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REVIEW

Tumor mutational burden for the prediction of PD-(L)1 blockade efficacy in cancer: challenges and opportunities

X. Wang[1], G. Lamberti[2], A. Di Federico[2], J. Alessi[2], R. Ferrara[3,4], M. L. Sholl[5], M. M. Awad[2], N. Vokes[6] & B. Ricciuti[2]*

[1]Harvard T.H. Chan School of Public Health, Boston; [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA; [3]Medical Oncology, University Vita-Salute San Raffaele, Milan; [4]Department of Medical Oncology, IRCCS San Raffaele, Milan, Italy; [5]Department of Pathology, Brigham and Women's Hospital, Boston; [6]Department of Thoracic Head and Neck Medical Oncology, MD Anderson Cancer Center, Houston, USA

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Tumor mutational burden (TMB) is a biomarker that measures the number of somatic mutations in a tumor's genome. TMB has emerged as a predictor of response to immune checkpoint inhibitors (ICIs) in various cancer types, and several studies have shown that patients with high TMB have better outcomes when treated with programmed death-ligand 1-based therapies. Recently, the Food and Drug Administration has approved TMB as a companion diagnostic for the use of pembrolizumab in solid tumors. However, despite its potential, the use of TMB as a biomarker for immunotherapy efficacy is limited by several factors. Here we review the limitations of TMB in predicting immunotherapy outcomes in patients with cancer and discuss potential strategies to optimize its use in the clinic.

Key words: TMB, PD-(L)1 blockade, neoantigens, biomarkers

INTRODUCTION

Immune checkpoint inhibitors (ICIs) that block programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), and/or anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have transformed the treatment landscape of multiple cancer types.[1-3] However, while ICIs can produce long-lasting responses in patients with advanced cancers, a significant fraction of patients do not respond to these therapies, and among responders acquired resistance frequently develops.[1,2] Therefore, there is an urgent need to develop clinically available biomarkers that identify those patients who derive clinically meaningful benefits from immune checkpoint inhibition.

Tumor mutational burden (TMB) is generally defined as the number of somatic mutations per coding area of the cancer genome analyzed.[4] TMB can be estimated using different methods and technologies, including targeted next-generation sequencing (NGS) assays, as well as whole genome (WGS) and whole exome sequencing (WES). While exome-wide sequencing methods of tumor DNA and matched normal DNA are considered optimal for assessing TMB, large, targeted panels targeting $\geq$0.8 Mb are increasingly being utilized to determine the somatic mutational load in the clinical setting, while accuracy significantly decreases when sequencing $<$0.5 Mb.[5,6]

Initial studies showed an association between mismatch repair deficiency or DNA polymerase deficiency and high tumor mutational load and increased sensitivity to immune checkpoint blockade.[4,7-9] Since then, extensive evidence has been produced suggesting that tumors with elevated TMB may be more likely to respond to immunotherapies.[4,10,11] A high TMB may result in the generation of cancer-specific neoantigens which can be processed and loaded on to major histocompatibility complexes (MHCs), recognized by the immune system, and elicit T-cell-mediated antitumor immune responses.[12,13] Initial studies reported that those tumors with the highest level of TMB, such as non-small-cell lung cancer (NSCLC), small-cell lung cancer (SCLC), and melanoma, derive the greatest benefit from PD-(L)1 blockade.[4,14-16] Nonetheless, other tumor types with low TMB such as renal cell carcinoma (RCC) display exquisite sensitivity to immunotherapies,[17] highlighting that TMB status alone is not sufficient to accurately predict ICI efficacy across tumor types.

Since the initial evidence that increased TMB levels were associated with improved benefit from immunotherapy in retrospective series, *post hoc* analyses from several clinical trials have tried to address this question, with inconsistent results.[4,10,11,18-20] More recently, the multi-cohort KEYNOTE-158 study showed a response rate of ~30% in patients with solid tumors and high TMB, defined as $\geq$10 mutations/megabase (mut/Mb) by F1CDx, and in only 6% of

*Correspondence to: Dr Biagio Ricciuti, Dana-Farber Cancer Institute, Harvard Medical School, Boston, 450 Brookline Avenue – DANA1230N, 02215 Boston, MA, USA. Tel.: +1-617-6326628
E-mail: biagio_ricciuti@dfci.harvard.edu (B. Ricciuti).

patients with low TMB (<10 mut/Mb).[18] While pembrolizumab monotherapy received regulatory approval for all cancers with high TMB (≥10 mut/Mb), the KEYNOTE-158 study did not fully address many of the questions surrounding the application of TMB as a universal biomarker, including whether PD-1 monotherapy is active across cancer types; Therefore, the use of TMB to optimize patient selection for immunotherapies has not been robustly implemented in clinical practice.

Here, we review the current evidence on the use of TMB for the prediction of clinical outcomes to PD-(L)1 blockade in patients with cancer, discuss the pitfalls of this biomarker, and describe potential strategies to improve its utility in the clinical setting to optimize patient selection for ICI and inform the design of biomarker-based clinical and translational studies.

TMB AS A PREDICTOR OF PD-(L)1 BLOCKADE EFFICACY IN PATIENTS WITH CANCER

Initial evidence for the association between increased TMB and ICI benefit derives from retrospective studies of whole exomes from patients with NSCLC and melanoma, in which higher TMB levels were found to correlate with significantly improved response rates and survival with PD-(L)1 blockade.[4,21] Since then, several studies including prospective clinical trials have interrogated TMB as a biomarker of response to immunotherapy in cancer. In NSCLC, an exploratory analysis of the CheckMate 026 comparing nivolumab monotherapy versus platinum-based chemotherapy as first-line therapy for patients with PD-L1 positive (≥5%) NSCLCs identified that patients with high TMB (>243 mut/exome) had the highest objective response rate (ORR), and the longest progression-free survival (PFS) with nivolumab.[22] Similarly, in the CheckMate 227 phase III trial which prospectively examined the efficacy of nivolumab plus ipilimumab versus chemotherapy in patients with high TMB (≥10 mut/Mb) as assessed by FoundationOne CDx, the combination of nivolumab and ipilimumab significantly improved PFS compared to standard chemotherapy in patients with elevated TMB,[20] although no overall survival (OS) benefit was reported in this study. An exploratory analysis of the IMpower 110 study showed longer OS in patients with PD-L1-positive NSCLC who had a blood-based TMB (bTMB) ≥14.5 mut/Mb (≥16 TMB score) receiving first-line atezolizumab versus chemotherapy, while there was no difference between treatment arms among patients with a bTMB <14.5 mut/Mb (<16 TMB score).[23] Additional non-randomized, phase II studies have shown that atezolizumab either alone or in combination with bevacizumab is effective in patients with high TMB [bTMB ≥16 mut/Mb, tissue-based TMB (tTMB) ≥10 mut/Mb], confirming the potential role of TMB in predicting outcomes to PD-(L)1 blockade in NSCLC.[24] However, a phase III study of atezolizumab versus chemotherapy in advanced or metastatic NSCLC with high bTMB did not meet its primary endpoint of PFS, highlighting the limitations of using bTMB to predict immunotherapy efficacy.[25] Similarly, in patients with SCLC, a high TMB in the top tertile (≥248 mutations/exome) was associated with the highest ORR and the longest PFS and OS with nivolumab monotherapy and nivolumab plus ipilimumab in the phase II CheckMate 032 trial.[26] Importantly, a Cochrane meta-analysis of 15 NSCLC clinical trials has also confirmed improved PFS and OS with PD-(L)1-based therapies versus chemotherapy in TMB-high NSCLC.[27] Lastly, a high TMB above the 50th percentile (>9.68 mut/Mb) as assessed by targeted NGS was also confirmed to be a predictor of improved response and survival in patients with SCLC receiving second- or subsequent-line PD-1 inhibition ± CTLA-4 blockade.[16]

In melanoma, the phase III IMspire150 study demonstrated significant PFS benefit with atezolizumab, vemurafenib, and cobimetinib versus placebo, vemurafenib, and cobimetinib in patients with *BRAF*^{V600} mutation and high TMB (≥10 mut/Mb) but not in tumors with low TMB (<10 mut/Mb).[28] Similarly, among patients enrolled in the CheckMate 067 study receiving nivolumab alone or in combination with ipilimumab or ipilimumab alone, an elevated TMB (≥50th percentile) was associated with significantly improved clinical outcomes in each of the three arms.[15] Consistent with these results, in a phase I study of atezolizumab in patients with metastatic melanoma, a TMB ≥16 mut/Mb was associated with higher ORR, and longer PFS and OS compared to a TMB <16 mut/Mb.[29]

Growing evidence indicates that TMB is predictive of immunotherapy benefit in other tumors types, including urothelial cancers,[30-32] head and neck carcinomas,[33] and gastrointestinal cancers, including microsatellite-stable tumors.[34-36] Based on these data, Marabelle and colleagues recently explored the association of high tTMB (≥10 mut/Mb by F1CDx) with outcomes in 10 tumor-type-specific cohorts from the phase II KEYNOTE-158 study, which assessed the activity of pembrolizumab monotherapy in patients with selected, previously treated, advanced solid tumors.[18] In this study, 790 patients had evaluable TMB data, and only a fraction ($N = 102$, 13%) were defined as TMB-high. As expected, the most common tumor types that exhibited high TMB levels included SCLC (33%) and squamous carcinomas of the uterine cervix (16%) and anus (14%). The ORR and PFS were superior in patients with TMB-high versus TMB-low tumors (ORR: 29% versus 6%; PFS at 12 months: 26% versus 13%). Based on these data, the Food and Drug Administration (FDA) approved pembrolizumab monotherapy for the treatment of patients with solid tumors that were TMB-high and had progressed following prior treatment and lacked alternative treatment options. While the KEYNOTE-158 contributed to the tumor-agnostic approval of pembrolizumab for patients with cancers harboring ≥10 mut/Mb, this treatment was not effective across all tumor types, suggesting that a single TMB threshold does not necessarily identify responders across all cancer types. This limitation has also been confirmed in a recent pan-cancer study, in which TMB was associated with improved outcomes with ICI only in those tumor types where CD8 T-cell levels positively correlated with neoantigen load, but not in cancer types that showed no relationship between CD8 T-cell levels and neoantigen

load.[37] In addition, highly prevalent tumors with known association with elevated TMB, such as NSCLC and melanoma, were not included in the KEYNOTE-158 study, further limiting the external validity of these results. Therefore, the incorporation of TMB in routine clinical practice remains elusive. A summary of selected clinical studies evaluating the impact of TMB on clinical outcomes of immunotherapies across cancer types is reported in Table 1.

