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High Tumor Mutational Burden Correlates with Longer Survival in Immunotherapy-Naïve Patients with Diverse Cancers

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ABSTRACT

Higher tumor mutational burden (TMB) has been correlated with response to checkpoint blockade immunotherapy. However, it is unclear whether TMB independently serves as a prognostic biomarker for outcomes in immunotherapy-naïve patients. Here, we evaluated the relationship between TMB and overall survival in 1,415 immunotherapy-naïve patients with diverse advanced malignancies. TMB was studied both as a tiered variable (low ≤5 mutations/Mb, intermediate >5 and <20, high ≥20 and <50, and very high ≥50) and as a continuous variable. Interestingly, we observed a parabolic correlation between TMB and overall survival, in which intermediate-range TMB correlated with decreased survival, whereas low and very high TMB correlated with improved outcomes (median survival: 238, 174, 195, and 350 weeks for low, intermediate, high, and very high TMB, respectively; multivariate $P < 0.01$). This corresponded to an HR of 1.29 (95% confidence interval, 1.07–1.54; $P < 0.01$) for intermediate-range TMB on multivariable survival analysis correcting for known confounders, including primary tumor of origin. These results demonstrate that TMB may have utility as a prognostic biomarker in immunotherapy-naïve patients, with a protective effect at higher TMBs, and that studies of survival in immunotherapy-treated patients may need to stratify or randomize by TMB in a nonlinear fashion to account for this confounding.

Introduction

TMB has been correlated with survival and responses to checkpoint blockade based off the hypothesis that a high mutational burden increases the probability of immunogenic tumor antigens which the immune system can recognize (1–3). However, the ability for TMB to serve as a prognostic biomarker for outcomes or survival in immunotherapy naïve patients is unclear. Given that many conventional and targeted cancer therapies are now known to function through immune-mediated mechanisms, we hypothesized that high TMB might similarly correlate with increased survival across a variety of cancers in patients who did not receive immunotherapy. In this study, we characterize the relationship between TMB and survival across a broad variety of cancers in a University of California San Diego immunotherapy-naïve patient cohort.

For this purpose we modeled TMB both tiered as low (≤5 mutations/megabase), intermediate (≥6 and <20), high (≥20 and <50), and very high (≥50), as per cut points from the literature (2), and also as a continuous variable, correcting for age, sex, ethnicity, smoking, and tissue of origin of the primary tumor in multivariate models. As prior work had demonstrated a linear relationship between TMB and response to PD-1/PD-L1 blockade (2, 4), our primary analyses studied 1,415 patients who had not received these immunotherapy agents (Supplementary Fig. S1).

Materials and Methods

Subject details

We studied 1,926 patients seen at the UC San Diego Moores Center for Personalized Cancer Therapy with reported sequencing starting from November 2012. Of these 1,926 patients, 1,526 could be included for TMB and survival analysis and 1,415 had never received immune checkpoint blockade. Patients were excluded if they were missing tumor mutational burden (TMB) evaluation, had biopsy samples with pathologic purity <20% by pathology review, had sequencing samples not meeting previously described NGS computational standards (e.g., <250× median exonic sequencing coverage; ref. 5), or if their cancer was not locally advanced or metastasized at the time of this study (unless they had brain tumors or hematologic cancers, in which case their data was retained). Median tumor purity in each of the four TMB tiers (low, intermediate, high and very high) was 30%. This study was performed in accordance with UCSD institutional review board guidelines for data analysis (NCT02478931) and for any investigational treatment for which patients gave informed and written consent. All survival and demographic data were collected by chart review of the electronic medical record.

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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Evaluation of tumor mutational burden

TMB (mutations per megabase) was calculated by interrogating 1.2 Mb of the genome to quantify somatic (defined by an industry-standard somatic-germline-zygosity algorithm), nondriver mutations (as listed in COSMIC) in coding regions, and extrapolating this value to the whole exome. Prior work (6) has demonstrated that whole-exome TMB (defined as any base substitution or indel mutation in a coding region) can be estimated very accurately and reliably ($R2 = 0.74$ and 0.98, respectively) across a broad variety of cancers using this method, allowing clinic-standard comprehensive genomic profiling to be applied to quantification of TMB.

