HHS Public Access

Author manuscript

Nat Genet. Author manuscript; available in PMC 2021 July 04.

Published in final edited form as:

Nat Genet. 2021 January ; 53(1): 11–15. doi:10.1038/s41588-020-00752-4.

The association between tumor mutational burden and prognosis is dependent on treatment context

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Concept and design: CV, ML, DH, TAC, VES, LGTM. *Acquisition, analysis, or interpretation of data*: CV, ML, DH, MAP, ANS, GP, VPB, JJS, AMC, KCLR, DWK, JW, ZN, NP, RMS, SR, IG, RJW, AAH, MFB, AZ, DBS, ML, NR. *Drafting of the manuscript*: CV, LGTM. *Critical revision of the manuscript for important intellectual content*: All authors. *Statistical analysis*: CV, VES, LGTM. *Obtained funding*: TAC, LGTM. *Administrative, technical, or material support*: DH, DWK. *Supervision*: TAC, VES, LGTM.

Data availability statement

All data needed to replicate the analyses in this study, including de-identified clinical data, TMB values, MSI status, and DNA damage repair gene mutations, have been deposited on Zenodo (DOI: 10.5281/zenodo.4074184; website: https://zenodo.org/record/4074184#.X5G-I-i6M2w). Original sequencing reads cannot be publicly deposited due to privacy restrictions, as sequencing was performed as part of clinical care.

Competing interests statement:

DH receives funding from AstraZeneca. **MAP** reports consulting fees from BMS, Merck, Array BioPharma, Novartis, Incyte, NewLink Genetics, and Aduro, honoraria from BMS and Merck, and research support from RGenix, Infinity, BMS, Merck, Array BioPharma, Novartis, and AstraZeneca. **ANS** reports advisory board position with BMS, Immunocore, and Castle Biosciences, and institutional research support from BMS, Immunocore, and Xcovery. **VPB** is a recipient of an immuno-oncology translational research grant from Bristol-Myers Squibb and is an inventor on a patent application related to work on neoantigen modelling. **JJS** has received travel support from Intuitive Surgical Inc. for fellow education and has served as a clinical advisor to Guardant Health, Inc. **AMC** reports advisory board position with Springworks Therapeutics. **AZ** reports honoraria from Illumina. **NR** reports research support from Pfizer and BMS, and consulting fees from REPARE Therapeutics, Mirati Therapeutics, and Illumina. **TAC** acknowledges grant funding from Bristol-Myers Squibb, AstraZeneca, Illumina, Pfizer, An2H, and Eisai, has served as an advisor for Bristol-Myers Squibb, Illumina, Eisai, and An2H, holds equity in An2H, and is a co-founder of Gritstone Oncology and holds equity in the company. **LGTM** reports laboratory research funding from Illumina and AstraZeneca. **RMS, TAC** and **LGTM** are inventors on a patent held by Memorial Sloan Kettering related to the use of TMB in cancer immunotherapy. The remaining authors declare no competing interests.

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Abstract

In multiple cancer types, high tumor mutational burden (TMB) is associated with longer survival after treatment with immune checkpoint inhibitors (ICI). The association of TMB with survival outside of the immunotherapy context is poorly understood. We analyzed 10,233 patients (80% non-ICI-treated, 20% ICI-treated) with 17 cancer types, before/without ICI treatment, or after ICI treatment. In non-ICI-treated patients, higher TMB (higher percentile within cancer type) was not associated with better prognosis; in fact, in many cancer types, higher TMB was associated with poorer survival, in contrast to ICI-treated patients, in whom higher TMB was associated with longer survival.

In multiple cancer types, high tumor mutational burden (TMB) is associated with higher rates of treatment response, and longer survival, among patients who receive treatment with immune checkpoint inhibitors (ICI).[1-5] This association has been attributed to higher numbers of potentially immunogenic neoantigens that may facilitate anti-tumor immune responses. However, an open question is whether this association might also reflect a general prognostic benefit to high TMB in cancer, irrespective of treatment with immunotherapy. To the best of our knowledge, there are no studies examining the association between TMB and survival, in cohorts including both non-ICI-treated and ICI-treated patients, using contemporary clinical and genomic data.

Interpreting the recent United States Food and Drug Administration approval of pembrolizumab for solid tumors of any histology with TMB ≥ 10 mutations/megabase, requires that we understand whether high TMB might also be associated with longer survival, in patients receiving other therapies, besides ICI. This understanding would be important, before concluding that it is rational to prioritize immunotherapy in tumors with high TMB.