LIMITATIONS OF TMB FOR THE PREDICTION OF IMMUNOTHERAPY EFFICACY IN CANCER

Technical limitations

Since the initial identification of TMB as a potential biomarker of response to PD-(L)1 blockade, several technical aspects have limited its use for patient selection in routine clinical practice (Figure 1). TMB was initially calculated as the number of somatic mutations identified in WES data derived from paired tumor-normal samples, reflecting mutations that might generate expressed neoantigens.[4] However, because of the complexity and high cost of WES, NGS has been increasingly used as a cost-effective substitute for WES for TMB assessment based on prespecified cancer-related gene sets. Examples of these commercial panels include F1CDx[6] (324 genes, 1.1 Mb of genome covered), Caris SureSelect XT (592 genes, 1.6 Mb), TEMPUS (648 genes, 2.4 Mb), and Guardant OMNI (550 genes, 2.2 Mb). Other institutional validated platforms such as the Memorial Sloan Kettering (MSK)-IMPACT[5] (468 genes, 1.2 Mb) and the Dana-Farber Cancer Institute (DFCI)-OncoPanel[38] (447 genes, 1.3 Mb) have been implemented to provide TMB estimates. However, despite the advantages of NGS platforms for assessing TMB for clinical use, the definition of TMB varies significantly across experimental and bioinformatic methodologies used (Table 2). For instance, in addition to nonsynonymous somatic mutations and small insertions/deletions (InDels), F1CDx also includes synonymous mutations and short InDels in intronic regions into TMB estimation, which are commonly excluded from other TMB estimation algorithms. However, evidence has shown that InDels can lead to new open reading frames and potentially more immunogenic neoantigens. This suggests that interrogating intronic regions and including InDels may be relevant for a correct estimation of TMB.[39] In addition, F1CDx does not carry out paired germline sequencing which may result in overestimation of the mutational load due to limited filtering of germline variants. By contrast, another FDA-approved assay, MSK-IMPACT, identifies somatic-only exonic mutations from ~500 cancer-related genes, by filtering out germline variants using matched blood sequencing. This highlights the heterogeneity of sequencing platform pipelines, which makes TMB estimation not easily reproducible across assays.

More recently, the development of bTMB based on circulating tumor DNA (ctDNA) shed by tumor cells has been proposed to be complementary to tTMB for the determination of TMB, with some arguing that bTMB may better capture tumor heterogeneity from ctDNA shed by different metastatic sites. Retrospective studies and clinical trials have provided compelling evidence that high bTMB may be associated with improved clinical response to ICIs.[19,24,25,40-42] However, variable degrees of correlation between bTMB and tTMB have been reported (Spearman's rho ranging from 0.20 to 0.93), suggesting an imperfect concordance between bTMB and tTMB, which can have significant implications for treatment selection and patient outcomes with ICIs[43,44] (Figure 2). In addition, the presence of ctDNA has been reported to be a poor prognostic factor across cancers,[45] which can further confound the predictive value of bTMB in patients receiving immunotherapies.

Regardless of whether TMB is estimated using blood or tissue samples, an important technical challenge that further limits the implementation of TMB for routine clinical use is the absence of robust standardization measures across platforms. Some efforts have been made to harmonize TMB through standardization to Z-scores and calibrations to ensure proper reproducibility.[46] Data from a multi-cohort study of patients with lung cancer suggested that harmonization of targeted panels and WES is possible through normal transformation followed by standardization to Z-scores, enabling integration of different datasets derived from different sequencing panels.[46] Another collaborative effort led by the 'Friends of Cancer TMB consortium' utilized a calibration tool based on a universal reference standard derived from The Cancer Genome Atlas data to enhance the harmonization of TMB distributions across 16 different panel assays, providing additional evidence that TMB alignment across different assays is possible and can be implemented to guide decision making in clinical practice.[47]

Spatial and temporal heterogeneity of TMB

In addition to technical limitations, there are also biological factors that impact the use of TMB as a biomarker for clinical ICI efficacy. One of the most relevant biological challenges is the spatial and temporal heterogeneity of TMB (Figure 1). In lung adenocarcinomas, the presence of subclonal events, including somatic mutations and copy number alterations, has been recently shown to correlate with high intratumoral spatial heterogeneity and heterogenous TMB values in different regions of the same sample, with significant spatial TMB variance in 30% of cases and a maximum absolute TMB difference of 14 mut/Mb.[48] In addition, TMB assessed in metastatic sites has been reported to be lower compared to primary lung tumors, possibly due to either a reduced cellular content or the oligoclonality of distant metastases,[48,49] which can impact the predictive power of TMB when this is assessed in samples from different sites.

In addition to spatial heterogeneity, changes in TMB over time due to the acquisition or loss of mutations can create a dimension of temporal heterogeneity in TMB estimates.[50,51] Although there are limited data on TMB variations over time, a recent study has identified a significant decrease in TMB after PD-(L)1 blockade, potentially as the result of clearance of responsive clones after immune checkpoint inhibition.[50] However, evidence is inconsistent, with some

Table 1. Summary of retrospective/observational and prospective studies that analyzed the impact of tumor mutational burden on immunotherapy efficacy

Refs	Setting	ICI	*N*	TMB cut-off	ORR	PFS	OS
Retrospective/observational							
[4]	Advanced NSCLC pretreated	Pembrolizumab	34	200 mut by WES	59% versus 12%; $P = 0.01$	NR versus 3.4 months; $P = 0.0004$	NA
[21]	Advanced melanoma	Ipilimumab, tremelimumab	25	100 mut by WES	NA	TMB higher in PFS >6 months; $P = 0.01$	Longer in TMB-high; $P = 0.04$
			39		NA	TMB higher in PFS >6 months; $P = 0.009$	No difference; $P = 0.10$
[16]	Pretreated SCLC	PD-1 ± CTLA-4 inhibitor	52	9.68 mut/Mb NGS	23.1% versus 7.7%; $P = 0.25$	3.3 versus 1.2 months; $P < 0.01$	10.4 versus 2.5 months; $P = 0.01$
[41]	Advanced NSCLC	PD-(L)1 inhibitors	50	6 mut/Mb by NGS	39.3% versus 9.1%; $P = 0.02$	NR versus 2.9 months; $P = 0.01$	NA
[43]	Advanced solid tumors	PD-(L)1 and CTLA-4 inhibitors	3775	10 mut/Mb by NGS	NA	7.6 versus 5.3 months[a]	NA
[43]	Advanced solid tumors	PD-(L)1 and CTLA-4 inhibitors	852	10 mut/Mb by NGS (blood)	NA	5.6 versus 5.1 months[a]	NA
[46]	Advanced NSCLC	PD-1 ± CTLA-4 inhibitor	499	z-score by NGS	Median TMB z-score: 0.47 (R) versus 0.14 (NR); $P = 0.002$	NA	NA
[58]	MMR-d CRC and esophageal cancer	PD-1 ± CTLA-4 inhibitor	33	Median by NGS	NA	NA	HR: 0.51; $P = 0.46$
[60]	MMR-d CRC	PD-1 inhibitor	22	>40 mut/Mb by NGS	Median TMB: 54 (R) versus 29 (NR) mut/Mb; $P < 0.001$	NR versus 2 months; $P < 0.001$	NR versus NR; $P = 0.003$
[61]	Advanced NSCLC	PD-(L)1 inhibitor	266	Continuous TMB by NGS	NA	HR: 0.96; $P < 0.001$	HR: 0.97; $P = 0.01$
[62]	Advanced urothelial carcinoma	PD-(L)1 inhibitor	60	Continuous TMB by NGS	NA	HR: 0.95; $P = 0.029$	HR: 0.95; $P = 0.052$
[65]	Non-melanoma advanced solid tumors	ICI	99	20 mut/Mb by NGS	13% versus 45%; $P = 0.0027$	NA	NA
[77]	Advanced NSCLC	PD-(L)1 inhibitor	309	WES	Median TMB: 14.0 (R) versus 7.4 (NR) mut/Mb; $P < 0.001$	NA	NA
[77]	Solid tumors ≥5% ARID1A mut	PD-(L)1 inhibitor	375	≥20 mut/Mb by NGS	NA	13.6 versus 3.7 months; $P < 0.001$	NA
[86]	Advanced NSCLC	PD-(L)1 inhibitor	198	272 mut (25th percentile by WES)	Median TMB 194 (R) versus 131 (NR) mut/Mb; $P = 0.0069$	HR: 0.67; $P = 0.043$	HR: 0.62; $P = 0.049$
	Advanced NSCLC with HLA-LOH		47	HLA-corrected TMB by WES	59.4% versus 20.0%; $P = 0.0363$	HR: 0.34; $P = 0.007$	HR: 0.29; $P = 0.004$
[117]	Melanoma, NSCLC, bladder, CRC	ICI[b]	995	13 mut/Mb by NGS	NA	NA	HR: 0.61; $P < 0.0001$
	Esophageal, Head and neck, glioma, breast, RCC		667		NA	NA	HR: 0.69; $P = 0.042$
	CUP		80	12 mut/Mb by NGS	NA	NA	HR: 0.47; $P = 0.033$
[87]	Advanced NSCLC	PD-(L)1 inhibitor	1552	90th percentile (by NGS)	49.1% versus 21.5; $P < 0.001$	11.4 versus 2.8 months; $P < 0.001$	36.1 versus 12.4 months; $P < 0.001$
[89]	Advanced untreated NSCLC	PD-(L)1 inhibitor	2165	<10 versus ≥20 mut/Mb by NGS	NA	17.2 versus 6.5 months; $P < 0.001$	26.9 versus 10.1 months; $P < 0.001$
[91]	Advanced solid tumors	ICI	151	20 mut/Mb by NGS	58% versus 20%; $P < 0.001$	12.8 versus 3.3 months; $P < 0.001$	NR versus 16.3 months; $P = 0.0036$
Prospective/*post hoc*							
[22]	Untreated NSCLC	Nivolumab	158	<100, 100-242, >242 mut (tertiles by WES)	47% versus 23%	9.7 versus 3.6 versus 4.2 months	18.3 (TMB-high) versus 12.7 months (TMB intermediate and low)
[20]	Untreated NSCLC	Nivolumab + ipilimumab	330	10 mut/Mb (by NGS)	45.3% (TMB-high)	7.2 versus 3.2 months	NA
[23]	Untreated NSCLC PD-L1 ≥1%	Atezolizumab	389	16 mut/1.1 Mb (blood-based by NGS)	NA	6.8 versus 4.5 months	13.9 versus 12.9 months
[24]	ICI-naïve NSCLC	Atezolizumab	119	16 mut/1.1 Mb (blood-based by NGS)	35.7% versus 5.5%; $P < 0.0001$	5.0 versus 3.5 months; $P = 0.35$	23.9 versus 13.4 months; $P = 0.18$