Analysis of TMB as a continuous variable was performed using natural log-transformation to correct for the nonnormal distribution of TMB in this cohort. TMB in binned analyses defined low TMB as ≤5, intermediate TMB >5 and <20, high TMB as ≥20 and <50, and very high TMB as ≥50 mutations/Mb with cutoffs as per prior publications (6). Patients were considered to have received immunotherapy if, at any point, they were given a checkpoint inhibitor or IL2. These patients were excluded in any analysis that specified "excludes patients treated with immunotherapy."

Quantification and statistical analysis

All clinical variables (date of diagnosis, tissue of origin, date of advanced disease, date of metastasis, treatment with immunotherapy, age, smoking status, gender, etc.) were obtained by chart review under UCSD PREDICT IRB protocol (NCT02478931). For all cancers, date of diagnosis was as defined by date of pathologic diagnosis. Locally advanced disease for brain and hematologic malignancies was also defined as date of diagnosis. For patients with radiologic evidence of metastasis or locally advanced disease prior to pathologic diagnosis, date of local advanced disease or metastasis was defined as date of diagnosis.

Patient age was defined as the age at time of diagnosis, and was treated as a binary (younger than sixty, or 60 and older) in analyses. Smoking status was as recorded by the physician, and patients with no recorded smoking status were treated as nonsmokers. Ethnicity was as self-reported by patients, and "Other" ethnicity included "Other" as described by patient as well as Pacific Islander, American-Indian, multiracial, unknown, and missing. Patients were recorded as dead either from UC San Diego electronic medical records, or via logged communications from family or outside residential or medical facilities. Reference groups were selected as follows: for ordinal variables, the lowest order; for exposures, the nonexposed; for all others, the most common group.

Time-to-event analyses were performed using Cox proportional hazards regression and/or Kaplan–Meier analysis as appropriate. Time was measured in weeks from locally advanced or metastatic disease unless otherwise specified. All analyses used all-cause death as the event of interest. If patients were alive at last follow up, they were censored for survival on that date. In tables in which multiple hypothesis tests were utilized, two-sided P values were bolded if found to be significant by Bonferroni-corrected α of ≤0.05 (7).

To visually represent the change in the OS HR with changes in the log(TMB), we fit a quadratic TMB model, and then plotted the predicted HR with respect to TMB on a semilogarithmic plot (with reference to TMB = 0) and with TMB ranging from the observed minimum to maximum TMB in our cohort (red curve, **Fig. 1B**). To represent the effect of age, ethnicity, smoking, and primary tissue on the quadratic TMB coefficients, we fit a new cox regression including these variables, calculating predicted HRs for each TMB value, subtracted the mean effect from the non-TMB predictors, and plotted these values (blue curve, **Fig. 1B**). Finally, to visualize both the distribution of TMB within each primary cancer type, and the relative effect of age, ethnicity, smoking, and primary cancer type, we plotted each individual patient's predicted HR (as compared with TMB = 0 and reference group for each of these **Table 1**) for OS on a semilogarithmic plot, color-coding based on the grouped cancer types (**Fig. 1C**).

For internal validation of our primary hypothesis, we utilized bootstrap resampling to generate P values. Bootstrapping functions by using random resampling of the original dataset to create a large number (in our case, 1,000) of "phantom datasets." Then, the same regression analysis is run on each of these new datasets to generate the output of interest (e.g., P-value), which is then averaged from each of these many analyses. This method uses a computationally-intensive approach to avoid pitfalls like assumptions of normal distribution, and also allow for the data from a single cohort to be more easily modeled to a larger population. Although external validation in an independent cohort remains the gold standard, bootstrapping can be utilized when or there is no such available cohort (as was the case in our study; ref. 8).

