool for ICPI treatment.

CANCER IMMUNOTHERAPY

Therapeutic manipulation of either the innate or adaptive arms of the immune system, or both, to optimize recognition and elimination of tumors is broadly known as cancer immunotherapy. The role of the immune system in cancer has been recognized for decades, and anticancer immunotherapy can be divided into three general categories: cytokine-targeting therapies (such as interleukins, interferons, and colony-stimulating factors), cell-based therapies (such as chimeric antigen receptor technologies), and ICPIs [14]. ICPIs, one of the most rapidly growing categories of immune-related agents developed for the treatment of cancer, are monoclonal antibodies that block key molecules in immune checkpoint pathways such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [14]. PD-L1, which is expressed on both tumor cells and immune cells and binds PD-1 to attenuate T-cell activity, is also an immunotherapy target [14].

At the most basic level, tumor cells differ from normal cells because of pathogenic changes in cellular function, which are often a result of genomic alterations that are considered to be “driver” mutations [15]. By contrast, the vast majority of alterations in tumoral DNA are considered “passenger” mutations, neither contributing to nor detracting from tumor growth [16]. However, a subset of these passenger alterations will generate neoantigens at the protein level, which may be recognized by the patient’s immune system as non-self or foreign. Although only a portion of overall immune response, neoantigenicity, correlated with response to checkpoint blockade and augmentation of the immune

response, is thought to be the mechanism by which ICPIs act on tumor tissue [17–19]. TMB is a surrogate measure of neoantigenicity, which allows it to serve as a predictive biomarker in this context.

MEASUREMENT AND ANALYTICAL VALIDATION OF TMB

TMB was originally measured using WES, and several studies demonstrated an association between WES-derived TMB and ICPI outcomes [10, 20–28]. Although these were encouraging results for patient selection, WES-derived TMB currently has limited clinical utility in many patient care settings owing to a 6–8-week sequencing time, the requirement for a matched normal sample, and associated costs. Estimating TMB using clinically validated, commercially available, targeted NGS-based panels that sequence a sufficient subset of the exome presents an attractive alternate method for calculating TMB [12, 13, 29] (Fig. 1; [30]). In general, WES methods typically count only non-synonymous base substitutions that alter the amino acid sequence of a protein, inferring that there is a direct link between protein coding changes and the number of potential neoantigens within a tumor genome [10, 20–27]. However, targeted NGS panels for estimating TMB have taken more sophisticated approaches, including the incorporation of non-synonymous and synonymous base substitutions and short insertion and deletion alterations in the calculation [13]. Synonymous variants, which are variants that do not alter the amino acid sequence of a protein, are not assumed to generate neoantigens. Their presence, however, is indicative of a mutational process also likely to result in nonsynonymous variants, and their inclusion in the TMB algorithm effectively improves assay sensitivity by increasing the number of qualifying variants into the calculation [13].

Although there are multiple platforms that have published data using TMB as a biomarker [31–33], to date only two products have gone through regulatory pathways: the FoundationOne CDx assay (Foundation Medicine, Inc., Cambridge, MA), which has been approved by the U.S. Food and Drug Administration (FDA) for the analytic calculation of TMB, and MSK-IMPACT (Memorial Sloan Kettering Cancer Center, New York, NY), which has been authorized by the 510K pathway [31, 33]. These panels have been optimized to identify all types of molecular alterations (i.e., single nucleotide variants, small and large insertion-deletion alterations, copy number alterations, and structural variants) in cancer-related genes, as well as genomic signatures such as microsatellite instability (MSI), loss of heterozygosity, and TMB, in a single test; this approach is collectively referred to as comprehensive genomic profiling (CGP) [34]. As a result of the broad and deep coverage of targeted NGS panels across several hundred tumor genes and nontumor tissue, studies have demonstrated that TMB measurement using a CGP approach has high statistical concordance with TMB measured from WES [13]. The two quality control metrics that should be considered when evaluating samples for TMB are median depth of sequencing coverage and coverage uniformity, as coverage is directly related to the sensitivity of calling both single nucleotide variants and indels, the two components that contribute to the TMB calculation [35]. Foundation Medicine utilizes a minimum sequencing coverage metric of

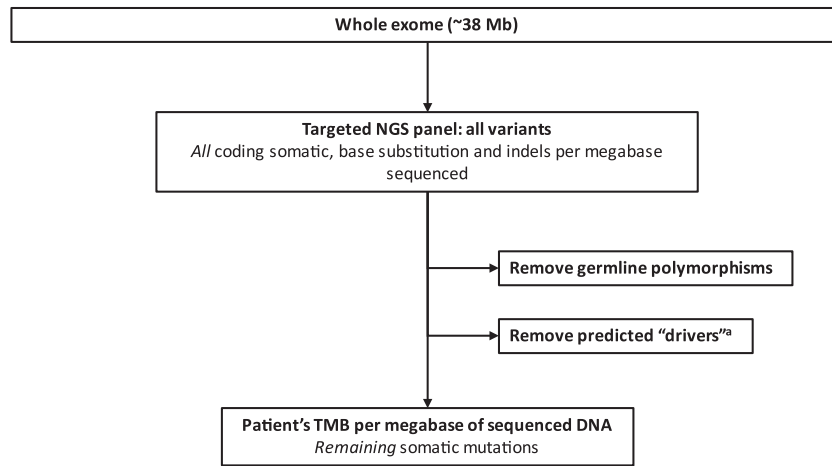


Figure 1. Example of targeted NGS panel-based TMB calculation. Adapted from Spigel et al. [30].

^aPredicted drivers are mutations thought to be responsible for oncogenesis in a tumor.

Abbreviations: Mb, megabase; NGS, next-generation sequencing; TMB, tumor mutational burden.