In the absence of therapy, the chronic antigen exposure associated with high TMB has been shown to cause dysfunction and terminal differentiation of T cells, leading to impaired immunologic surveillance.[6] Results of prior studies have suggested that high TMB may be associated with poorer outcomes in some cancer types, but these studies have not controlled for ICI therapy.[7-11] We hypothesized that, in some cancer types, high TMB would have opposite associations with survival, depending on immunotherapy context.

A caveat to simply comparing the effect of TMB in patients receiving or not receiving ICI treatment is that immunotherapy has now become integrated into standard of care for many

types of cancer, in some cases as first-line therapy. Thus, a selected cohort of patients never treated with ICI would be unrepresentative of patients with cancer types where ICI is now standard of care. To analyze the effect of high TMB with and without ICI, we undertook an analysis of overall survival (OS) among a large contemporary cohort of cancer patients, rather than a selected cohort of those never treated with ICI. We used a Cox proportional hazards regression model that included ICI treatment as a time-dependent covariate together with high vs. low TMB and their interaction ($h(t) = h0(t)$ $e^{(\beta1\times TMB + \beta2\times ICI(t) + \beta3\times TMB\times ICI(t))}$, stratified by cancer type). The time-dependent covariate in this model allowed us to isolate the effect of high TMB on survival in cancer patients, without/before ICI therapy versus after ICI therapy. Because TMB distributions differ across cancer types,[1,10] high TMB was defined as the top 20th percentile within cancer type, as previously described.[1] Because survival times differ among various cancer types, OS in the model was stratified by cancer type. OS was calculated from time of diagnosis (for the analyses before/without ICI) or from ICI first dose (for the analyses after ICI) to death of any cause; patients alive at time of analysis were censored at last contact (last known to be alive). The hazard ratio (HR) of OS for high TMB without/before ICI was defined as $e^{(\beta1)}$, and the HR of TMB after ICI was $e^{(\beta1 + \beta3)}$.

In addition, we addressed potential immortal time bias due to left truncation (defined as a type of selection bias that results from only studying patients who have survived long enough to fulfill certain conditions, such as being alive in the era of tumor sequencing), by limiting the cohort to patients followed after receiving a cancer diagnosis during the period when tumor sequencing at our center was routinely performed. This consideration, and the time-dependent covariate for ICI, were not employed in prior initial analyses of non-ICI-treated patients.[1]

The study population consisted of 10,233 patients with 17 types of cancer. Tumors and normal DNA underwent targeted next-generation sequencing with the MSK-IMPACT panel (Figure 1).[12] The majority of patients never received ICI (n = 8,211; 80%), and only 542 patients (5%) received ICI as first-line therapy. Patient and tumor characteristics are shown in Table 1 and Supplementary Table 1.

Some cancer types, mainly colorectal and endometrial cancers, include a subset of tumors with defects in DNA mismatch repair, leading to microsatellite instability (MSI). These tumors tend to have very high TMB and, overall, a more favorable prognosis.[13,14] Although less common, MSI has also been associated with better outcomes in other cancer types, outside the context of ICI treatment.[15,16] To avoid the possible confounding effect of MSI on survival, we first analyzed the effect of TMB excluding MSI-high (n = 264; identified with MSISensor[17]), and MSI unknown (n = 1,613) tumors across all cancer types.

Among all 8,356 patients with microsatellite-stable tumors without/before ICI therapy, high TMB was associated with worse overall survival (HR 1.26, 95% CI, 1.12-1.43; $P<.001$). Among all patients who did receive ICI therapy, high TMB was associated with better survival (HR = 0.74, 95% CI 0.63-0.88; $P<.001$). The HR for ICI treatment (HR = 4.45, 95% CI 4.02-4.93; $P<.001$) reflects ICI therapy generally being given in later lines of therapy, when risk of death is higher. We then conducted a multivariable analysis that included TMB,

ICI, and the clinicopathologic covariates that, on univariate analysis, were significantly associated with OS (patient age, sex, cancer type, and tumor stage). This multivariable analysis yielded similar hazard ratios for TMB: HR = 1.26 (95% CI, 1.11-1.43; *P*=.001) without/before ICI, and HR = 0.62 (95% CI, 0.52-0.74; *P*<.001) after ICI (Table 2).

We caution that these overall associations do not imply that the effect is identical in all cancer types. We therefore examined these associations within each cancer type, noting that statistical power is limited in those with smaller sample size. In most cancer types, high TMB was associated with numerically poorer survival without/before ICI therapy, and in many cancers, contrasted with better survival after ICI therapy (Figure 2). Directionally opposite associations of high TMB with OS (depending on immunotherapy context) were observed in non-small cell lung cancer, breast cancer, cutaneous melanoma, gastric, esophageal, and head and neck cancers.