Data utilized in this study involves protected healthcare information. A supplementary data spreadsheet is available with de-identified data used in for this publication. All analyses were performed with R (www.r-project.org) using publicly available packages, and the methods verified by our biostatistician (DAB). We have included the code for our figures in a supplement (Supplementary Script S1) for authors seeking to produce similar figures in the future.

Results

Of our 1,526 patients, we found that only 111 (7%) had received immunotherapy. A total of 634 (42%) of patients were 60 years of age or older at time of sequencing, and 775 (51%) patients were women. Non-Hispanic white (NHW) ethnicity was the most common ($N =$ 1052, 69%), followed by Hispanic ($N = 207$, 14%), Asian ($N = 147$, 10%), African-American ($N = 55$, 4%), and Other ($N = 65$, 4%). A total of 611 (40%) patients had a history of tobacco smoking. Regarding the primary site of malignancy, "other" was the most common tumor type which included (alphabetically): adrenal gland ($N = 3$, 0.2% of patients overall), ampulla ($N = 1$, 0.1%), anus ($N = 8$, 0.5%), appendix ($N = 42$, 2.8%), bladder ($N = 18$, 1.2%), cervix ($N = 3$, 0.2%), choroid ($N = 1$, 0.1%), endometrium ($N = 20$, 1.3%), esophagus ($N = 22$, 1.4%), eye ($N = 4$, 0.3%), gallbladder ($N = 12$, 0.8%), head/neck ($N = 71$, 4.7%), kidney ($N = 16$, 1.0%), liver ($N = 39$, 2.6%), mesentery ($N = 2$, 0.1%), ovary ($N = 43$, 2.8%), pancreas ($N = 38$, 2.5%), peritoneum ($N = 12$, 7.9%), prostate ($N = 16$, 1.0%), small intestine ($N = 24$, 1.6%), soft tissue ($N = 34$, 2.2%), stomach ($N = 31$, 2.0%), testis ($N = 1$, 0.1%), thymus ($N = 3$, 0.2%), thyroid ($N = 38$, 2.5%), vulva ($N = 7$, 0.5%), and unknown primary ($N = 28$, 1.8%). Outside of this category, hematologic malignancies were the most common ($N = 205$, 13%), followed by lung ($N = 171$, 11%), brain ($N = 160$, 10%), breast ($N = 158$, 10%), colon/rectum ($N = 148$, 10%), and cutaneous ($N = 98$, 6%; **Table 1**).

Of our 1,415 immunotherapy-naïve patients, 68% had low TMB, 25% intermediate, 4% high, and 3% very high (**Table 2**), similar to previously published data in over 62,000 patients (6). Median age was 57 (interquartile range 45–66.5). Age ≥60 years, NHW ethnicity, smoking, and primary cutaneous and lung cancers were all associated with significantly higher distributions of TMBs (with Bonferroni correction for multiple comparisons), whereas primary brain and hematologic cancers were associated with lower TMBs (**Table 2**). Median survival decreased from 238 weeks in the low TMB group to 174 weeks in the intermediate group, after which it increased to