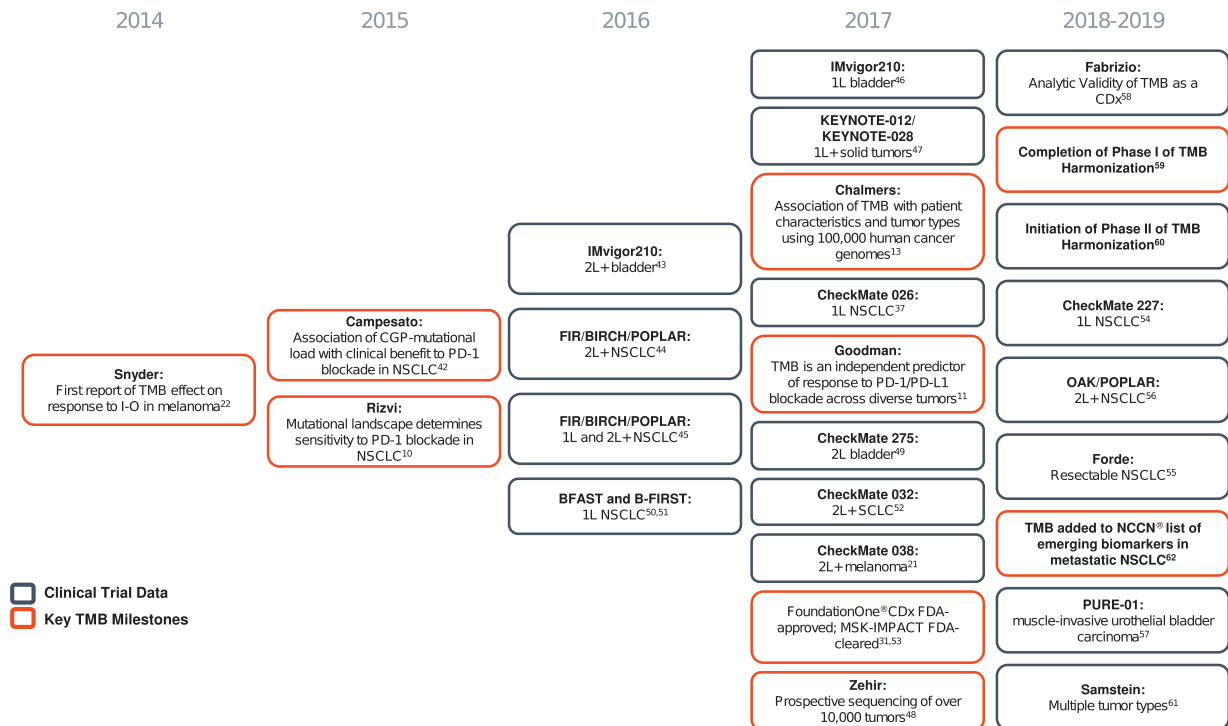


Figure 2. Timeline of TMB biomarker development.

Abbreviations: 1L, first-line; 2L, second-line; CGP, comprehensive genomic profiling; FDA, U.S. Food and Drug Administration; I-O, immune-oncology; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SCLC, small cell lung cancer; TMB, tumor mutational burden.

250× median exon coverage and a uniformity metric of ≥95% of exons with at least 100× coverage [36].

Chalmers and colleagues [13] reported that a key determinant for the accuracy of the targeted NGS-based TMB measurement is the number of megabases sequenced in the genome and that sequencing approximately 1.1 Mb over 315 genes resulted in a TMB estimate that was similar to the reference standard of WES. In this study, samples with 300× median exon coverage or greater were included. The study also estimated that sampling approximately 0.5 Mb or less resulted in an

unacceptable degree of difference from the WES reference standard, suggesting that more limited assays may result in an inaccurate TMB calculation [13]. Additional in silico analyses demonstrated acceptable agreement between targeted NGS-derived and WES-derived TMB data [37–39]. In addition to establishing in silico accuracy against WES, additional key performance metrics such as reproducibility and repeatability of the TMB classifier, limit of detection according to the minimum tumor purity, and empirical accuracy against WES should be established to validate any TMB measurement currently being

reported as a biomarker upon which therapeutic decisions will be based.

TMB AS A PREDICTIVE BIOMARKER

There are several known carcinogenic processes linked to elevated TMB [10, 13]. Exposure to environmental carcinogens, such as cigarette smoke and ultraviolet radiation, has been shown to cause cancers with the highest number of somatic mutations [13, 40, 41]. Alterations in DNA mismatch repair (MMR) pathway-associated genes such as *MSH2*, *MSH6*, *MLH1*, and *PMS2*, which typically result in MSI, and alterations in DNA polymerase genes (*POLE/POLD1*) contribute to high TMB in some cancers [13]. Chalmers and colleagues [13] have reported that a proportion of tumors across multiple cancer types have high TMB, showing that TMB has broad clinical validity as a biomarker. Figure 2 [10, 11, 13, 21, 22, 31, 37, 42–62] illustrates a timeline of the emergence of TMB as a biomarker.

Clinical, pathologic, and molecular features influence the likely course for a given patient with cancer and predict the chance of response to a given therapy. An optimal therapeutic biomarker has analytic validity (accurate, reproducible, and reliable), clinical utility, economic feasibility, and a biologic basis [63]. Retrospective analyses have demonstrated that a greater clinical benefit following treatment with ICPIs has been observed in patients with high TMB compared with those without high TMB; however, these studies used a variety of testing platforms and differing TMB cutoffs to define “high” levels rather than standardized, prospectively defined cutoffs [11, 25, 46, 54, 64–66]. Early reporting of TMB included both the quantitative metric as mutations per Mb and qualitative description of high, intermediate, and low. Discrete cutoffs that define the biological subtype of cancer are currently being pursued, and such cutoffs will likely vary based on tumor type and therapeutic intervention [59, 60, 67, 68]. In an analytic validation of FoundationOne CDx in patients with NSCLC, reproducibility and repeatability for TMB were shown to be 97.3% and 95.3% [58]. Although the reproducibility and standardization of TMB calculations via NGS have yet to be thoroughly validated across other tumor types, efforts are currently underway to do so [60, 69].