Continued

Table 1. Continued

Refs	Setting	ICI	*N*	TMB cut-off	ORR	PFS	OS
[26]	Pretreated SCLC	Nivolumab Nivolumab + ipilimumab	133 78	<143, 143-247, >247 mut (tertiles by WES)	21.3% versus 6.8% versus 4.8% 46.2% versus 16.0% versus 22.2%	1.4 versus 1.3 versus 1.3 months 7.8 versus 1.3 versus 1.5 months	5.4 versus 3.9 versus 3.1 months 22.0 versus 3.6 versus 3.4 months
[28]	BRAF V600-mutant melanoma	Atezolizumab (+ vemurafenib + cobimetinib)	215	10 mut/Mb by NGS	NA	16.6 versus 11.1 months	NA
[15]	Untreated melanoma	Ipilimumab Nivolumab Nivolumab + ipilimumab	178 176 184	203.5 mut by WES	25.5% versus 14.3% 62.1% versus 31.5% 64.8% versus 51.0%	HR: 0.60 HR: 0.45 HR: 0.55	HR: 0.52 HR: 0.46 HR: 0.53
[15]	Untreated melanoma	Nivolumab	52	157 mut by WES	60.9% versus 27.6%	HR 0.34	HR 0.69
[29]	Advanced melanoma	Atezolizumab	23	16 mut/Mb by NGS	50.0% versus 0.0%	20 versus 1 months; $P = 0.002$	NR versus 7 months; $P = 0.0009$
[30]	Urothelial carcinoma	Neoadjuvant pembrolizumab	114	Continuous, by NGS	OR: 1.06; $P = 0.03$	NA	NA
[31]	Pretreated urothelial carcinoma	Nivolumab	139	<85 versus 85-169 versus >169 mut (tertiles by WES)	31.9% versus 19.6% versus 13.0%; OR: 2.13; $P < 0.05$	3.5 versus 1.8 versus 1.9 months; HR: 0.75; $P < 0.05$	11.6 versus 9.7 versus 5.7 months; HR: 0.73; $P < 0.05$
[32]	Pretreated urothelial carcinoma	Atezolizumab	150	Continuous TMB by NGS	R: 12.4 versus NR: 6.4 mut/Mb; $P < 0.001$	NA	NA
[34]	MMR-d tumors; MMR-p CRC	Pembrolizumab	15	Continuous TMB by WES	OR: 3.31; $P = 0.214$	HR: 0.63; $P = 0.021$	HR: 0.71; $P = 0.077$
[35]	Pretreated gastric cancer	Toripalimab	54	12 mut/Mb WES	3.3% versus 7.1%; $P = 0.017$	2.5 versus 1.9 months; $P = 0.055$	14.6 versus 4.0 months; $P = 0.038$
[18]	Advanced solid tumors	Pembrolizumab	790	10 mut/Mb by NGS	29% versus 6%	2.1 versus 2.1 months	11.7 versus 12.8 months
[101]	Advanced NSCLC	Pembrolizumab	164	175 mut by WES	23.5% versus 16.9%; $P = 0.009$	4.2 versus 3.7 months; $P = 0.001$	14.1 versus 9.3 months; $P = 0.006$
[101]	Advanced NSCLC	Pembrolizumab	423	175 mut by WES	34.4% versus 18.8%; $P < 0.001$	6.3 versus 4.1 months; $P < 0.001$	21.9 versus 12.0 months; $P < 0.001$
[113]	PD-L1-positive advanced solid tumors	Pembrolizumab	77	Continuous by WES	R > NR; $P = 0.018$	$P = 0.051$	NA
[114]	Advanced solid tumors	Pembrolizumab	119	102.5 mut by WES	R > NR; $P < 0.001$	3.8 versus 1.9 months	NA

Only studies with at least one available outcome comparison between the TMB-high and TMB-low group or arm (by any study-specific definition) were included. In the ORR, PFS, and OS columns, comparison is always reported as 'outcome in the TMB-high group' versus 'outcome in the TMB-low group'.

CRC, colorectal cancer; CTLA, cytotoxic T-lymphocyte-associated protein 4; CUP, carcinoma of unknown primary; HLA LOH, HLA loss of heterozygosity; HR, hazard ratio; ICI, immune checkpoint inhibitors; Mb, megabase; MMR-d/p, mismatch repair deficient/proficient; Mut, mutations; NA, not available/not reported; NGS, next-generation sequencing; NR, non-responders; NR, not reached; NSCLC, non-small-cell lung cancer; OR, odds ratio; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; R, responders; RCC, renal cell carcinoma; SCLC, small-cell lung cancer; TMB, tumor mutational burden; WES, whole exome sequencing.

[a]Time to treatment failure.

[b]Include multiple checkpoint inhibitors ± immunomodulating agents.

studies showing significant changes in TMB estimates over time,[49,52] and others demonstrating that TMB is instead generally stable and not subject to significant temporal variations.[48] Because multiple variables can impact tumor genomics, such as cancer type, site of tumor sequencing, and type of treatment received before sequencing, it is also possible variations in TMB estimates over time may be dependent on other factors, rather than time intervals *per se*.[53]

Genomic and non-genomic factors that impact TMB as a biomarker

Targeted NGS panels generally survey mutations and copy number alterations of hotspot genomic regions of cancer-related genes. Therefore, the sole quantitative count of nonsynonymous mutations in the tumor genome might not necessarily capture all mutations that are relevant for a correct TMB estimation. For instance, *in silico* evidence suggests that TMB variability increases by ~10% when using 1-Mb panels interrogating oncogenes and tumor suppressor genes compared to panels of 1 Mb that instead query random genes.[54] Therefore, TMB can be artificially inflated by clinically validated NSG panels. In addition, evidence suggests that not all mutations are equally immunogenic and lead to the production of neoantigens. As an example, RCCs display high sensitivity to immune checkpoint blockade despite an overall low TMB. However, these cancers have higher rates of InDels which are 3-9 times more likely to generate neoantigens than point mutations.[40]

TMB can also be an indirect measure of different underlying mutational processes that can mediate tumor immunogenicity. Increased TMB has been reported in mismatch repair-deficient (dMMR)/microsatellite instability (MSI)-high (MSI-H) cancers,[55] with >80% of MSI-H cancers

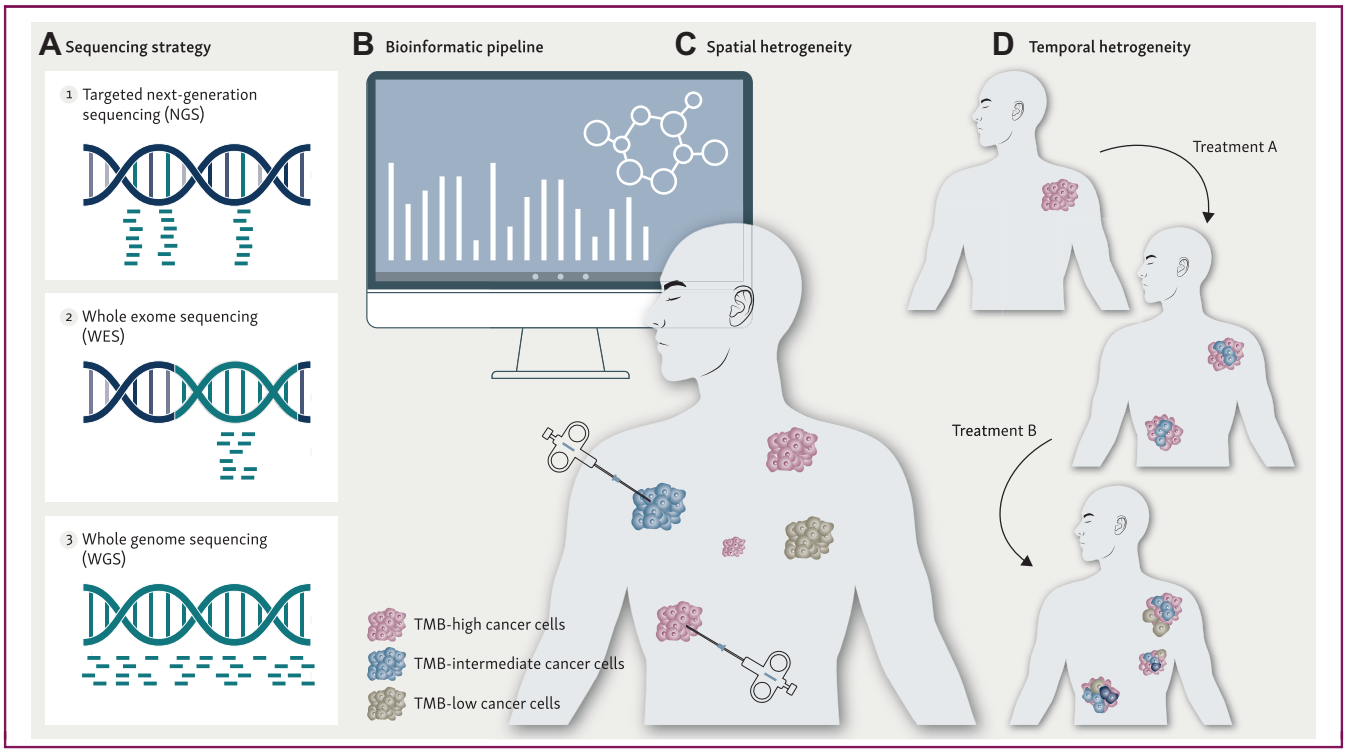

Figure 1. Technical and biological limitations to the assessment of TMB in clinical practice. Technical and biological limitations to the assessment of tumor mutational burden in clinical practice include differences in sequencing strategies (A), bioinformatic pipelines used (B), intratumoral and inter-lesional spatial heterogeneity (C), and temporal heterogeneity (D).
NGS, next-generation sequencing; TMB, tumor mutational burden; WES, whole exome sequencing; WGS, whole genome sequencing.

having $\geq$20 mut/Mb. Several studies have confirmed that dMMR and MSI-H tumors are very sensitive to immune checkpoint blockade.[56-58] Nonetheless, only a fraction of TMB-high tumors are MSI-H,[59] suggesting that while these measures are interconnected, TMB and MSI status may be two complementary biomarkers. In a small retrospective study of 22 patients with advanced MSI-high colorectal cancer, median TMB was significantly higher among responders to immunotherapies, compared to non-responders (54 versus 29 mut/Mb, $P < 0.001$) indicating that increasing TMB levels can further stratify outcomes among patients with MSI-H tumors.[60] In addition to dMMR, increasing evidence indicates that cancers with deleterious alterations in other DNA damage response and repair (DDR) genes have increased mutational load and may benefit more from PD-(L)1-based therapies.[61,62] Importantly, the impact of DDR pathogenic variants on immunotherapy efficacy has been shown to be independent of TMB levels,[61] suggesting that additional mechanisms, such as increased cGAS-STING and interferon-γ (IFN-γ) response activation,[63,64] may contribute to the increased tumor immunogenicity of DDR-deficient tumors.

Increased TMB is also correlated with specific mutational signatures that associate with improved ICI efficacy such as apolipoprotein B mRNA-editing cytidine-deaminase (APOBEC) signature, which is characterized by C>G and C>T substitutions, increased TMB, increased PD-L1 expression and IFN-γ,[65-68] and ultraviolet (UV) signatures (e.g. melanoma) which in *in silico* simulations have shown to produce highly hydrophobic neopeptides.[69]

Lastly, several cancer-specific genomic alterations that are associated with immunotherapy response and resistance can occur in TMB-high tumors. In NSCLC, loss-of-function mutations in *STK11*, *KEAP1*, and *SMARCA4* are highly prevalent (10%-20% of cases) and are associated with low response rates and poor survival on PD-(L)1 inhibition.[70-73] However, NSCLCs harboring these alterations develop more commonly in patients who had a history of smoking, and are associated with increased tumor mutational load, but decreased PD-L1 expression levels and decreased CD8+ T-cell count.[70,71,73,74] By contrast, tumors with pathogenic variants in *ARID1A*, *PBRM1* (clear cell renal carcinoma), *CDK12* (prostate cancer), or concurrent *ATM*/*TP53* loss (NSCLC) tend to have increased PD-L1 expression, high TMB levels, and better outcomes to PD-(L)1 blockade.[75-79] Together, these data suggest that the predictive accuracy of high TMB assessed by clinically validated targeted NGS assay may be limited in cancers harboring concurrent mutations that are associated with resistance to ICI regimens.