We next performed this analysis including patients with MSI-high tumors or unknown MSI status (total n = 10,233). The results were similar in the overall analyses and for most of the cancer types (Supplementary Table 2 and Extended Data 1). The main differences were seen in colorectal and endometrial cancer, where including MSI-high tumors led to an association between high TMB and longer overall survival in both the non-ICI and ICI contexts. We note that the overall survival model became unstable for endometrial and bladder cancers, when MSI-high tumors were excluded, and therefore, data are only shown for these cancer types in the analysis with MSI-high tumors included. Considering these data, we conclude that high TMB may have prognostically favorable associations with survival in colorectal, endometrial and bladder cancer. However, in all other cancer types analyzed, high TMB was associated with unchanged or poorer prognosis, among patients not receiving ICI therapy.

We considered that, in some patients, other factors (such as prior therapies or other mutational processes) might be associated with the number of mutations in the tumor and confound associations with survival. We therefore performed several sensitivity analyses to examine these possibilities.

Temozolomide (TMZ) is an alkylating agent known to increase the number of somatic mutations in a tumor.[18] Excluding patients with TMZ therapy prior to IMPACT tissue sample collection (n = 660), we repeated the analysis and obtained similar results (see Supplementary Table 3). We also repeated the analysis, excluding patients who received any cytotoxic chemotherapy or radiotherapy prior to tumor sequencing (n = 3,255), to eliminate these potentially mutagenic therapies,[19,20] and again found that results were essentially unchanged (see Supplementary Table 4).

Additionally, we examined other mechanisms associated with high TMB, such as carcinogen exposure and DNA damage repair deficiency.[21,22] Excluding tumors with dominant mutational signatures reflecting smoking or UV mutagenesis (n = 927) (see Supplementary Table 5) did not alter associations observed in multivariable analyses. Similarly, excluding tumors with mutations in DNA damage repair pathway genes (n = 3,078) (see Supplementary Table 6) did not alter the associations in multivariable analyses.

Lastly, an analysis was performed limited to patients who only received one line of therapy (n = 2,451) – either ICI or a non-ICI drug. Of these patients, 227 (9%) received first-line ICI, and 2,224 (91%) did not receive ICI. In the multivariable analysis, among patients who received a non-ICI therapy, high TMB was associated with worse OS (HR 1.34, 95% CI, 1.06-1.70; P=.016), and in patients who did receive first-line ICI therapy only, high TMB was associated with better OS (HR = 0.45, 95% CI 0.24-0.85; P=.014) (Supplementary Table 7). These findings, using a simpler model, are consistent with the results of our Cox model with the time-dependent covariate. However, an important caveat is that patients who only receive one line of therapy will tend to be enriched for those with the best responses to that therapy, and this analysis is therefore subject to potential bias.

Taken together, these data indicate that the effect of high TMB in cancer depends on treatment context; specifically, whether the patient has received checkpoint inhibitor immunotherapy or not. Among patients who have not received ICI treatment, high TMB was associated with, on average, poorer overall survival in most, but not all, cancer types. After ICI therapy, high TMB was associated with, on average, better overall survival in most, but not all, cancer types.

High TMB may have negative prognostic implications in cancer patients for a number of reasons. High TMB could (1) increase the likelihood of the tumor harboring oncogenic drivers or mutations that could mediate therapeutic resistance,[23] (2) increase the degree of intratumor genetic heterogeneity and ability of a tumor to evolve under selective pressure, [24,25] or (3) represent underlying chromosomal instability.[26] Moreover, recent studies have suggested that persistent antigen exposure due to high TMB drives differentiation skewing and T cell dysfunction, partly explaining the negative effect on outcomes seen in patients with high TMB outside the context of immunotherapy.[6,27]

There are several important caveats to these findings. First, in contrast to the protective effect of high TMB in ICI-treated patients, the negative effect of high TMB in non-ICI treated patients was modest. Second, although this trend was seen in many cancer types, it was not observed in all cancer types, especially those with smaller sample size, or where MSI was prevalent. Third, in this large cohort of over 10,000 patients, we did not have the ability to accurately record and analyze how specific therapies might have mediated the observed associations between TMB and survival, and how specific tumor factors, such as histologic subtype or specific genetic alterations, might have modified these associations.

With respect to immunotherapy, these data validate and expand upon prior pan-cancer MSK-IMPACT data showing an association between high TMB and superior survival after ICI for multiple cancer types,[1] now in a larger cohort of 2,022 patients treated with ICI. The comparison data among non-ICI treated patients indicates that this association between TMB and improved outcome after ICI is not attributable to a general positive prognostic effect of high TMB. In fact, high TMB appears to often be associated with modestly poorer survival in many cancers, based on these data from over 10,000 patients analyzed without or before ICI therapy.