Clinical Validation of TMB as an Independent Predictive Biomarker

Establishing whether any biomarker test, including a test for TMB, accurately and reliably separates patients into groups with distinct clinical or biological outcomes or differences (i.e., clinical validation) is an important factor for defining clinical use of a test [63].

The evidence of ICPI efficacy in MSI-high disease led to the first tumor-agnostic FDA approval for pembrolizumab for MSI-high or MMR-deficient tumors independent of anatomic origin [27, 28, 70]. Additionally, the same study also found that high TMB was independently associated with longer progression-free survival (PFS) [27]. Across cancers, MSI-high tumors are rare (~1% as measured retrospectively by NGS, 5% in late stage colorectal cancer [CRC], 15% in endometrial/uterine cancer), and MSI assessment alone will fail to identify all patients who may benefit from ICPIs [13, 71, 72]. Nearly all MSI-high samples have high

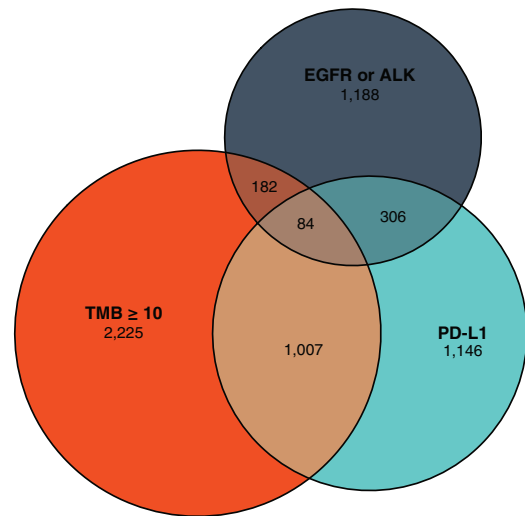


Figure 3. Interaction of high TMB with other cancer biomarkers. An analysis of Foundation Medicine’s FoundationCore database (data on file) was undertaken to understand the relative prevalence of biomarkers that play a predictive role in immunotherapy decisions for patients with non-small cell lung cancer (NSCLC). Through September 2018, there were 9,347 NSCLC samples with Foundation Medicine testing (FoundationOne and FoundationOne CDx) that also underwent PD-L1 testing. The relative distribution of *EGFR* and/or *ALK* alterations, TMB ≥ 10 mutations per megabase, and PD-L1 positive is shown here. Prevalence of each of the biomarkers in all patients with NSCLC ($n = 35,370$), regardless of PD-L1 testing, was determined with *EGFR* alterations found in 14.1% and *ALK* alterations in 2.9%; this appears similar to the rates observed in the smaller subset of patients with concurrent PD-L1 assessment. Overall, the overlap is limited, indicating a need to assess each of these biomarkers when making immunotherapy decisions in the NSCLC setting. Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden.

TMB and represent a subset of high TMB tumors across anatomic sites [13]. However, MSI-high is not sufficient to explain all instances of high TMB, even in tumor types where MSI is a well-established biomarker such as CRC [73]. For example, a TMB score of 12 mutations per Mb was shown to include 99.7% of all MSI-high cases in patients with CRC, while also identifying an additional 3% of the much larger microsatellite-stable population [73]. As such, reclassification of CRC according to TMB effectively increased the number of patients eligible for ICPI therapy by more than 50% compared with MSI status alone [73]. Although PD-L1 overexpression is associated with improved ICPI response and is common among patients with high TMB levels, several studies have concluded that TMB and PD-L1 expression are independent predictive biomarkers [12, 25, 26, 74].

The culmination of these clinical validation efforts is the identification of TMB cutoffs that predict ICPI outcome, demonstrating that TMB augments both MSI and PD-L1 as an independent predictive biomarker for ICPI treatment. This was demonstrated in the NSCLC CheckMate-227 trial, which found that TMB was clearly predictive of the PFS benefit observed in the combination nivolumab/ipilimumab arm and was not associated with improved PFS among patients receiving only chemotherapy [54]. Elevated TMB was also observed