Among non-genomic factors that impact TMB as a biomarker for ICI response, MHC expression plays an important role in shaping the mutational landscape of several cancers and determining the sensitivity of these tumors to PD-(L)1 blockade.[80-82] For neoantigens derived from somatic mutations to induce robust antitumor T-cell responses, intact MHC expression is necessary for neoantigen recognition by T-cell receptors (TCRs). In this context, increased human leukocyte antigen (HLA diversity) (e.g. germline HLA heterozygosity, high HLA evolutionary

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Table 2. Summary of commercial and Institutional sequencing assays that quantify tumor mutational burden

Type of TMB	Biospecimen	Advantage	Disadvantage	Laboratory/ institution	Panel name	Number of genes	TMB region covered (Mb)	Total region covered (Mb)	Type of exonic mutations included in TMB estimation
Tumor-based TMB	Paired tumor and blood samples	• High accuracy • High clinical validity • Potential of refined TMB based on germline information	• Invasive • Less able to reflect tumor heterogeneity • Sample availability and quality	OrigiMed	CSYS assay	450	1.28	3.07	Nonsynonymous, synonymous
				Memorial Sloan Kettering Cancer Center	MSK-IMPACT	468	1.14	1.53	Nonsynonymous
				TEMPUS	TEMPUS Xt	648	2.40	2.40	Nonsynonymous
	Tumor sample only	• High accuracy • High clinical validity	• Invasive • Less able to reflect tumor heterogeneity • Sample availability and quality • Potential of misclassification for germline variants • Potential of inflated TMB in underrepresented populations	ACTGenomics	ACTOnco+	440	1.12	1.80	Nonsynonymous, synonymous
				Caris	SureSelect XT	592	1.40	1.60	Nonsynonymous
				Foundation Medicine	FoundationOne CDx	324	0.80	2.20	Nonsynonymous, synonymous
				Illumina	TSO500 (TruSight Oncology 500)	523	1.33	1.97	Nonsynonymous, synonymous
				NeoGenomics	NeoTYPE Discovery Profile for Solid Tumors	372	1.03	1.10	Nonsynonymous, synonymous
				Personal Genome Diagnostics	PGDx elio tissue complete	507	1.33	2.20	Nonsynonymous, synonymous
				QIAGEN	QIAseq TMB panel	486	1.33	1.33	Nonsynonymous
				Thermo Fisher Scientific	Oncomine Tumor Mutation Load Assay	409	1.20	1.70	Nonsynonymous
				Dana-Farber Cancer Institute	OncoPanel	447	1.30	1.94	Nonsynonymous
				John Hokpins University	JHOP2	432	—	1.14	Nonsynonymous, synonymous
Blood-based TMB	Blood sample only	• Less invasive • Potential for early detection and longitudinal progression monitoring • Reflective of tumor heterogeneity	• Low sensitivity • Low clinically validity	Guardant Health	GuardantOMNI	500	1.00	2.15	Nonsynonymous, synonymous
				Foundation Medicine	FoundationOne Liquid CDx	324	0.80	2.20	Nonsynonymous, synonymous
				Personal Genome Diagnostics	PGDx elio plasma complete	521	—	2.10	Nonsynonymous, synonymous

TMB, tumor mutational burden.

divergence) enhances the likelihood that a tumor's neoantigen will be efficiently presented to T cells.[81] Nonetheless, reversible (e.g. decreased MHC expression with intact euploid HLA loci) or irreversible (e.g. *B2M* loss-of-function mutations, HLA mono- and bi-allelic deletions) somatic defects leading to reduced MHC expression have been documented in 90% of cancers,[83-85] and ∼25%-30% of NSCLCs with high TMB have a loss of expression in HLA genes. Therefore, these patients whose tumors have high TMB or high neoantigen burden but lack MHC expression have a lower probability to respond to PD-(L)1 inhibition, while patients with intermediate TMB or low neoantigen burden, but intact MHC expression, may also respond, suggesting that HLA-adjusted TMB estimations may be necessary to refine the predictive accuracy of TMB.[86]

STRATEGIES TO IMPROVE THE ACCURACY OF TMB TO PREDICT RESPONSE TO PD-(L)1 BLOCKADE

Identification of optimal TMB thresholds to predict response to PD-(L)1 blockade

The identification of optimal TMB thresholds is another essential factor for using TMB as a biomarker for immunotherapy efficacy in the clinic. However, there is no consensus on whether a universal TMB cut-off that identifies responders to ICI across cancers exists. Initial studies defined TMB-high as TMB greater than the median, or in the upper tertile or quartile, and consistently showed benefit from ICIs among patients with increasing TMB levels.[4,14,22] In the phase II KEYNOTE-158 study, objective responses to pembrolizumab were observed in 29% of patients with high TMB (≥10 mut/Mb) and only 6% of patients with low TMB[18] across multiple cancer types. However, this trial did not demonstrate the consistent clinical activity of PD-1 monotherapy across all tumor types, nor did it validate that a TMB threshold of 10 was predictive in all histologies. Therefore, despite the ensuing FDA approval, it does not establish whether a threshold of 10 mut/Mb is appropriate for every patient with cancer. In fact, growing evidence suggests that TMB acts more as a continuous variable, with increasing levels correlating with an increased probability of benefiting from PD-(L)1 inhibition. A recent pan-cancer analysis of 1662 patients treated with ICI showed that a high TMB in the top 20% of each histology was independently associated with improved OS.[10] This study also noted that patients with a TMB in the

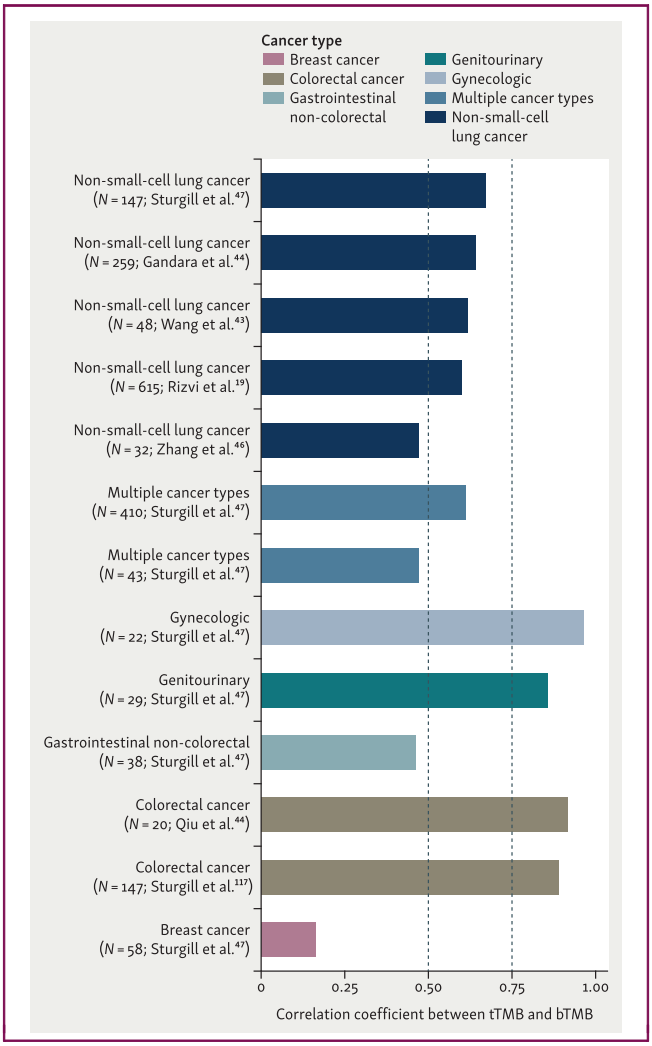

Figure 2. Histogram showing the correlation between tissue and blood tumor mutational burden by cancer type.
bTMB, blood-based tumor mutational burden; tTMB, tissue tumor mutational burden.

top decile within specific histology had even better clinical outcomes with ICI, suggesting that histology-specific TMB thresholds may inform more precise clinical decisions. Additionally, in this analysis, TMB was predictive of improved survival in only a fraction of cancer types, including bladder cancer, colorectal cancer, head and neck carcinoma, and NSCLC.[10] In a similar study conducted on 8356 patients (80% non-ICI-treated, 20% ICI-treated) with 17 microsatellite-stable cancer types, a TMB within the top 20th percentile was also confirmed to predict improved survival of ICI, but not with chemotherapy.[11] Again, improved clinical outcomes of ICI in patients with high TMB were only noted in a fraction of patients with NSCLC, and the impact of TMB on survival after ICI treatment was inconsistent across different tumor types. Therefore, available data seem to suggest cancer-specific, rather than cancer-agnostic, thresholds are likely necessary to accurately predict outcomes of ICI. In NSCLC, a large analysis from three independent cohorts has recently established a TMB threshold above the 90th percentile, corresponding to $\geq$19 mut/Mb by targeted NGS and to a harmonized TMB $\geq$16 mut/Mb by WES, as a robust and independent predictor of PFS and OS of ICI.[87] Of note, NSCLCs with a TMB greater than this cut-off had increased CD8+ T cells, increased MHC class II expression, and derived significant benefit from immunotherapy regardless of PD-L1 expression levels. This same threshold was recently found to predict improved outcomes to first-line chemo-immunotherapy in patients with advanced NSCLC, suggesting that a very high TMB can predict outcomes to PD-(L)1 blockade not only alone but also in combination with other treatment modalities.[88] Importantly, this TMB cut-off was validated by an independent analysis of 3127 patients with advanced NSCLC who received ICI and whose tumors were profiled with F1CDx.[89]

Although large-scale studies dedicated to establishing optimal cut-off of TMB in other tumor types are still lacking, evidence from pan-cancer analyses that used absolute TMB values rather than cancer-specific percentiles has shown that cancers with $\geq$20 mut/Mb may be more likely to respond to immune checkpoint therapies.[90,91] However, the interpretation of pan-tumor ICI studies is often limited by the heterogeneity of patients included, type of immunotherapy received, patient performance status, disease burden, and other important cancer-specific biomarkers of ICI response. Therefore, whether a universal TMB cut-off can be used to accurately predict ICI efficacy across cancer types remains unclear, and studies aimed at establishing optimal TMB levels that can robustly predict improved outcomes with immunotherapy in each cancer type are necessary.

Finally, it will be important to clarify the proposed role of TMB in clinical practice. If the goal of TMB is to identify ICI responders, the above data suggest that the application of a higher TMB threshold will better identify those patients. However, this comes at the cost of excluding progressively larger proportions of patients (80%-90% of the population), many of whom may still benefit from ICIs. Conversely, lower TMB thresholds are more inclusive, but there is less evidence that TMB is truly predictive of response at thresholds between 30% and 80%. One strategy going forward may be to use TMB not to exclude patients but to use it within the context of prospective clinical trials utilizing novel strategies to boost antitumor immunity in those patients with lower TMB that are less likely to achieve durable long-term responses with PD-(L)1 inhibitors alone.