METHODS

Patient selection

After receiving Memorial Sloan Kettering institutional review board (IRB) approval, we initially selected patients with solid tumors who were first diagnosed during 2015 through 2018, whose tumors underwent next-generation DNA sequencing with MSK-IMPACT, and who received subsequent cancer therapy at Memorial Sloan Kettering Cancer Center (n = 14,577). MSK-IMPACT sequencing came into broad use starting in 2015. Next, we excluded patients with a history of more than 1 primary cancer (n = 3,425), cancer types with < 100 cases (n = 797), and cancers of unknown primary origin (n = 122), leaving a final cohort of 10,233 patients with 17 different cancer types.

All patients provided informed consent to a Memorial Sloan Kettering institutional review board-approved protocol, permitting return of results from sequencing analyses for research. To identify somatic tumor mutations, germline DNA from peripheral blood was sequenced at the same time as the tumor samples for all patients. Tissue processing and next-generation sequencing analysis were previously described.[12] Tumor mutational burden (TMB) was defined as the total number of somatic, non-synonymous mutations normalized to the exonic coverage of the respective MSK-IMPACT panel in megabases (mutations/megabase).[1] Dominant mutational signatures were assigned based on mutational patterns and nucleotide context as previously described.[22]

Clinical and genomic data collected

The primary outcome was overall survival (OS), calculated from time of diagnosis (for the analyses before/without treatment with immune checkpoint inhibitors; ICI) or from ICI first dose (for the analyses after ICI) to death of any cause; patients alive at time of analysis were censored at last contact (last known to be alive).

TMB was the main variable of interest and was analyzed as the percentile within cancer type; high TMB was defined as the top 20th percentile within cancer type. If a patient received more than one instance of MSK-IMPACT sequencing during the course of their care, we used the earliest sample analyzed. In most cases, sequencing was performed prior to ICI. Out of the 2,022 patients who received ICI, 1,888 (93%) patients had sequencing performed on tumor samples before ICI start, and in the remaining 134 patients (7%), sequencing was performed on tumor samples gathered after ICI start.

Treatment with ICI (anti PD-1/PD-L1, anti CTLA-4, or combination of both) was the main covariate in the models. We limited the cohort of patients categorized as non–immunotherapy-treated to patients who received all cancer treatment at MSK to ensure that they did not receive ICI therapy elsewhere. The other covariates analyzed were patient age at diagnosis, sex, cancer type, stage at diagnosis, and year of diagnosis. Microsatellite instability (MSI) status was determined from next-generation sequencing data with MSISensor, as previously described.[17]

To additionally control for the possibility that prior therapies might influence TMB, we examined whether chemotherapy and/or radiation therapy were received before the

collection of the MSK-IMPACT tissue sample. For patients treated with ICI, if patients had not received any specific treatment at our institution prior to MSK-IMPACT sequencing, but received any prior treatment elsewhere, those patients were considered "unknown" for prior chemotherapy or radiation therapy and were excluded from the corresponding subanalysis.

Statistical analyses

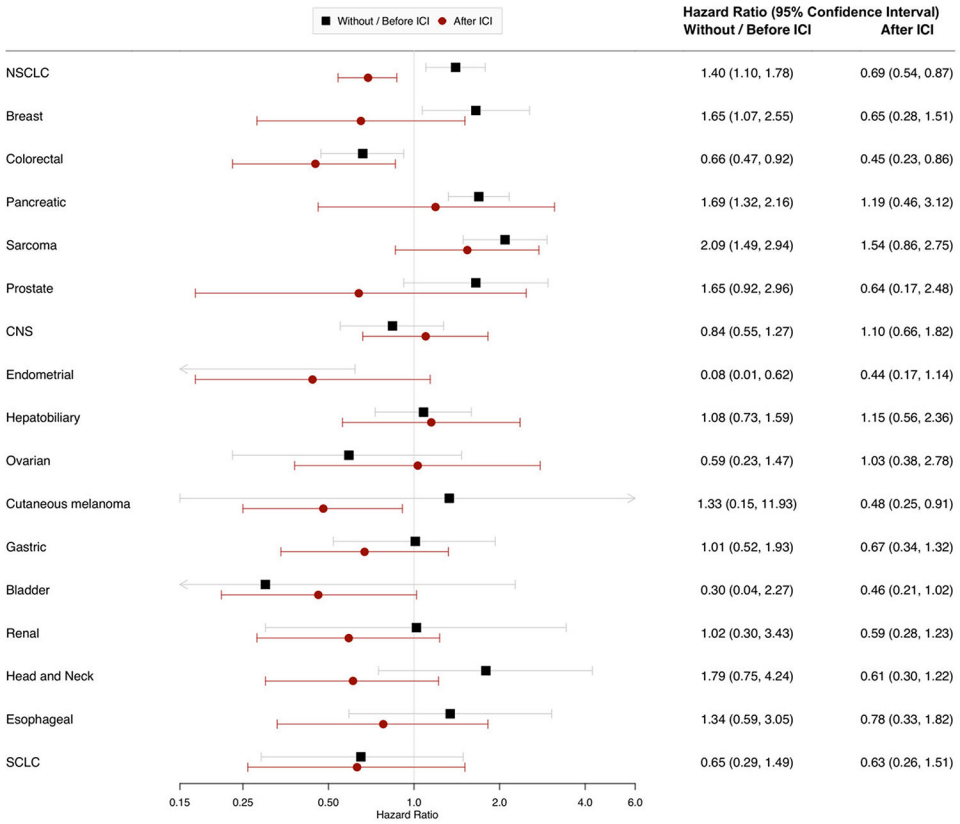
Cox proportional hazards regression was used with OS as the endpoint. The initial regression model included TMB, ICI treatment as a time-dependent covariate, and the interaction between ICI(t) and TMB. The time-dependent ICI covariate is assigned a value of 0 before (or without) ICI treatment and 1 after ICI treatment (if received). The pan-cancer model is expressed as $h(t) = h_0(t)\, e^{(\beta 1 \times TMB + \beta 2 \times ICI(t) + \beta 3 \times TMB \times ICI(t))}$, stratified by cancer type. The hazard ratio (HR) of OS for high TMB without/before ICI was defined as $e^{(\beta 1)}$, and the HR of TMB after ICI was $e^{(\beta 1 + \beta 3)}$.