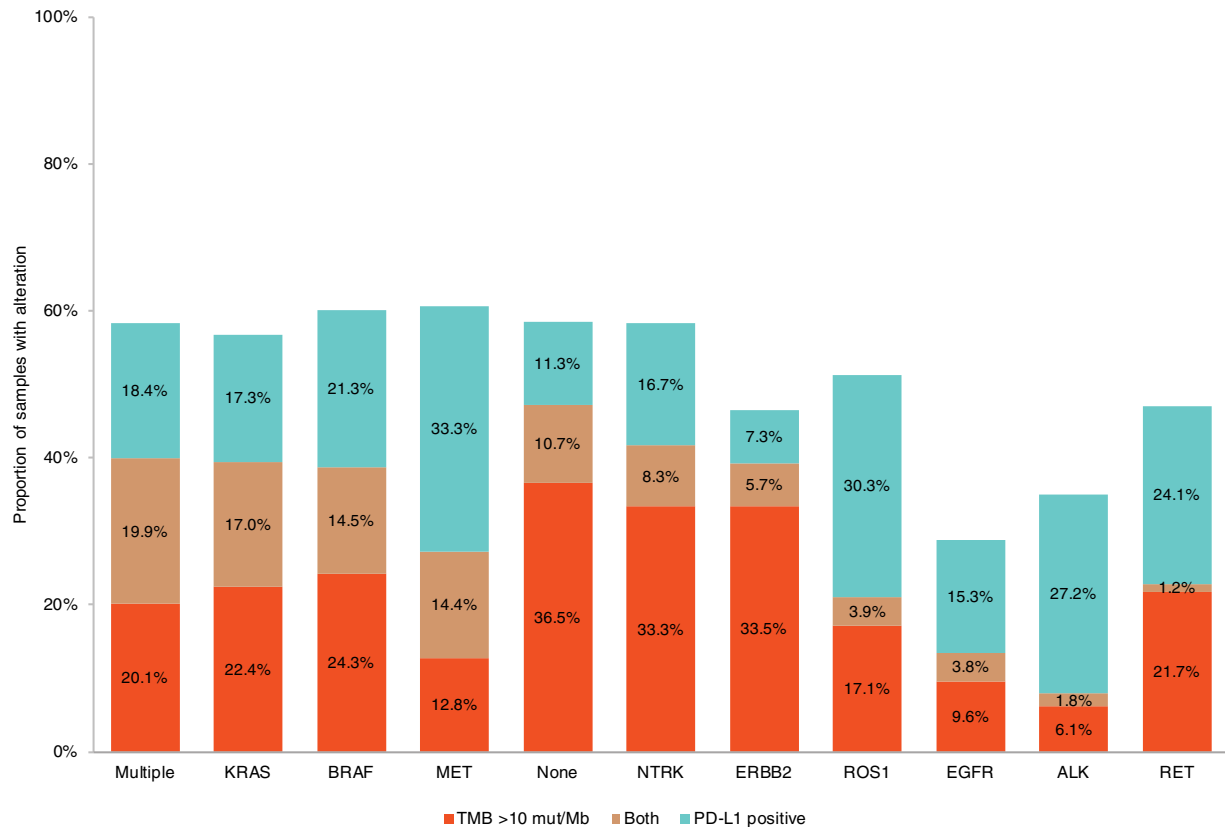


Figure 4. Degree of overlap between high TMB and PD-L1 varies based on the presence of other alterations among patients with non-small cell lung cancer (NSCLC). Among NSCLC samples with Foundation Medicine testing that also underwent PD-L1 testing ($n = 9,347$; described in Fig. 3), the relative overlap between TMB ≥ 10 mutations per megabase and PD-L1 is highest in patients with multiple genomic alterations as well as *KRAS*, *BRAF*, and *MET* alterations and lowest in patients with *ALK* and *RET* alterations. Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden.

to be predictive of improved survival in patients with any tumor type receiving ICPIs, although the TMB cutoffs varied markedly between cancer types [61, 73].

Additional Clinically Relevant Genomic Biomarkers Associated with TMB

TMB is an important treatment selection tool that complements existing molecular testing methodologies, PD-L1, and other established and emerging oncogenes. Recurrent genomic alterations associated with elevated TMB, which together can be identified using a CGP approach, may provide additional biologic insights and inform therapy in select scenarios. For example, mutations in *POLE* are an emerging immunotherapy-related biomarker that have been associated with very high TMB in multiple solid tumor types, including endometrial, CRC, gastric, melanoma, lung, and pediatric cancers [75–78]. *POLE*-mutated, MSI-high, and DNA MMR-deficient CRC have each been associated with high TMB and favorable outcomes following treatment with ICPIs [27, 73, 79, 80]. Therefore, tumors with pathogenic *POLE* mutations leading to elevated TMB may be good candidates for ICPI therapy independent of tumor type. Furthermore, as with MSI-high, *POLE*-mutated cancers represent only a subset of high TMB cancers, emphasizing the need to evaluate a broad set of biomarkers in order to capture all mechanisms of hypermutation.

Advanced NSCLC provides a particularly strong case for the use of CGP given that well-established biomarkers, such as epidermal growth factor receptor (*EGFR*) mutations, anaplastic lymphoma kinase (*ALK*) alterations, and PD-L1 expression, are present at different rates based on TMB levels. Each one of these biomarkers should be considered independently as well as together when making treatment decisions. In a sample of 9,347 NSCLC samples that underwent CGP (FoundationOne and FoundationOne CDx) and concurrent PD-L1 testing, 18.0% were shown to be positive for *ALK* or *EGFR* alterations, 37.4% were TMB-high (≥ 10 mutations/Mb), and 6.4% were PD-L1 positive (data on file). However, there was minimal overlap between these molecular markers (Figs. 3 and 4). Because *EGFR* and *ALK* mutations are associated with low TMB and attenuated response rates to ICPIs, patients with tumors that are *EGRF* or *ALK* positive are ineligible for ICPI therapy in the first-line setting according to FDA-approved labeling. As discussed above, PD-L1 and TMB are not mutually inclusive; thus both are needed to identify all patients who are likely to respond to ICPIs, whereas *EGRF/ALK* biomarker status will be needed to rule out those less likely to respond in the first-line setting [12, 81–83].

Additionally, *KRAS* mutations have been associated with improved treatment outcomes in NSCLC [30, 82, 84, 85], and certain classes of alterations in *JAK1*, *MDM2/MDM4*, *ARID1A*, and *STK11* have predicted a lack of response to ICPIs in a high