Refining TMB estimation prioritizing immunogenic mutations

Although high TMB is associated with improved clinical outcomes to ICI, not every mutation quantified by TMB generates an immunogenic neoepitope, limiting its predictive accuracy. To improve the classic TMB, prioritization should be given to mutations with a higher likelihood for giving rise to neoantigens that T cells can recognize. Frameshift deletion and insertion that are more likely to generate large stretches of neopeptides may play a more important role compared to other types of mutations. Several efforts

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have been made to better quantify the burden of immunogenic mutations that are determined by patients' somatic mutations and HLA and TCR diversity. A lower level of revised TMB prioritizing truncating mutations (i.e. nonsense, frame-shift deletion, frame-shift insertion, and splice-site mutations) has been reported to associate with increased naive B cells, regulatory T cells, memory resting CD4 T cells, memory B cells, activated mast cells, and resting natural killer cells.[92] Harmonic-mean best rank (PHBR) score, measuring the ability of a patient's MHC-I to efficiently present mutated driver neoantigens, has been developed and a higher score was observed in patients with extended median PFS of ICI (PHBR score <0.5 versus ≥ 0.5; mPFS: 5.1 months versus 4.4 months, $P = 0.04$) with the potential ability to further stratify patients with TMB > 10 mut/Mb.[93] More recently, a new metric using the burden of HLA class II immunogenic mutations (IMMs) based on germline HLA class II alleles and missense mutations has been proposed. A higher burden of HLA class II IMMs was shown in melanomas and NSCLCs that responded to ICI. Higher HLA class II IMM burden was also significantly associated with prolonged survival of ICI ($P < 0.001$) in patients with NSCLC in the second study, especially in the context of low intratumoral heterogeneity.[94] An effective immune response is also influenced by T-cell recognition of the neoantigens presented on MHC. Increased CD8+ T-cell effector function and TCR diversity with enrichment of certain TCR clonotypes in the tumor have also been associated with antitumor effects and tumor suppression in murine melanoma models.[95] Consistently, a higher TCR clonality was associated with improved survival of ICI in patients with melanoma,[96] highlighting the importance of factoring these additional metrics in TMB estimate interpretation.

Integration of TMB with other biomarkers

Although TMB has emerged as an independent biomarker for immunotherapy response, integrating TMB estimates with other biomarkers can further stratify patients for clinical decision making and potentially overcome some of the inherent limitations of using TMB alone as an ICI biomarker. Several biomarkers have been studied in conjunction with TMB, including PD-L1 expression, tumor-infiltrating lymphocytes (TILs), RNA gene expression, aneuploidy levels, blood-derived neutrophil-to-lymphocyte ratio (dNLR), and HLA variations, among others (Figure 3).

In general, TMB and PD-L1 expression have a weak but positive association.[87,97-99] However, this correlation can vary significantly across tumor types and is stronger in cervical, endometrial, neuroendocrine, gastric, pancreatic, NSCLC, head and neck squamous cancers, and sarcomas.[98,99] Given that they are two potentially independent predictors of immunotherapy efficacy, it has been suggested that tumors with increased TMB and high PD-L1 expression levels may derive the greatest benefit from ICI. In the CheckMate 026 study, patients with advanced NSCLC and with high levels of both TMB ($\geq$243 somatic missense mutations/exome) and PD-L1 tumor proportion score (TPS) ($\geq$50%) had the highest probability of radiographic response to nivolumab, while those with low/medium TMB and low PD-L1 expression (1%-49%) had the lowest ORR.[22] Similar findings were reported by subsequent randomized clinical trials, including KEYNOTE-010, KEYNOTE-042, IMpower 110, CheckMate 227, and MYSTIC studies, which demonstrated improved outcomes of ICI in patients with high TMB and high PD-L1 expression levels.[19,23,100,101] However, the definition of high TMB varied across these studies and how to optimally integrate these two biomarkers for the prediction of immunotherapy efficacy in NSCLC is unclear. In an NSCLC multi-cohort study, a harmonized very high TMB ($\geq$19 mut/Mb by NGS, and $\geq$16 mut/Mb by WES) was independently associated with improved ORR, PFS, and OS in each clinically relevant PD-L1 expression subgroups of $<$1%, 1%-49%, and $\geq$50%.[87] However, patients with high TMB and high PD-L1 TPS had the highest ORR (56.5%) and the longest survival outcomes (median PFS: 18.1 months, median OS: 47.7 months) with ICI, again suggesting that combining these biomarkers can identify patients with the greatest likelihood of responding to ICI. Using cut-offs of TMB $\geq$20 mut/Mb and PD-L1 TPS $\geq$50%, another study independently validated this association and found a greater magnitude of difference in PFS (median 21.9 versus 6.5 months) and OS (median 32.4 months versus 10.4 months) between the double biomarker-positive cohort versus the double biomarker-negative cohort.[89]

In addition to PD-L1 expression, high levels of TMB are generally associated with a greater neoantigen load, higher CD8+ TILs, and therefore response to immunotherapy.[4,37,87,102,103] However, this association does not necessarily apply to all cancer types. A recent pan-cancer analysis showed that, among patients with cancer types where CD8+ T-cell levels positively correlated with neoantigen load, including melanoma, lung, and bladder cancers, a high TMB was associated with an ORR to ICI of 39.8% which was significantly higher than the ORR observed in patients with low TMB (OR: 4.1, $P < 0.0001$). By contrast, in cancer types that showed no relationship between CD8+ T-cell levels and neoantigen load, such as breast cancer, prostate cancer, and glioma, patients with high TMB tumors had lower ORR compared to those with low TMB tumors (OR: 0.46, $P = 0.02$). These data suggest that the predictive accuracy of TMB is in part derived from the presence of high basal immune cell infiltration levels, which is different across cancer types. Integrating TMB levels with tumor microenvironment parameters is therefore essential to refine TMB as a biomarker for patient selection for immunotherapy.

Emerging evidence highlight how germline and somatic HLA variations dictate antigen presentation and possibly cancer sensitivity to ICI. Therefore, integrating HLA status with TMB may be another strategy to refine our ability to predict ICI efficacy in patients with cancer. Somatic HLA-I LOH has been reported to have a nonlinear relationship with TMB, being most prevalent in tumors with intermediate

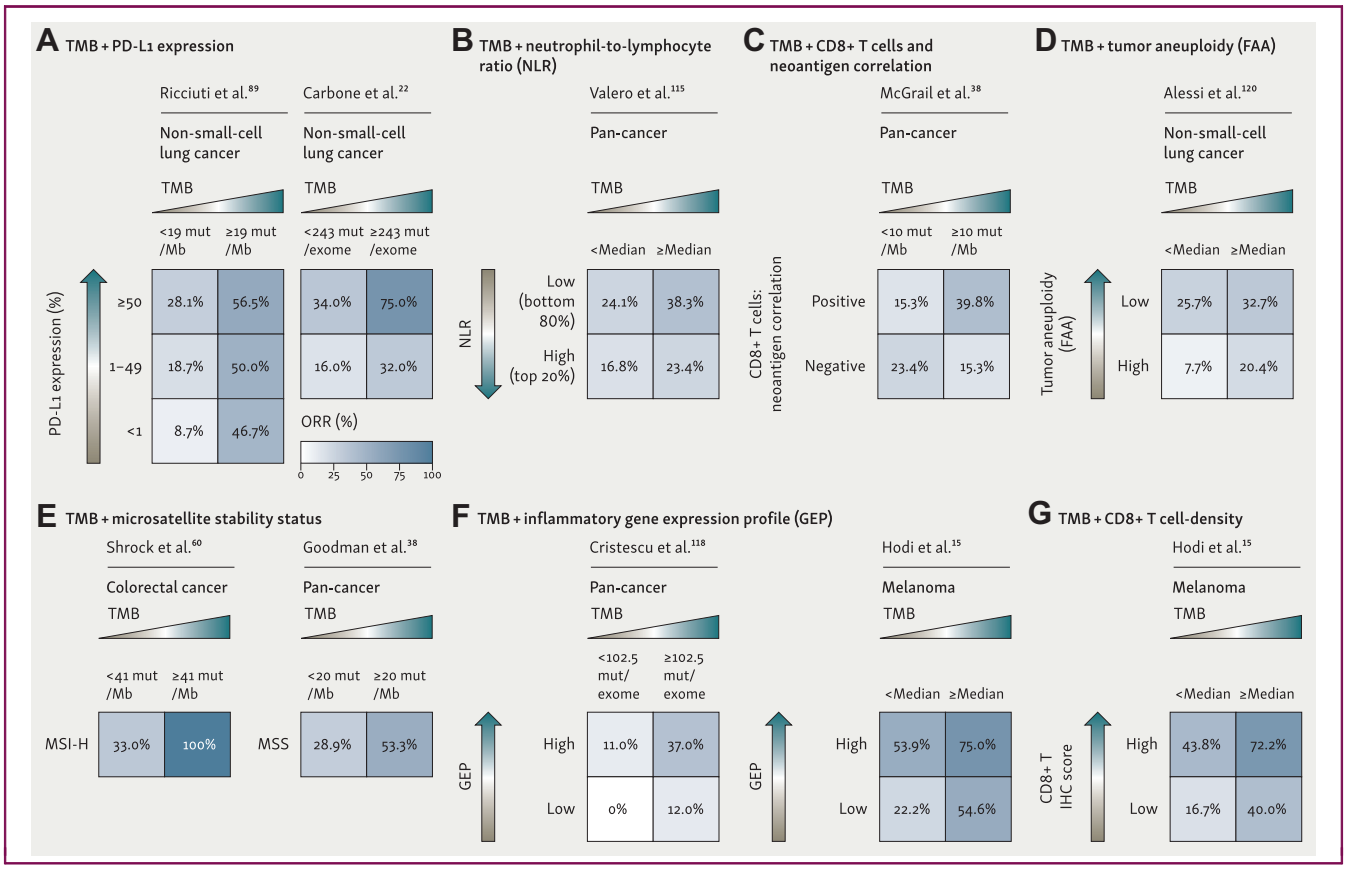

Figure 3. Integration of TMB and other biomarkers to predict response to PD-(L)1 blockade in cancer. Heatmap showing the objective response rate in patients with advanced cancers treated with PD-(L)1 blockade according to tumor mutational burden integrated with other biomarkers, including (A) PD-L1 expression, (B) dNLR, (C) CD8+ T cells/neoantigen correlation, (D) tumor aneuploidy, (E) MSI status, (F) GEP, and (G) CD8+ cell density.
CRC, colorectal cancer; dNLR, blood-derived neutrophil-to-lymphocyte ratio; FAA, fraction of chromosomal arm altered; GEP, gene expression profile; IHC, immunohistochemistry; MSI-H, microsatellite instability high; MSS, microsatellite stable; NSCLC, non-small-cell lung cancer; ORR, objective response rate; PD-L1, programmed cell death-ligand 1; TMB, tumor mutational burden.