The proportional hazards (PH) assumption was tested using Schoenfeld residuals (see Extended Data 2). Even though the residual plots showed no obvious or systematic violation of PH, when we performed the overall PH test, significant differences were observed ($P<.001$), suggesting that the PH assumption was not met. In clinical studies, hazards may not be perfectly proportional. In such a scenario, interpretation is important – it would not be valid to conclude that there is a constant increase or decrease in mortality with time based on TMB; rather, it should be concluded that TMB is associated with, on average, an increase or decrease in mortality during the follow-up period.[28] To confirm the robustness of model fit, we calculated 95% CIs of parameter estimates using a bootstrap procedure with 5,000 replicates, which were similar to the standard variance estimates (see Supplementary Table 8).

In summary, while the PH test is significant for PH assumption violation, the Schoenfeld residual plots do not indicate any systematic association between the covariate and time. In this study, the estimated hazard ratio is a weighted average of the time varying hazard ratio and summarizes the treatment effect. Bootstrapping confirmed accuracy of our variance estimates and robustness of the statistical model. Therefore, the average hazard ratio estimates from the model are indeed reasonable and indicate that there is a significant effect of TMB and ICI on survival.

Additional clinical and genomic variables were tested for association with OS in univariate analysis, and the variables significant in these univariate analyses, along with TMB and ICI, were included in the subsequent multivariable analyses. A *P* value of less than 0.05 was considered statistically significant, and all the hypothesis tests were 2-sided. All statistical analyses were conducted using Stata (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

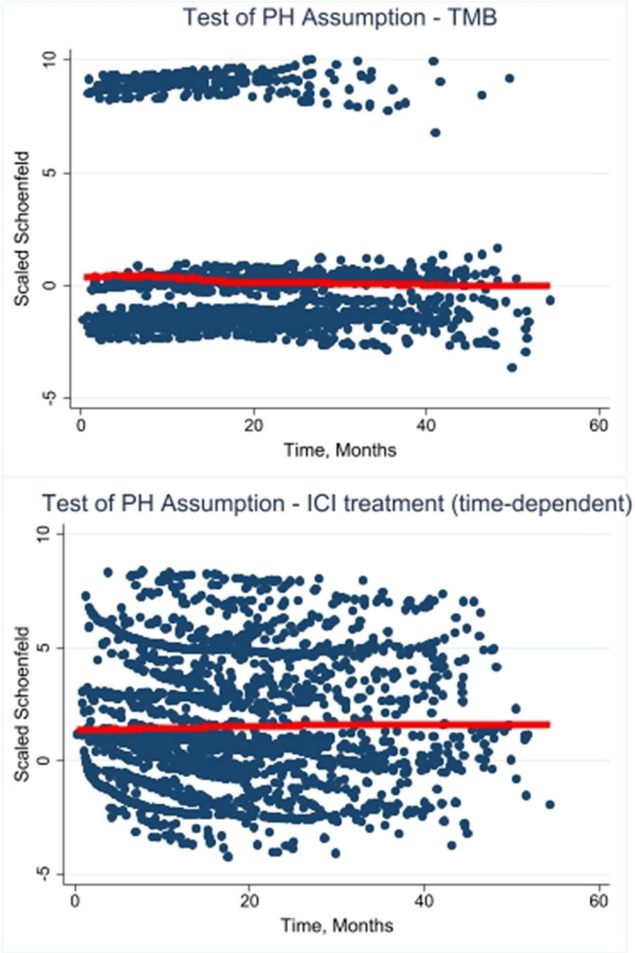
Extended Data



Extended Data Figure 1. Association between tumor mutational burden (TMB) and overall survival (OS) with and without immune checkpoint inhibitor (ICI) treatment in the entire cohort (10,233 patients analyzed)