Table 1. ICPI clinical trials that have evaluated TMB as a biomarker

Trial [reference]/study design/population	Intervention(s)	Type of sequencing for TMB	TMB cutpoint or highest threshold	TMB-related results
CheckMate-227 [54] Open-label, randomized, phase III trial Advanced NSCLC <i>n</i> = 1,739	1st-line nivolumab plus ipilimumab vs. platinum-doublet chemotherapy	CGP	≥10 mutations per Mb	Nivolumab plus ipilimumab vs. chemotherapy—with TMB ≥10 mutations/Mb: <ul style="list-style-type: none"> • 1-year PFS: 42.6% vs. 13.2% (<i>p</i> < .001) • Median PFS: 7.2 vs. 5.5 months (<i>p</i> < .001) • Disease progression or death: HR, 0.58 (97.5% CI: 0.41–0.81; <i>p</i> < .001) • ORR: 45.3% vs. 26.9% Nivolumab plus ipilimumab vs. chemotherapy—with TMB <10 mutations/Mb: <ul style="list-style-type: none"> • Median PFS, 3.2 vs. 5.5 months • Disease progression or death: HR, 1.07 (95% CI, 0.84–1.35) Treatment difference in TMB ≥10 mutations/Mb was consistent across PD-L1 expression subgroups (<1% vs. ≥1%)
CheckMate-012 [25] Prospective study Advanced NSCLC <i>n</i> = 75	1st-line nivolumab plus ipilimumab	WES	> median of 158 mutations	TMB > median vs. TMB < median: <ul style="list-style-type: none"> • ORR: 51% vs. 13% (<i>p</i> = .0005) • DCB: 65% vs. 34% (<i>p</i> = .011) • PFS: HR, 0.41 (<i>p</i> = .0024) TMB was independent of PD-L1 expression (<i>r</i> = .087; <i>p</i> = .48) and most strongly associated with ORR (<i>p</i> = .001) and PFS (<i>p</i> = .002) (multivariable analysis).
CheckMate-026 [37, 74] Open-label, randomized, phase III trial Advanced NSCLC with PD-L1 ≥ 1% <i>n</i> = 541	1st-line nivolumab vs. doublet platinum chemotherapy	CGP	≥243 somatic missense mutations per sample	Nivolumab vs. chemotherapy—TMB ≥243 mutations subgroup: <ul style="list-style-type: none"> • ORR: 47% vs. 28% • Median PFS: 9.7 vs. 5.8 months (HR, 0.62; 95% CI: 0.38–1.00) • OS: no between-group differences Nivolumab vs. chemotherapy—TMB <243 mutations subgroup: <ul style="list-style-type: none"> • ORR: 23% vs. 33% • Median PFS: 4.1 vs. 6.9 months (HR, 1.82; 95% CI: 1.30–2.55) • OS: 12.7 vs. 13.2 months (HR, 0.99; 95% CI: 0.71–1.40) There was no association between PD-L1 and TMB (all patients had PD-L1 ≥ 1%).
CheckMate-032 [25] Single-arm, randomized phase I/II trial Advanced SCLC <i>n</i> = 211	Nivolumab plus ipilimumab, nivolumab alone	WES	≥248 mutations (high tertile)	The high vs. low TMB tertile were compared: <ul style="list-style-type: none"> • Nivolumab plus ipilimumab <ul style="list-style-type: none"> ◦ ORR: 46.2% vs. 22.2% ◦ 1-year PFS: 30.0% vs. 6.2% ◦ 1-year OS: 62.4% vs. 23.4% • Nivolumab alone <ul style="list-style-type: none"> ◦ ORR: 21.3% vs. 4.8% ◦ 1-year PFS: 21.2% vs. NE ◦ 1-year OS: 62.4% vs. 23.4% PD-L1 expression ≥1% was rare and evenly distributed among the TMB tertiles. There was no association between PD-L1 expression and TMB.
CheckMate-568 [93] Single-arm, phase II trial Advanced NSCLC <i>n</i> = 288	1st-line nivolumab plus ipilimumab vs. platinum-doublet chemotherapy	CGP	≥10 mutations per Mb	ORR at TMB cutpoints for nivolumab plus ipilimumab: <ul style="list-style-type: none"> • <5 mutations/Mb: 4% • <10 mutations/Mb: 10% • ≥10 mutations/Mb: 44% • ≥15 mutations/Mb: 39% TMB ≥10 mutations/Mb was associated with enhanced response to nivolumab plus ipilimumab regardless of PD-L1 expression

(continued)

Table 1. (continued)

Trial [reference]/study design/population	Intervention(s)	Type of sequencing for TMB	TMB cutpoint or highest threshold	TMB-related results
BIRCH/FIR/POPLAR/OAK, IMvigor 210/211, PCD4989g [94] Retrospective study of tumor tissue samples from 7 monotherapy studies NSCLC (<i>n</i> = 342), advanced UC (<i>n</i> = 400), advanced solid tumors (<i>n</i> = 245)	Atezolizumab	CGP	≥16 mutations per Mb	Biomarker-evaluable population vs. TMB ≥16 mutations/Mb vs. <16 mutations/Mb: • ORR: 16.4% vs. 29.7% vs. 13.5% • DOR: 16.6 months vs. 29.0 months vs. 13.8 months
PURE-01 [57] Single-arm, open-label, phase II trial Muscle-invasive urothelial bladder cancer <i>n</i> = 43	Neoadjuvant pembrolizumab before radical cystectomy	CGP	NA	Mean TMB in transurethral resection of the bladder resection samples was identical between patients with and without pathologic complete response 11.2 mutations/Mb vs. 11.2 mutations/Mb.
Apache [95] Open-label, randomized, 3-stage, phase II trial Advanced germ cell tumors <i>n</i> = 18	Durvalumab alone or with tremelimumab	CGP	NA	Median TMB was 4 mutations/Mb and was not related to efficacy.
IMvigor210 [46, 64] Single-arm, phase II trial Advanced UC <i>n</i> = 310	1st-line atezolizumab	CGP	≥16 mutations per Mb ^a	There was a greater proportion of TMB patients with ≥16 mutations/Mb among responders vs. nonresponders. • Consistent across TCGA luminal and basal subtypes • Associated with significantly longer OS. TMB ≥16 mutations/Mb was associated with increased expression of • APOBEC3A: <i>r</i> = 0.18 (<i>p</i> = .0025) • APOBEC3B: <i>r</i> = 0.22 (<i>p</i> = .00046) Responders exhibited higher mean APOBEC3 expression.
Le et al. (2015) [27] Single-arm, phase II study Advanced pan-tumor <i>N</i> = 41	Pembrolizumab	WES	NA	Tumors with MSI-high had high TMB levels vs non-MSI tumors (<i>p</i> = .007). High TMB was associated with • Longer PFS: HR, 0.628 (95% CI: 0.424–0.931; <i>p</i> = .021) • Trend toward higher ORR (<i>p</i> = .214).
POPLAR and OAK [56] Randomized, phase II trial Previously treated NSCLC OAK, <i>n</i> = 425; POPLAR, <i>n</i> = 287	2nd + line atezolizumab vs. docetaxel	Blood-based CGP	≥10 mutations per Mb	PFS and OS in patients with bTMB ≥10 mutations/Mb were higher than in the overall population.
BIRCH/FIR, POPLAR [45] Single-arm (BIRCH/FIR) and randomized (POPLAR) phase II trials NSCLC OAK, <i>n</i> = 425; POPLAR, <i>n</i> = 287; FIR, <i>n</i> = 138	1st/2nd + line atezolizumab (single-arm) in BIRCH/FIR, 2nd-line atezolizumab vs. docetaxel in POPLAR	CGP	BIRCH/FIR: In 1st line, ≥13.5 mutations/Mb; in 2nd line or later, ≥17.1 mutations/Mb POPLAR: ≥15.8 mutations/Mb	RR and OS were higher in patients with both TMB ≥9 mutations/Mb (median) and ≥ 13.5 mutations/Mb (high) vs <9 and <13.5 mutations/Mb, respectively.
Samstein et al. (2019) [61] Retrospective study Multiple tumor types Treated with ICPI, <i>n</i> = 1,662; Non-ICPI treated, <i>n</i> = 5,371	Atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab, pembrolizumab or tremelimumab (monotherapy or in combination)	CGP	NA	OS was higher in patients with high TMB (highest 20% in each cancer type), across entire cohort. TMB cutoff associated with the top 20% varied markedly between cancer types.