levels of TMB but less so in highly mutated tumors (above 30 mut/Mb), suggesting alternative mechanisms of immune evasion in high TMB tumors.[104] In this study, HLA-I intact was associated with significantly prolonged OS [hazard ratio (HR) = 0.65, $P = 0.01$] in patients treated with ICI with non-squamous NSCLC, and outcomes prediction improved when HLA status was combined with TMB.[104] In addition to somatic HLA alterations, studies have explored the impact of germline HLA polymorphisms on ICI-based treatment, yielding varied results. HLA-A*03 has been suggested to predict reduced OS in an additive manner (HR = 1.48 per HLA-A*03 allele, $P < 0.001$) in patients treated with various ICI agents (anti-PD-1, anti-PD-L1, and anti-CTLA-4 inhibitors).[105] HLA-B44 supertype has been shown to associate with improved OS with ICI whereas the HLA-B62 supertype or somatic loss of heterozygosity at HLA-I was associated with poor outcomes to immunotherapy.[106] A recent study also indicates that a higher germline HLA class I evolutionary divergence (HED) is significantly associated with improved ORR (HED^{high} versus HED^{low} 81.8% versus 53.2%, $P = 0.03$), PFS (HR = 0.47, $P = 0.02$), and OS (HR = 0.39, $P = 0.02$) in patients receiving first-line PD-1 blockade plus chemotherapy in the CameL trial independent of TMB and PD-L1 TPS.[107] Moreover, previous work has suggested that patients with both high TMB and high HED are most likely to benefit from ICIs, and both TMB and HED should be considered to optimize patient selection for ICI.[81] Nonetheless, a recent meta-analysis of 17 clinical trials of pembrolizumab across eight different tumor types and one basket trial in over 3500 patients revealed that germline HLA landscape alone is not a significant and independent determinant of pembrolizumab efficacy.[108] All of these highlight the complexity and importance of HLA variations in determining effective immune response, and the need for a better understating of HLA contribution to these responses.

dNLR has been proposed as a clinically available and inexpensive biomarker of ICI efficacy, as it may reflect the relationship between cancer pro-inflammatory status and host immune response.[109-111] A recent study showed that combining TMB and dNLR enhances the predictive accuracy of each of these factors when used alone. In this study, patients were assigned to four categories based on dNLR [low (bottom 80^{th} percentile) versus high (top 20th percentile)] and TMB [low (<median) versus high (≥median)]. Patients with high dNLR and low TMB had the poorest clinical outcomes of ICI, while patients with low dNLR and high TMB had the highest ORR, and the longest survival with immunotherapy, suggesting that a combined dNLR/TMB score can be a robust biomarker of benefit from PD-(L)1-based therapies across cancers.[112]

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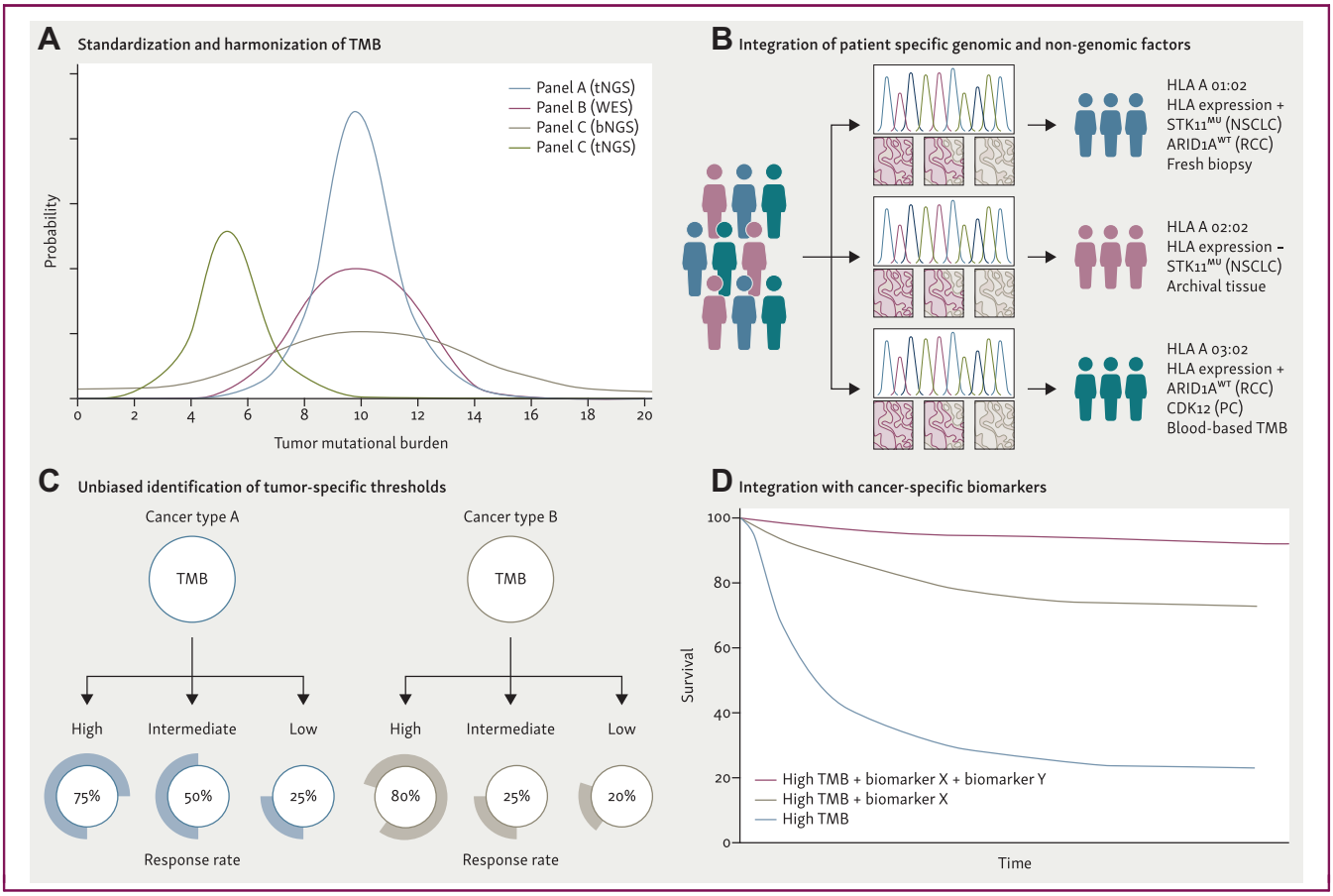

Figure 4. Schematic representation of potential strategies to improve the accuracy of tumor mutational burden to predict response to PD-(L)1 blockade. (A) Standardization and harmonization of TMB across platforms. (B) Factoring of patients or cancer-specific factors to interpret TMB estimates. (C) Identification of cancer-specific thresholds to categorize TMB-high and TMB-low tumors. (D) Integration of TMB with other biomarkers of immunotherapy response.
bNGS, blood next-generation sequencing; HLA, human leukocyte antigens; NSCLC, non-small-cell lung cancer; PC, prostate cancer; PD-L1, programmed death-ligand 1; RCC, renal cell carcinoma; TMB, tumor mutational burden; tNGS, tissue next-generation sequencing; WES, whole exome sequencing.

MSI is also a known a predictor of response to immunotherapy across cancer types.[9,34] Tumors with MSI-H have a deficient DNA repair machinery that makes them more prone to accumulate somatic mutations, resulting in significantly high TMB levels. However, as discussed in the preceding text, even among MSI-H tumors, increasing TMB levels can discriminate patients with a higher likelihood of responding to and benefiting longer from ICIs,[60] suggesting a role for combining TMB and MSI status for an accurate prediction of immunotherapy efficacy in MSI-H cancers.

Gene expression signature (GEP) and tumor-associated immune cells are also emerging as potential predictors of ICI efficacy in cancer. A retrospective analysis of the CheckMate 066 and 067 studies of nivolumab, nivolumab plus ipilimumab, and ipilimumab alone in patients with stage III (unresectable) or stage IV melanoma has shown that patients with high TMB and high expression of a four-gene inflammatory signature (*CD274*, *CD8A*, *LAG3*, and *STAT1*) had the highest ORR with nivolumab (75.0%) and nivolumab plus ipilimumab (66.7%) versus ipilimumab (27.6%), while the lowest ORR was observed in patients whose tumors were low for both TMB and the four-gene inflammatory signature score.[15] In this same analysis, the authors also noted greater ORR in patients with high TMB and high CD8+ T-cell expression treated with nivolumab and nivolumab plus ipilimumab (72.2% and 69.6%) versus ipilimumab alone (23.3%). Similarly, a biomarker analysis from different cohorts of patients with advanced solid tumors treated with pembrolizumab monotherapy across 22 tumor types from 4 clinical trials of pembrolizumab has shown that patients with both high TMB and high T-cell-inflamed GEP score or PD-L1 expression were more likely to respond to pembrolizumab, suggesting that the highest likelihood of clinical efficacy with pembrolizumab may be expected in tumors with both high TMB and high levels of tumor inflammation as measured by either GEP or PD-L1 expression.[113,114]

Lastly, merging evidence suggests that high aneuploidy levels are also associated with decreased CD8+ T-cell density and poor outcomes of PD-(L)1 blockade in cancer,[115,116] and that a weak but positive correlation between TMB and aneuploidy exists. Therefore, the predictive effect of high TMB in cancers with high aneuploidy levels may be more limited. Interestingly, in a recent multi-center study conducted in NSCLCs treated with ICI, cases with low aneuploidy levels [as the fraction of chromosomal arm altered (FAA)] and high TMB (≥median) had the highest ORR (32.7%) and the longest median PFS (5.3 months) and

OS (24.5 months) with PD-(L)1 inhibitor as monotherapies, while tumors with low TMB and high aneuploidy levels had the lowest ORR (7.7%) and the shortest median PFS (1.8 months) and OS (7.8 months).[115]

Overall, these data suggest that integrating TMB with other biomarkers of immunotherapy efficacy, such as PD-L1 expression, MSI status, immune infiltration, HLA variations and neoantigen landscape, and aneuploidy levels, holds significant promise for enhancing the accuracy of predicting patient response to immunotherapy. A comprehensive assessment considering multiple biomarkers can guide treatment decisions, identify patients who are likely to benefit, and contribute to personalized cancer care. A summary of the potential strategies to improve TMB as a biomarker for ICI response and implementing its use in the clinic is represented in Figure 4.

CONCLUSION

Although TMB shows promise as a predictive biomarker, its clinical utility and value in routine practice are still being investigated. While high TMB has been associated with better outcomes, not all patients with high TMB experience clinical benefits from immunotherapy. Additionally, some patients with low TMB still respond to immunotherapy, indicating that TMB alone may not capture the complete complexity of tumor-immune interactions. Strategies to optimize the use of TMB as a biomarker for immunotherapy efficacy include standardization of TMB measurement to ensure that TMB results are comparable across different laboratories and studies. Standardization can involve using the same sequencing platform, same panel of genes, same variant calling and filtering methods, following standardized protocols for sample preparation, or harmonization across multiple platforms. TMB can also vary within and between tumors due to the presence of subclonal mutations and may evolve with treatment. Identification of appropriate TMB cut-offs will also enhance the clinical utility of TMB. Different studies have used different thresholds for defining high TMB, and there is no consensus on the optimal cut-off for predicting response to immunotherapy across cancer types. Therefore, large prospective clinical trials to establish the most predictive cut-offs that also fully interrogate the influence of specific tumor types and treatment regimens when setting these cut-offs will be necessary. An additional critical consideration is whether we can use the same or similar cut-off of tTMB and bTMB to predict the outcomes of ICI. Although bTMB and tTMB are generally correlated, high bTMB may also reflect increased disease burden and has been shown to be a poor prognostic factor. Therefore, to implement the use of bTMB in clinical practice, we will need to establish bTMB cut-offs that account for this potential bias.