The forest plots compare the hazard ratios for OS for patients with TMB-high versus TMB-low tumors (using top 20th percentile within cancer type as cutoff) for patients who never received ICI treatment or before receiving ICI (black), and after receiving ICI (red). Error bars represent the 95% confidence intervals (95% CI). Cox proportional hazards regression was used with overall survival as the endpoint. All the hypothesis tests were 2-sided. No adjustments for multiple comparisons were made.

Abbreviations: NSCLC, Non–small cell lung cancer; CNS, central nervous system; SCLC, small cell lung cancer.



Extended Data Figure 2. Proportional hazards (PH) assumption testing using Schoenfeld residuals

Abbreviations: TMB, Tumor mutational burden; ICI, immune checkpoint inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are grateful to our patients and their families for their bravery and their support of cancer research. We thank members of the Molecular Diagnostics Service in the Department of Pathology and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, and members of the Morris and Chan Laboratories for helpful discussions.

We thank J. Novak for editing the manuscript.

This study was funded by Fundación Alfonso Martín Escudero (to **CV**), NIH K08 DE024774 and R01 DE027738, the Jayme and Peter Flowers Fund, the Sebastian Nativo Fund, the Catherine and Frederick Adler Junior Faculty Chair, and Cycle for Survival (to **LGTM**), Pershing Square Sohn Cancer Research Foundation, The PaineWebber Chair, Stand Up To Cancer, the STARR Cancer Consortium, NIH R01 CA205426 and R35 CA232097 (to **TAC**), and the NIH/NCI Cancer Center Support Grant P30 CA008748.

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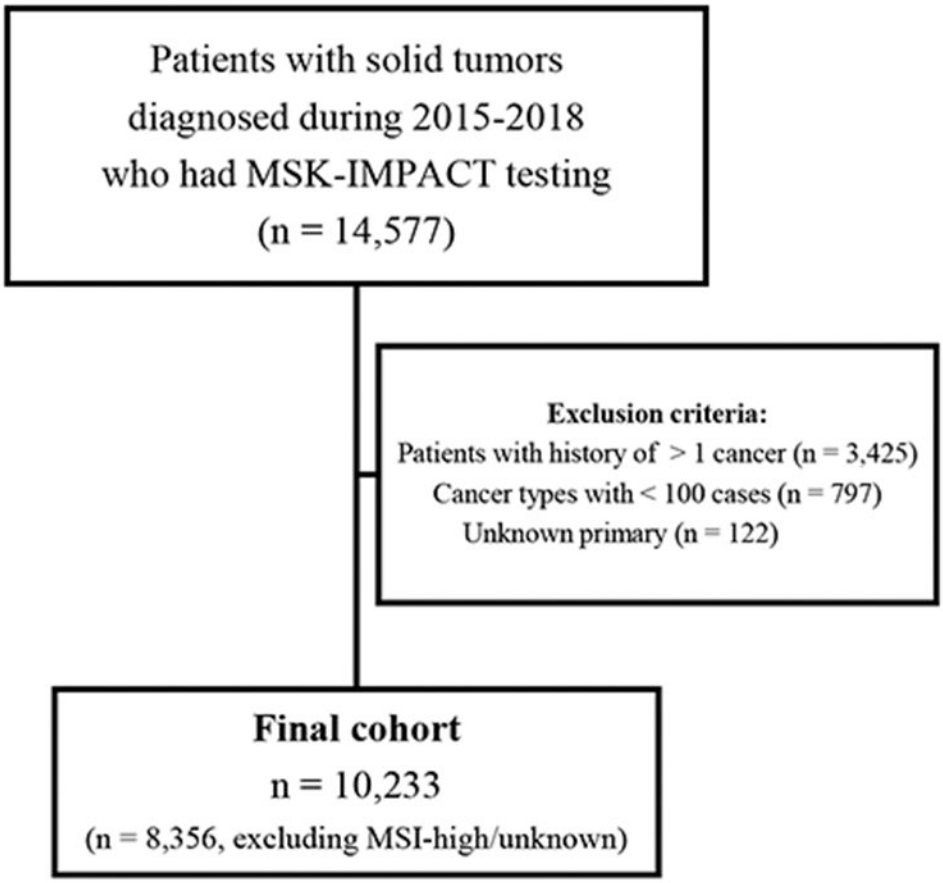

Figure 1. Flow diagram of the study
Abbreviations: MSI, Microsatellite instability.