^aCutpoint only given in Balar et al. [46].

Abbreviations: bTMB, blood-based TMB; CGP, comprehensive genomic profiling; CI, confidence interval; DCB, durable clinical benefit; HR, hazard ratio; ICPI, immune checkpoint inhibitor; Mb, megabase; MSI, microsatellite instability; NA, not applicable; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PD-L1, programmed death ligand 1; PFS, progression-free survival; RR, response rate; TCGA, The Cancer Genome Atlas; TMB, tumor mutational burden; UC, urothelial cancer; WES, whole-exon sequencing.

TMB setting [12, 85–91]. Initial data from studies utilizing targeted NGS panels have also suggested that certain *BRAF* and *MET* alterations are associated with longer duration of ICPI treatment, regardless of TMB status [88].

Overall, appropriate treatment selection in the era of genomically targeted therapies and immunotherapies will require insight into PD-L1, TMB, MSI, as well as alterations in several individual genes. Looking at only a subset of these biomarkers

Table 2. Studies demonstrating the relationship between TMB and treatment outcome in patients with cancer

Trial [reference]/study design/population	Intervention(s)	Type of sequencing for TMB	TMB cutpoint or highest threshold	TMB-related results
Eroglu et al. (2018) [20] Retrospective review of pathology reports Advanced desmoplastic melanoma <i>n</i> = 60	PD-1 or PD-L1 blockade therapy	WES	NA	Patients with desmoplastic melanoma had substantial clinical benefit from PD-1 or PD-L1 immune checkpoint blockade therapy likely resulting from high TMB, increased CD8 density, and high expression of PD-L1 in tumor invasive margin (median follow-up of 22 months). <ul style="list-style-type: none"> • ORR: 70% (95% CI: 57–81) • CR: 32% • PR: 38% • OS: 74% (95% CI: 60–84)
Rizvi et al. (2018) [12] Prospective and retrospective study Advanced NSCLC <i>n</i> = 240	1st/2nd/3rd + line immunotherapy: Anti-PD-1 or anti-PD-L1	CGP and WES	NA	Median TMB: <ul style="list-style-type: none"> • DCB vs. NDB: 8.5 vs. 6.6 SNVs/Mb (<i>p</i> = .006) • CR/PR vs. SD vs. PD: 8.5 vs. 6.6 vs. 66 SNVs/Mb (<i>p</i> = .015) TMB was stratified into increasing thresholds above vs. below the 50th percentile in patients treated with immunotherapy: <ul style="list-style-type: none"> • DCB: 38.6% vs. 25.1% (<i>p</i> = .009) • PFS: HR, 1.38 (<i>p</i> = .024) TMB was independent of PD-L1 expression (<i>r</i> = .1915; <i>p</i> = .08).
Greally et al. (2018) [65] Retrospective study of tumor tissue samples Esophagogastric cancer <i>n</i> = 120	Various immune checkpoint inhibitors	CGP	≥7.4 mutations per Mb	High TMB vs. low TMB, OS: 27.1 months vs. 8.4 months (<i>p</i> = .063)
Goodman et al. (2017) [11] Retrospective study of clinical records Locally advanced or metastatic pan-tumor <i>n</i> = 151	Various immune checkpoint inhibitors	CGP	≥20 mutations per Mb	TMB ≥20 mutations/Mb vs. <20 mutations/Mb: <ul style="list-style-type: none"> • RR: 58% vs. 20% (<i>p</i> = .0001) • Median PFS: 12.8 vs. 3.3 months (<i>p</i> ≤ .0001) • Median OS: not reached vs. 16.3 months (<i>p</i> = .0036) High TMB was independently associated with better outcome parameters (multivariable analysis).
Rozenblum et al. (2017) [96] Retrospective cohort study Advanced lung cancer <i>n</i> = 33	Nivolumab or pembrolizumab	CGP	NA	Response rate in patients treated with immunotherapy: <ul style="list-style-type: none"> • PR: 11% • SD: 11% • PD: 78% Patients who were not carrying any treatment-associated driver (<i>n</i> = 17) had the highest mean TMB (11.8 ± 5 mutations/Mb) and the highest ORR to immunotherapy (33%).
Johnson et al. (2016) [66] Retrospective study of tumor tissue samples Metastatic melanoma Initial cohort: <i>n</i> = 32; validation cohort: <i>n</i> = 33	2nd-line immune checkpoint inhibitors	CGP	>23.1 mutations per Mb (high)	High TMB vs. intermediate vs. low: <ul style="list-style-type: none"> • ORR: 85% vs. 29% vs. 14% (<i>p</i> < .001) • Median PFS: not reached vs. 89 days vs. 86 days (<i>p</i> < .001) • Median OS: not reached vs. 300 days vs. 375 days (<i>p</i> < .001)
Rizvi et al. (2015) [10] Prospective study NSCLC Discovery cohort: <i>n</i> = 16; validation cohort: <i>n</i> = 18	Pembrolizumab	CGP	>median mutations per sample within cohort	TMB > median vs. others, both cohorts: <ul style="list-style-type: none"> • PFS: HR, 0.19 (95% CI: 0.08–0.47; <i>p</i> = .0004) TMB > median vs. others, discovery cohort: <ul style="list-style-type: none"> • DCB: 73% vs. 13% (<i>p</i> = .04) • ORR: 63% vs. 0% (<i>p</i> = .03)
Van Allen et al. (2015) [24] Retrospective study of tumor tissue samples Metastatic melanoma <i>n</i> = 110	Ipilimumab	WES	NA	TMB was significantly associated with CB from ipilimumab (<i>p</i> = .0076).
Snyder et al. (2014) [22] Retrospective study of tumor tissue samples Malignant melanoma Discovery cohort: <i>n</i> = 25; validation cohort: <i>n</i> = 39	Ipilimumab or tremelimumab	WES	NA	Higher TMB in long-term benefit subgroup vs. minimal benefit subgroup (<i>p</i> = .009) OS correlated with higher TMB, discovery cohort (<i>p</i> = .04)