Integrating TMB with additional biomarkers may improve its predictive accuracy for immunotherapy responses, recognizing that TMB only reflects a facet of the complex biology underlying ICI response. Beyond TMB, biomarkers such as PD-L1 expression, MSI, mutations in DDR genes, dNLR, HLA variations, and gene expression signatures also provide valuable information about a patient's likelihood of responding to immunotherapy. Therefore, it is important to consider these other biomarkers in addition to TMB when making treatment decisions. Further research and validation studies are essential to establish standardized guidelines for the integration of these biomarkers in clinical practice.

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REFERENCES

1. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350-1355.
2. Sharma P, Siddiqui BA, Anandhan S, et al. The next decade of immune checkpoint therapy. *Cancer Discov*. 2021;11(4):838-857.
3. Yap TA, Parkes EE, Peng W, Moyers JT, Curran MA, Tawbi HA. Development of immunotherapy combination strategies in cancer. *Cancer Discov*. 2021;11(6):1368-1397.
4. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348(6230):124-128.
5. Cheng DT, Mitchell TN, Zehir A, et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn*. 2015;17:251-264.
6. Milbury CA, Creeden J, Yip W-K, et al. Clinical and analytical validation of FoundationOne®CDx, a comprehensive genomic profiling assay for solid tumors. In: Vousden G, editor. *PLoS One*. 2022;17(3):e0264138.
7. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413.
8. Chung J, Maruvka YE, Sudhaman S, et al. DNA polymerase and mismatch repair exert distinct microsatellite instability signatures in normal and malignant human cells. *Cancer Discov*. 2021;11(5):1176-1191.
9. Dudley JC, Lin M-T, Le DT, Eshleman JR. Microsatellite instability as a biomarker for PD-1 blockade. *Clin Cancer Res*. 2016;22(4):813-820.

10. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*. 2019;51(2):202-206.
11. Valero C, Lee M, Hoen D, et al. The association between tumor mutational burden and prognosis is dependent on treatment context. *Nat Genet*. 2021;53:11-15.
12. Sha D, Jin Z, Budczies J, Kluck K, Stenzinger A, Sinicrope FA. Tumor mutational burden as a predictive biomarker in solid tumors. *Cancer Discov*. 2020;10(12):1808-1825.
13. McGranahan N, Swanton C. Neoantigen quality, not quantity. *Sci Transl Med*. 2019;11(506):eaax7918.
14. Rizvi H, Sanchez-Vega F, La K, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol*. 2018;36(7):633-641.
15. Hodi FS, Wolchok JD, Schadendorf D, et al. TMB and inflammatory gene expression associated with clinical outcomes following immunotherapy in advanced melanoma. *Cancer Immunol Res*. 2021;9(10):1202-1213.
16. Ricciuti B, Kravets S, Dahlberg SE, et al. Use of targeted next generation sequencing to characterize tumor mutational burden and efficacy of immune checkpoint inhibition in small cell lung cancer. *J Immunother Cancer*. 2019;7(1):87.
17. Strickler JH, Hanks BA, Khasraw M. Tumor mutational burden as a predictor of immunotherapy response: is more always better? *Clin Cancer Res*. 2021;27(5):1236-1241.
18. Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol*. 2020;21(10):1353-1365.
19. Rizvi NA, Cho BC, Reinmuth N, et al. Durvalumab with or without tremelimumab vs standard chemotherapy in first-line treatment of metastatic non-small cell lung cancer: the MYSTIC phase 3 randomized clinical trial. *JAMA Oncol*. 2020;6(5):661-674.
20. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med*. 2018;378(22):2093-2104.
21. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371(23):2189-2199.
22. Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage IV or recurrent non—small-cell lung cancer. *N Engl J Med*. 2017;376:2415-2426.
23. Herbst RS, Giaccone G, de Marinis F, et al. Atezolizumab for first-line treatment of PD-L1—selected patients with NSCLC. *N Engl J Med*. 2020;383(14):1328-1339.
24. Kim ES, Velcheti V, Mekhail T, et al. Blood-based tumor mutational burden as a biomarker for atezolizumab in non-small cell lung cancer: the phase 2 B-F1RST trial. *Nat Med*. 2022;28(5):939-945.
25. Peters S, Dziadziuszko R, Morabito A, et al. Atezolizumab versus chemotherapy in advanced or metastatic NSCLC with high blood-based tumor mutational burden: primary analysis of BFAST cohort C randomized phase 3 trial. *Nat Med*. 2022;28(9):1831-1839.
26. Hellmann MD, Callahan MK, Awad MM, et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. *Cancer Cell*. 2018;33(5):853-861.e4.
27. Ferrara R, Imbimbo M, Malouf R, et al. Single or combined immune checkpoint inhibitors compared to first-line platinum-based chemotherapy with or without bevacizumab for people with advanced non-small cell lung cancer. *Cochrane Database Syst Rev*. 2021;4(4):CD013257.
28. Robert C, Lewis KD, Gutzmer R, et al. Biomarkers of treatment benefit with atezolizumab plus vemurafenib plus cobimetinib in BRAFV600 mutation—positive melanoma. *Ann Oncol*. 2022;33(5):544-555.
29. Hamid O, Molinero L, Bolen CR, et al. Safety, clinical activity, and biological correlates of response in patients with metastatic melanoma: results from a phase I trial of atezolizumab. *Clin Cancer Res*. 2019;25(20):6061-6072.
30. Necchi A, Raggi D, Gallina A, et al. Updated results of PURE-01 with preliminary activity of neoadjuvant pembrolizumab in patients with muscle-invasive bladder carcinoma with variant histologies. *Eur Urol*. 2020;77(4):439-446.
31. Galsky MD, Saci A, Szabo PM, et al. Nivolumab in patients with advanced platinum-resistant urothelial carcinoma: efficacy, safety, and biomarker analyses with extended follow-up from CheckMate 275. *Clin Cancer Res*. 2020;26(19):5120-5128.
32. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet*. 2016;387(10031):1909-1920.
33. Hanna GJ, Lizotte P, Cavanaugh M, et al. Frameshift events predict anti—PD-1/L1 response in head and neck cancer. *JCI Insight*. 2018;3(4):e98811.
34. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.
35. Wang F, Wei XL, Wang FH, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432. *Ann Oncol*. 2019;30(9):1479-1486.
36. Fabrizio DA, George TJ Jr, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *J Gastrointest Oncol*. 2018;9(4):610-617.
37. McGrail DJ, Pilié PG, Rashid NU, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann Oncol*. 2021;32(5):661-672.
38. Garcia EP, Minkovsky A, Jia Y, et al. Validation of OncoPanel: a targeted next-generation sequencing assay for the detection of somatic variants in cancer. *Arch Pathol Lab Med*. 2017;141(6):751-758.
39. Turajlic S, Litchfield K, Xu H, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol*. 2017;18(8):1009-1021.
40. Gerratana L, Movarek M, Wehbe F, et al. Genomic landscape of advanced solid tumors in circulating tumor DNA and correlation with tissue sequencing: a single institution's experience. *JCO Precis Oncol*. 2022;(6):e2100289.
41. Wang Z, Duan J, Cai S, et al. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non—small cell lung cancer with use of a next-generation sequencing cancer gene panel. *JAMA Oncol*. 2019;5(5):696.
42. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med*. 2018;24:1441-1448.
43. Sturgill EG, Misch A, Jones CC, et al. Discordance in tumor mutation burden from blood and tissue affects association with response to immune checkpoint inhibition in real-world settings. *Oncologist*. 2022;27(3):175-182.
44. Qiu P, Poehlein CH, Marton MJ, Laterza OF, Levitan D. Measuring tumor mutational burden (TMB) in plasma from mCRPC patients using two commercial NGS assays. *Sci Rep*. 2019;9(1):114.
45. Reichert ZR, Morgan TM, Li G, et al. Prognostic value of plasma circulating tumor DNA fraction across four common cancer types: a real-world outcomes study. *Ann Oncol*. 2023;34(1):111-120.
46. Vokes NI, Liu D, Ricciuti B, et al. Harmonization of tumor mutational burden quantification and association with response to immune checkpoint blockade in non—small-cell lung cancer. *JCO Precis Oncol*. 2019;3. https://doi.org/10.1200/po.19.00171.
47. Vega DM, Yee LM, McShane LM, et al. Aligning tumor mutational burden (TMB) quantification across diagnostic platforms: phase II of