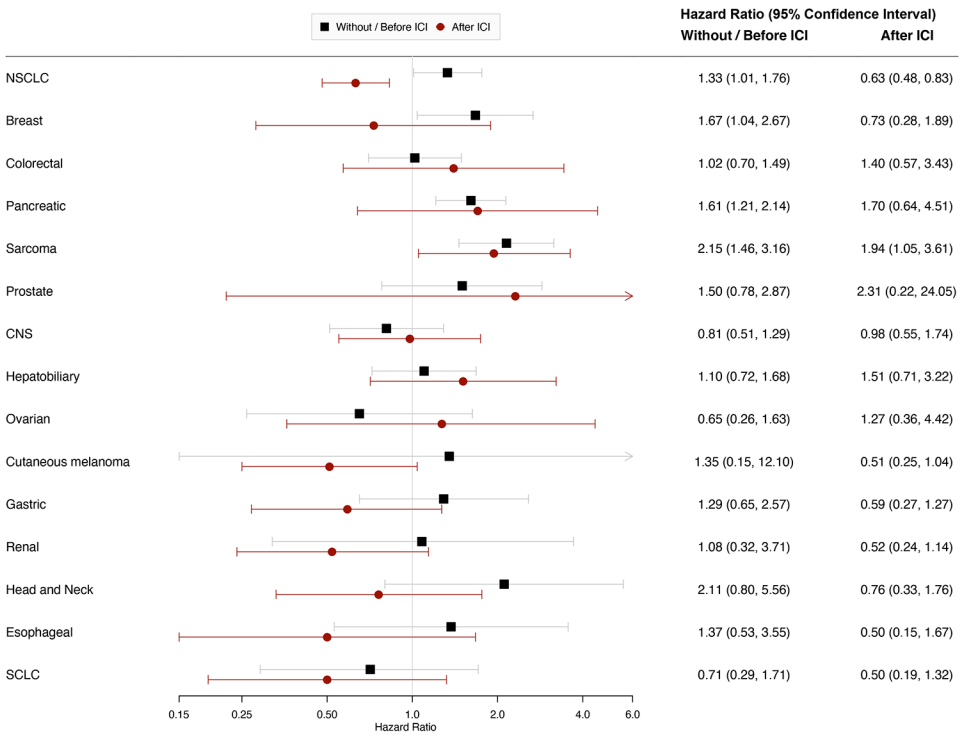

Figure 2. Association between high tumor mutational burden (TMB) and overall survival (OS) with and without immune checkpoint inhibitor (ICI) treatment in 8,356 microsatellite-stable tumors.

Patients with microsatellite instability high (MSI-high) or MSI unknown status were excluded but are analyzed along with microsatellite-stable tumors in Extended Data 1. Endometrial and bladder cancer could not be plotted due to instability of the model when MSI-high cases were excluded but are shown in Extended Data 1. The forest plots compare the hazard ratios for OS for patients with TMB-high versus TMB-low tumors (using top 20th percentile within cancer type as cutoff) for patients who never received ICI treatment or before receiving ICI (black), and after receiving ICI (red). Error bars represent the 95% confidence intervals (95% CI). Cox proportional hazards regression was used with OS as the endpoint. All the hypothesis tests were 2-sided. No adjustments for multiple comparisons were made.

Abbreviations: NSCLC, Non–small cell lung cancer; CNS, central nervous system; SCLC, small cell lung cancer.

Table 1.

Patients' characteristics

Characteristic	No. patients	%
Total cohort	10233	100
Age at diagnosis, median, years (IQR)	61	51-69
Sex		
Female	5781	56
Male	4452	44
Cancer type		
NSCLC	2084	20
Breast	1552	15
Colorectal	1353	13
Pancreatic	849	8
Sarcoma	741	7
Prostate	569	6
CNS	511	5
Endometrial	427	4
Hepatobiliary	408	4
Ovarian	325	3
Cutaneous Melanoma	298	3
Gastric	249	2
Bladder	232	2
Renal	201	2
Head and Neck	174	2
Esophageal	138	1
SCLC	122	1
Year of diagnosis		
2015-2016	5334	52
2017-2018	4899	48
Stage at diagnosis[a]		
I-III	5001	49
IV	4499	44
Non-applicable/Unknown	733	7
Time between diagnosis and MSK-IMPACT sample, median, days (IQR)	22	0-58
TMB, median, mutations/Mb (IQR)	4	2-7
MSI		
Stable	8162	80
Indeterminate	194	2
Unstable	264	3
Unknown	1613	16
Type of treatment		