Abbreviations: CB, clinical benefit; CGP, comprehensive genomic profiling; CI, confidence interval; CR, complete response; DCB, durable clinical benefit; DOR, duration of response; HR, hazard ratio; Mb, megabase; NA, not applicable; NDB, no durable benefit; NE, not estimable; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PD, partial disease; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; PR, partial response; RR, response rate; SD, stable disease; SNV, single-nucleotide variant; TMB, tumor mutational burden; WES, whole-exome sequencing.

Table 3. Examples of planned or ongoing clinical trials evaluating TMB as a biomarker

Trial	Study design	TMB-related design elements	Completion date, estimated
B-F1RST [97] NCT02848651	Phase II, single-arm trial of atezolizumab in advanced NSCLC	Primary biomarker endpoint is bTMB; clinical outcomes (OS, PFS, OR) will be evaluated according to bTMB	2018
B-FAST [98] NCT03178552	Phase II/III nonrandomized multiple cohort trial of atezolizumab, alectinib, pemetrexed, or gemcitabine in advanced NSCLC	Enrollment by actionable genomic alterations or positive bTMB	2020 (primary) 2022 (study)
CAPTUR [99] NCT03297606	Phase II basket trial of various targeted therapies in advanced cancer (pan-tumor)	Study group: Nivolumab ± ipilimumab in patients with high TMB and/or alterations in <i>POLE/POLD1</i>	2021
Javelin Parp Medley [100] NCT03330405	Phase Ib/II dose-finding trial of avelumab plus talazoparib in advanced cancer (pan-tumor)	Secondary endpoint: TMB at baseline	2020
My Pathway [101] NCT02091141	Phase II basket trial of various targeted therapies in advanced cancer (pan-tumor)	Study group: Atezolizumab in patients with high TMB/MSI-high and/or alterations in <i>PD-L1</i> , <i>POLE</i> , or <i>POLD1</i>	2019
PECULIAR [102]	Phase II, single-arm trial of neoadjuvant pembrolizumab and epacadostat in muscle-invasive urothelial bladder cancer	Endpoint: TMB among biomarkers to be assessed	Unknown
TAPUR [109] NCT02693535	Phase II basket trial of various targeted therapies in advanced cancer (pan-tumor)	Study group: Pembrolizumab or nivolumab + ipilimumab in patients with high TMB and/or alterations in <i>POLE/POLD1</i>	2019 (primary)

Abbreviations: bTMB, blood-based TMB; MSI, microsatellite instability; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; TMB, tumor mutational burden.

could potentially result in suboptimal treatment among a considerable portion of patients and lead to inefficiencies in our health care ecosystem. Utilizing a CGP approach has the advantage of providing the data to generate composite biomarkers, which can be used collectively to further stratify patient populations most likely to derive maximal clinical benefit from both ICPIs and other genomically matched targeted treatments.

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SUMMARY OF OUTCOMES ASSOCIATED WITH TMB

Clinical studies and observational data have further reinforced the notion of TMB as an independent biomarker that is predictive of ICPI outcomes [92]. Increased nonsynonymous TMB from WES was first demonstrated as a predictor for ICPI treatment outcome by Snyder and colleagues [22] and Rizvi and colleagues [10] for CTLA-4 and PD-1/PD-L1 inhibition, respectively. The relationship between WES-derived TMB was further explored in the CheckMate-032 study, wherein patients with tumors in the top tertile of TMB experienced 46.2% objective response rate (ORR) with nivolumab plus ipilimumab compared with 16.0% and 22.2% in the medium and low

tertiles, respectively [26]. As mentioned above, PD-L1 expression and TMB have not been significantly correlated in most ICPI studies (Table 1). Reported and ongoing clinical trials and observational studies that have analyzed or will analyze TMB outcomes are summarized in Table 1 [25, 27, 37, 45, 46, 54, 57, 61, 64, 74, 93–95], Table 2 [10–12, 20, 22, 24, 65, 66, 96], and Table 3 [97–102].