the Friends of Cancer Research TMB Harmonization Project. *Ann Oncol*. 2021;32(12):1626-1636.
48. Kazdal D, Endris V, Allgäuer M, et al. Spatial and temporal heterogeneity of panel-based tumor mutational burden in pulmonary adenocarcinoma: separating biology from technical artifacts. *J Thorac Oncol*. 2019;14(11):1935-1947.
49. Zhou KI, Peterson B, Serritella A, et al. Spatial and temporal heterogeneity of PD-L1 expression and tumor mutational burden in gastroesophageal adenocarcinoma at baseline diagnosis and after chemotherapy. *Clin Cancer Res*. 2020;26(24):6453-6463.
50. Riaz N, Havel JJ, Makarov V, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell*. 2017;171(4):934-949.e16.
51. Warth A, Endris V, Stenzinger A, et al. Genetic changes of non-small cell lung cancer under neoadjuvant therapy. *Oncotarget*. 2016;7(20):29761-29769.
52. de Bruin EC, McGranahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science*. 2014;346(6206):251-256.
53. Pham TV, Goodman AM, Sivakumar S, Frampton G, Kurzrock R. Intrapatient stability of tumor mutational burden from tissue biopsies at different time points in advanced cancers. *Genome Med*. 2021;13(1):159.
54. Budczies J, Allgäuer M, Litchfield K, et al. Optimizing panel-based tumor mutational burden (TMB) measurement. *Ann Oncol*. 2019;30(9):1496-1506.
55. Mardis ER. Neoantigens and genome instability: impact on immunogenomic phenotypes and immunotherapy response. *Genome Med*. 2019;11(1):71.
56. André T, Shiu K-K, Kim TW, et al. Pembrolizumab in microsatellite-instability—high advanced colorectal cancer. *N Engl J Med*. 2020;383(23):2207-2218.
57. Bouffet E, Larouche V, Campbell BB, et al. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. *J Clin Oncol*. 2016;34(19):2206-2211.
58. Mandal R, Samstein RM, Lee K-W, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti—PD-1 immunotherapy response. *Science*. 2019;364(6439):485-491.
59. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9(1):34.
60. Schrock AB, Ouyang C, Sandhu J, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. *Ann Oncol*. 2019;30(7):1096-1103.
61. Ricciuti B, Recondo G, Spurr LF, et al. Impact of DNA damage response and repair (DDR) gene mutations on efficacy of PD-(L)1 immune checkpoint inhibition in non—small cell lung cancer. *Clin Cancer Res*. 2020;26(15):4135-4142.
62. Teo MY, Seier K, Ostrovnaya I, et al. Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers. *J Clin Oncol*. 2018;36(17):1685-1694.
63. Reisländer T, Groelly FJ, Tarsounas M. DNA damage and cancer immunotherapy: a STING in the tale. *Mol Cell*. 2020;80(1):21-28.
64. Sen T, Rodriguez BL, Chen L, et al. Targeting DNA damage response promotes antitumor immunity through STING-mediated T-cell activation in small cell lung cancer. *Cancer Discov*. 2019;9(5):646-661.
65. Boichard A, Pham TV, Yeerna H, et al. APOBEC-related mutagenesis and neo-peptide hydrophobicity: implications for response to immunotherapy. *Oncoimmunology*. 2019;8(3):1550341.
66. Wang S, Jia M, He Z, Liu X-S. APOBEC3B and APOBEC mutational signature as potential predictive markers for immunotherapy response in non-small cell lung cancer. *Oncogene*. 2018;37(29):3924-3936.
67. Shi R, Wang X, Wu Y, et al. APOBEC-mediated mutagenesis is a favorable predictor of prognosis and immunotherapy for bladder cancer patients: evidence from pan-cancer analysis and multiple databases. *Theranostics*. 2022;12(9):4181-4199.
68. Boichard A, Tsigelny IF, Kurzrock R. High expression of PD-1 ligands is associated with kataegis mutational signature and APOBEC3 alterations. *Oncoimmunology*. 2017;6(3):e1284719.
69. Pham TV, Boichard A, Goodman A, et al. Role of ultraviolet mutational signature versus tumor mutation burden in predicting response to immunotherapy. *Mol Oncol*. 2020;14(8):1680-1694.
70. Skoulidis F, Goldberg ME, Greenawalt DM, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov*. 2018;8(7):822-835.
71. Ricciuti B, Arbour KC, Lin JJ, et al. Diminished efficacy of programmed death-(ligand)1 inhibition in STK11- and KEAP1-mutant lung adenocarcinoma is affected by KRAS mutation status. *J Thorac Oncol*. 2022;17(3):399-410.
72. Arbour KC, Jordan E, Kim HR, et al. Effects of co-occurring genomic alterations on outcomes in patients with KRAS-mutant non-small cell lung cancer. *Clin Cancer Res*. 2018;24:334-340.
73. Alessi JV, Ricciuti B, Spurr LF, et al. SMARCA4 and other SWItch/Sucrose Nonfermentable family genomic alterations in NSCLC: clinicopathologic characteristics and outcomes to immune checkpoint inhibition. *J Thorac Oncol*. 2021;16(7):1176-1187.
74. Koyama S, Akbay EA, Li YY, et al. STK11/LKB1 deficiency promotes neutrophil recruitment and proinflammatory cytokine production to suppress T-cell activity in the lung tumor microenvironment. *Cancer Res*. 2016;76(5):999-1008.
75. Ravi A, Hellmann MD, Arniella MB, et al. Genomic and transcriptomic analysis of checkpoint blockade response in advanced non-small cell lung cancer. *Nat Genet*. 2023;55:807-819.
76. Chen Y, Chen G, Li J, et al. Association of tumor protein p53 and ataxia-telangiectasia mutated comutation with response to immune checkpoint inhibitors and mortality in patients with non-small cell lung cancer. *JAMA Netw Open*. 2019;2(9):e1911895.
77. Okamura R, Kato S, Lee S, Jimenez RE, Sicklick JK, Kurzrock R. ARID1A alterations function as a biomarker for longer progression-free survival after anti-PD-1/PD-L1 immunotherapy. *J Immunother Cancer*. 2020;8(1):e000438.
78. Miao D, Margolis CA, Gao W, et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. *Science*. 2018;359(6377):801-806.
79. Wu Y-M, Cieślik M, Lonigro RJ, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. *Cell*. 2018;173(7):1770-1782.e14.
80. Hazini A, Fisher K, Seymour L. Deregulation of HLA-I in cancer and its central importance for immunotherapy. *J Immunother Cancer*. 2021;9(8):e002899.
81. Chowell D, Krishna C, Pierini F, et al. Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy. *Nat Med*. 2019;25(11):1715-1720.
82. Marty R, Kaabinejadian S, Rossell D, et al. MHC-I genotype restricts the oncogenic mutational landscape. *Cell*. 2017;171(6):1272-1283.e15.
83. Seliger B. Molecular mechanisms of HLA class I-mediated immune evasion of human tumors and their role in resistance to immunotherapies. *HLA*. 2016;88(5):213-220.
84. Bukur J, Jasinski S, Seliger B. The role of classical and non-classical HLA class I antigens in human tumors. *Semin Cancer Biol*. 2012;22(4):350-358.
85. Filip I, Wang A, Kravets O, et al. Pervasiveness of HLA allele-specific expression loss across tumor types. *Genome Med*. 2023;15(1):8.
86. Shim JH, Kim HS, Cha H, et al. HLA-corrected tumor mutation burden and homologous recombination deficiency for the prediction of response to PD-(L)1 blockade in advanced non-small-cell lung cancer patients. *Ann Oncol*. 2020;31(7):902-911.
87. Ricciuti B, Wang X, Alessi JV, et al. Association of high tumor mutation burden in non—small cell lung cancers with increased immune infiltration and improved clinical outcomes of PD-L1 blockade across PD-L1 expression levels. *JAMA Oncol*. 2022;8(8):1160.

88. Alessi JV, Elkrief A, Ricciuti B, et al. Clinicopathologic and genomic factors impacting efficacy of first-line chemoimmunotherapy in advanced non-small cell lung cancer (NSCLC). *J Thorac Oncol*. 2023;18(6):731-743.
89. Huang RSP, Carbone DP, Li G, et al. Durable responders in advanced NSCLC with elevated TMB and treated with 1L immune checkpoint inhibitor: a real-world outcomes analysis. *J Immunother Cancer*. 2023;11(1):e005801.
90. Palmeri M, Mehnert J, Silk AW, et al. Real-world application of tumor mutational burden-high (TMB-high) and microsatellite instability (MSI) confirms their utility as immunotherapy biomarkers. *ESMO Open*. 2022;7(1):100336.
91. Goodman AM, Kato S, Bazhenova L, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol Cancer Ther*. 2017;16(11):2598-2608.
92. Zhang C, Dang D, Liu C, Wang Y, Cong X. Identification of tumor mutation burden-related hub genes and the underlying mechanism in melanoma. *J Cancer*. 2021;12(8):2440-2449.
93. Goodman AM, Castro A, Pyke RM, et al. MHC-I genotype and tumor mutational burden predict response to immunotherapy. *Genome Med*. 2020;12(1):45.
94. Shao XM, Huang J, Niknafs N, et al. HLA class II immunogenic mutation burden predicts response to immune checkpoint blockade. *Ann Oncol*. 2022;33(7):728-738.
95. Hosoi A, Takeda K, Nagaoka K, et al. Increased diversity with reduced "diversity evenness" of tumor infiltrating T-cells for the successful cancer immunotherapy. *Sci Rep*. 2018;8(1):1058.
96. Weber J, Horak C, Hodi FS, et al. Baseline tumor T cell receptor (TcR) sequencing analysis and neo antigen load is associated with benefit in melanoma patients receiving sequential nivolumab and ipilimumab. *Ann Oncol*. 2016;27:vi359.
97. Lamberti G, Spurr LF, Li Y, et al. Clinicopathological and genomic correlates of programmed cell death ligand 1 (PD-L1) expression in nonsquamous non-small-cell lung cancer. *Ann Oncol*. 2020;31:807-814.
98. Yarchoan M, Albacker LA, Hopkins AC, et al. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. *JCI Insight*. 2019;4(6):e126908.
99. Chen Y, Wang Y, Luo H, et al. The frequency and inter-relationship of PD-L1 expression and tumour mutational burden across multiple types of advanced solid tumours in China. *Exp Hematol Oncol*. 2020;9(1):17.
100. Hellmann MD, Paz-Ares L, Bernabe Caro R, et al. Nivolumab plus ipilimumab in advanced non—small-cell lung cancer. *N Engl J Med*. 2019;381:2020-2031.
101. Herbst RS, Lopes G, Kowalski DM, et al. Association between tissue TMB (tTMB) and clinical outcomes with pembrolizumab monotherapy (pembro) in PD-L1-positive advanced NSCLC in the KEYNOTE-010 and -042 trials. *Ann Oncol*. 2019;30:v916-v917.
102. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. *Br J Cancer*. 2021;124(2):359-367.
103. Li F, Li C, Cai X, et al. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: a systematic review and meta-analysis. *eClinicalMedicine*. 2021;41:101134.
104. Montesion M, Murugesan K, Jin DX, et al. Somatic HLA class I loss is a widespread mechanism of immune evasion which refines the use of tumor mutational burden as a biomarker of checkpoint inhibitor response. *Cancer Discov*. 2021;11(2):282-292.
105. Naranbhai V, Viard M, Dean M, et al. HLA-A*03 and response to immune checkpoint blockade in cancer: an epidemiological biomarker study. *Lancet Oncol*. 2022;23(1):172-184.
106. Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science*. 2018;359(6375):582-587.
107. Jiang T, Jin Q, Wang J, et al. HLA-I evolutionary divergence confers response to PD-1 blockade plus chemotherapy in untreated advanced non—small cell lung cancer. *Clin Cancer Res*. 2023;29(23):4830-4843.
108. Chhibber A, Huang L, Zhang H, et al. Germline HLA landscape does not predict efficacy of pembrolizumab monotherapy across solid tumor types. *Immunity*. 2022;55(1):56-64.e4.
109. Alessi JV, Ricciuti B, Alden SL, et al. Low peripheral blood derived neutrophil-to-lymphocyte ratio (dNLR) is associated with increased tumor T-cell infiltration and favorable outcomes to first-line pembrolizumab in non-small cell lung cancer. *J Immunother Cancer*. 2021;9(11):e003536.
110. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *JNCI J Natl Cancer Inst*. 2014;106(6). https://doi.org/10.1093/jnci/dju124.
111. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil—lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol*. 2013;88(1):218-230.
112. Valero C, Lee M, Hoen D, et al. Pretreatment neutrophil-to-lymphocyte ratio and mutational burden as biomarkers of tumor response to immune checkpoint inhibitors. *Nat Commun*. 2021;12(1):729.
113. Ott PA, Bang Y-J, Piha-Paul SA, et al. T-cell—inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J Clin Oncol*. 2019;37(4):318-327.
114. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade—based immunotherapy. *Science*. 2018;362(6411). https://doi.org/10.1126/science.aar3593.
115. Alessi JV, Wang X, Elkrief A, et al. Impact of aneuploidy and chromosome 9p loss on tumor immune microenvironment and immune checkpoint inhibitor efficacy in non-small cell lung cancer. *J Thorac Oncol*. 2023;18(11):1524-1537.
116. Spurr LF, Weichselbaum RR, Pitroda SP. Tumor aneuploidy predicts survival following immunotherapy across multiple cancers. *Nat Genet*. 2022;54(12):1782-1785.
117. Zheng M. Tumor mutation burden for predicting immune checkpoint blockade response: the more, the better. *J Immunother Cancer*. 2022;10(1):e003087.