Characteristic	No. patients	%
Never received ICI	8211	80
Received ICI as first line of therapy	542	5
Received ICI as subsequent lines of therapy	1267	12
Received ICI in unknown sequence	21	2
Follow-up time, median, months (IQR)	25	17-35

[a]Stage based on American Joint Committee on Cancer (AJCC) using the edition that was current at the time of diagnosis

Abbreviations: NSCLC, Non–small cell lung cancer; CNS, central nervous system; SCLC, small cell lung cancer; TMB, tumor mutational burden; Mb, megabase; MSI, microsatellite instability; ICI, immune checkpoint inhibitors.

Table 2.

Factors associated with overall survival

	Initial model[a]			Additional univariate analyses			Complete multivariable model		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
TMB[b]									
Bottom 80% without/before ICI	Ref	Ref					Ref	Ref	
Top 20% without/before ICI	1.264	1.117-1.431	<.001				1.258	1.105-1.432	.001
Bottom 80% after ICI	Ref	Ref					Ref	Ref	
Top 20% after ICI	0.741	0.627-0.875	<.001				0.621	0.520-0.741	<.001
ICI treatment[c]									
No	Ref	Ref					Ref	Ref	
Yes	4.447	4.015-4.926	<.001				3.615	3.240-4.035	<.001
Age at diagnosis, years				1.015	1.012-1.018	<.001	1.013	1.010-1.016	<.001
Sex									
Female				Ref	Ref		Ref	Ref	
Male				1.384	1.283-1.493	<.001	1.100	1.008-1.200	.033
Cancer type									
NSCLC				Ref	Ref		Ref	Ref	
Breast				0.180	0.145-0.222	<.001	0.438	0.350-0.548	<.001
Colorectal				0.641	0.560-0.733	<.001	0.967	0.834-1.122	.658
Pancreatic				1.592	1.403-1.808	<.001	2.796	2.439-3.205	<.001
Sarcoma				0.661	0.560-0.780	<.001	1.195	0.990-1.441	.063
Prostate				0.226	0.170-0.298	<.001	0.284	0.212-0.379	<.001
CNS				1.489	1.275-1.738	<.001	–	–	–
Endometrial				0.444	0.345-0.571	<.001	0.826	0.639-1.068	.145
Hepatobiliary				1.659	1.413-1.949	<.001	2.359	2.000-2.783	<.001
Ovarian				0.429	0.335-0.549	<.001	0.511	0.396-0.659	<.001
Cutaneous Melanoma				0.498	0.381-0.650	<.001	0.439	0.332-0.580	<.001
Gastric				1.145	0.929-1.413	.205	1.178	0.953-1.458	.131
Bladder				0.535	0.404-0.708	<.001	0.786	0.591-1.040	.091
Renal				0.696	0.533-0.909	.008	0.654	0.499-0.857	.002

Nat Genet. Author manuscript; available in PMC 2021 July 04.

	Initial model[a]			Additional univariate analyses			Complete multivariable model		
	HR	**95% CI**	**P**	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
Head and Neck				0.789	0.595-1.047	.101	0.670	0.503-0.892	.006
Esophageal				1.266	0.974-1.647	.078	1.664	1.273-2.175	<.001
SCLC				2.348	1.832-3.011	<.001	1.854	1.445-2.378	<.001
Stage at diagnosis[d]									
I-III				Ref	Ref		Ref	Ref	
IV				3.448	3.152-3.772	<.001	3.162	2.878-3.475	<.001
Year of diagnosis									
2015-2016				Ref	Ref		–	–	–
2017-2018				0.954	0.876-1.038	.274	–	–	–

Factors associated with overall survival among 8,356 patients with microsatellite-stable cancers. Patients with microsatellite instability high (MSI-high) or MSI unknown status were excluded but are analyzed along with microsatellite-stable tumors in Supplementary Table 2.

[a] TMB and ICI were analyzed together as described in the text; In this analysis, overall survival was stratified by cancer type.

[b] TMB percentiles were calculated within each cancer type.

[c] Time-dependent covariate.

[d] Stage based on American Joint Committee on Cancer (AJCC) using the edition that was current at the time of diagnosis. Cox proportional hazards regression was used with overall survival as the endpoint. All the hypothesis tests were 2-sided. No adjustments for multiple comparisons were made.

Abbreviations: HR, Hazard ratio; CI, confidence interval; P, p value; TMB, tumor mutational burden; Ref, reference; ICI, immune checkpoint inhibitors; NSCLC, non–small cell lung cancer; CNS, central nervous system; SCLC, small cell lung cancer.