It should be noted that TMB cutoffs have been defined differently across studies, testing platforms, and in various patient populations, and it is also important to acknowledge that cutoffs might differ by tumor type and ICPI agent (e.g., >16 mutations/Mb for atezolizumab in urothelial carcinoma; >23.1 mutations/Mb for pembrolizumab in NSCLC; and ≥13.5, ≥15.8, or ≥17.1 mutations/Mb for atezolizumab in NSCLC; Tables 1, 2) [45, 46, 61, 66]. Goodman and colleagues [11] suggested a pan-tumor cutoff of 20 mutations per Mb, and Yarchoan and colleagues [103] have reported a nearly linear relationship between TMB and ORR. A TMB cutoff of 10 mutations per Mb for treatment outcome among patients with advanced NSCLC treated with nivolumab plus ipilimumab was recently validated in the CheckMate-568 trial [93], which demonstrated ORRs of 4% and 10% at cutoffs of <5 and <10 mutations per Mb, respectively, compared with 44% at ≥10 mutations per Mb. These findings informed the cutoff for the phase III, randomized, placebo-controlled CheckMate-227 study, in which the treatment group with TMB ≥10 mutations per Mb experienced 1-year PFS of 42.6% compared with 13.2% in the chemotherapy group [54]. It remains to be seen how TMB cutoffs will be applied broadly across tumor types in a clinical setting, but the possibility exists for TMB to redefine therapeutic approaches agnostic of tumor type, similar to MSI.

CURRENT VALUE OF TMB TO THE ONCOLOGY COMMUNITY

The value of TMB is intrinsically tied to the value of identifying patients who are likely to have a clinical benefit from ICPIs given that the magnitude of such benefit is often considerable. As discussed above, immuno-oncology biomarkers are not mutually inclusive. Comprehensive assays capable of measuring TMB are likely to identify information about other biomarkers and alterations associated with targeted therapies, allowing care providers to make fully informed therapeutic decisions. By developing and refining this stratification, certain patients with low TMB or other alterations predictive of lack of response or hyper-progression may avoid costly ineffective treatment, whereas others who are strong candidates for ICPIs may become eligible to receive these agents at earlier lines of therapy.

There is a recognized need for standardization of clinically valid TMB assays across testing platforms. Lessons can be learned from the challenges of validation for PD-L1 testing [7], which led to an acknowledgment of the need for improved harmonization for biomarker testing. International studies and a coalition of organizations are currently working on methods to ensure the standardization of TMB across assays in order to confirm that accurate clinical decisions are being made for patients with cancer [60]. At the time of this writing, the TMB Harmonization Working Group is reviewing the current methods of TMB calculation and reporting as well as developing a consensus on how best to standardize these measurements (phase I has been completed; phase II is underway; Fig. 2) [59, 60, 68].

Improved patient selection for immunotherapy is also likely to enhance the economic value of ICPIs. In particular, health-related quality of life outcomes among patients with NSCLC and urothelial cancer who were treated with pembrolizumab have demonstrated a substantial improvement compared with chemotherapy, thus showing the potential of TMB to increase incremental quality-adjusted life-years in economic analyses [104, 105]. Although there is limited direct evidence evaluating the economic value of using TMB as a biomarker for treatment stratification, the potential impact of incorporating TMB testing into routine clinical practice might lead to improved outcomes and greater stratification of patients who undergo effective immunotherapy for a longer duration. In this scenario, testing costs are likely to remain stable when TMB is provided in the context of an existing CGP panel, while clinical value improves.

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FUTURE DIRECTIONS

Collectively, the current data suggest that measuring TMB for all patients with cancer has the potential to increase access to

life-extending therapies while improving the overall clinical and economic value of ICPIs. Several ongoing or planned ICPI studies are using TMB to enroll patients and thereby increase the proportion of patients who are likely to benefit (Table 3). Additionally, TMB measurement from circulating tumor DNA, derived from blood specimens (bTMB), was recently shown to be predictive of survival in patients with NSCLC who were treated with atezolizumab, and several ongoing trials are evaluating the prospective efficacy of bTMB in a first-line NSCLC setting [56, 97, 98, 106] (Table 3).

There are many more avenues of research to be explored in order to better understand the relationship between TMB and ICPI outcomes. For example, recent findings have suggested that TMB could be useful for patient stratification in trials assessing ICPI use at earlier stages of cancer [55, 107]. Important questions regarding the role of concurrent genomic alterations in high TMB tumors that may negatively predict the impact ICPI responsiveness, such as pre-existing *STK11*, *JAK1/2*, *MDM2* or *B2M* alterations, remain unclear. In addition, the potential for treatment to affect a patient's TMB status, and whether such a change in TMB over time has any clinical significance, is not yet known.

Finally, the role of TMB should be considered in combination therapy trials, including combinations of ICPIs as well as ICPIs with conventional therapies. As noted above, the first prospective clinical validation of a TMB cutoff was with nivolumab plus ipilimumab, which are PD-1 and CTLA-4 inhibitors, respectively [54]. Furthermore, a benefit from chemotherapy-based approaches was seen in patients with TMB >8 in the phase III SWOG 80405 trial [108]. Although there is limited research evaluating TMB as a predictive biomarker for response to non-ICI treatment such as chemotherapy, this represents an area of future investigation [54, 61]. Utilizing TMB and other biomarkers to select patients for specific ICPI-based combinations could be critical in subsets of patients, and the effect of such combinations on the TMB threshold for clinical benefit should be examined.

CONCLUSION

Here we provide an overview of the current evidence for TMB as a clinically relevant predictive biomarker of ICPI outcomes in several tumor types. Targeted NGS assays have been validated against WES for accurate TMB measurement. Current research to establish appropriate TMB cutoffs is ongoing, and these cutoffs are likely to be ICPI- and tumor type-specific. The standardization of TMB and clinical studies of its use in varying disease states and drug regimens are expected to result in the approval of TMB as a companion diagnostic for ICPIs.

Collectively, current and future trials utilizing CGP to inform enrollment will further elucidate the value of TMB as a biomarker alone and in context with other biomarkers and genomic data. With ongoing study, TMB alongside other genomic biomarkers can direct appropriate patients to ICPI or other targeted therapies at earlier lines of treatment and potentially identify those likely to continue to have durable responses after short-term treatment, while simultaneously sparing those unlikely to benefit. Overall, the measurement of and

appropriate use of TMB has the potential to add substantially to both the clinical and economic value of ICPI agents in oncology.

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