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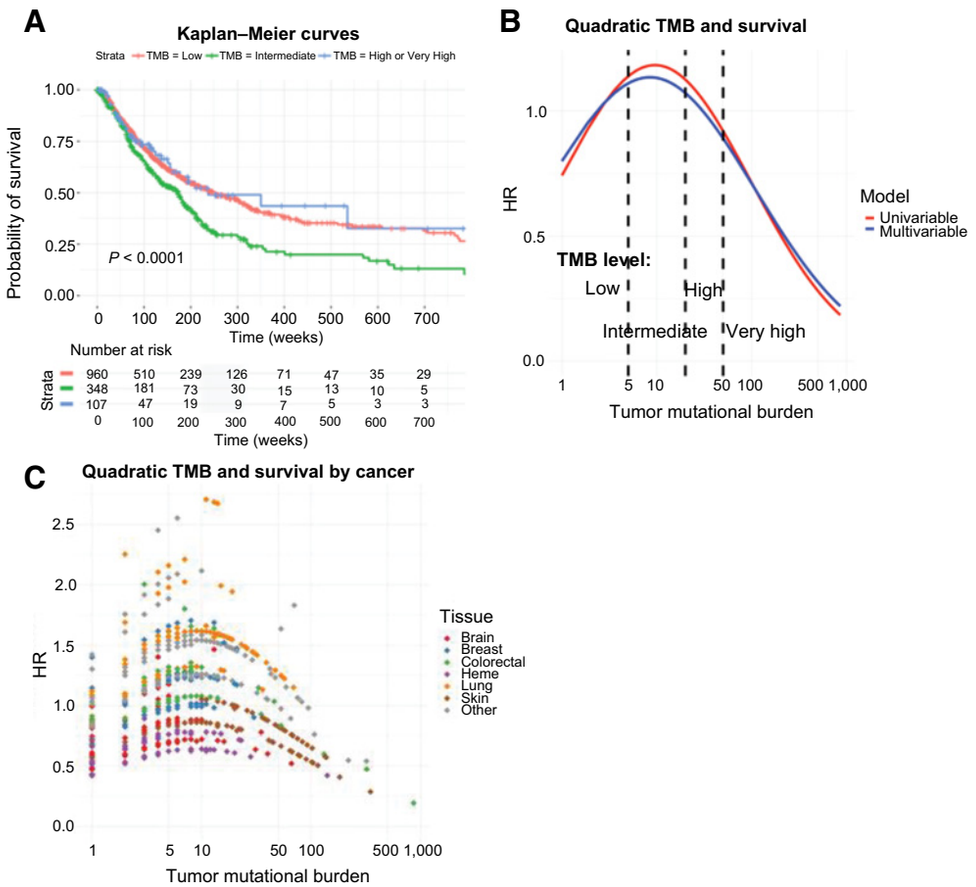

Figure 1.

Survival and TMB (excluding immunotherapy-treated patients; $N = 1{,}415$ patients). **A,** Survival curve with Kaplan–Meier analysis comparing low (≤5 mutations/Mb), intermediate (>5 and ≤20 mutations/Mb), and high/very-high tiered (>20 mutations/Mb) TMB. Low and high/very-high TMB tiers find increased survival compared with intermediate tiered TMB. **B,** HR plotted against log-adjusted TMB from univariate and multivariate polynomial Cox regressions. The HR initially increases with higher TMB and then decreases in an inverted U-shape relationship. Linear log and quadratic log TMB are correlated with HR ($P = 6.23 \times 10^{-3}$ and 2.83×10^{-3}, respectively). Low TMB defined as ≤5 mutations/Mb, intermediate TMB defined as >5 and ≤20/Mb, high TMB defined as >20 and ≤50 Mb, and very high TMB defined as >50 mutations/Mb. **C,** HR plotted against log-adjusted TMB from multivariate polynomial Cox proportional hazards. Points represent the log(TMB) from the patient population. The inverted U curve showing increasing HR for death with increasing TMB followed by a decreasing HR is maintained regardless of the covariate that was analyzed. Intermediate TMB fares significantly worse than low and high/very high TMB for OS ($P < 0.001$ and $P = 0.018$, respectively). See Supplementary Table S2 for coefficients.

195 weeks in the high TMB group and 350 in the very high TMB group. A similar effect was observed when measuring survival from the date of diagnostic biopsy (**Table 2**).

To quantitatively evaluate this apparent nonlinear relationship between survival and TMB, we first studied TMB as a discreet variable organized into four tiers as described above (**Table 3**). Our primary endpoint was survival from time of advanced disease. Statistically significant variables in univariable models (by log-rank test with Bonferroni-corrected $\alpha < 0.05$) were incorporated into the multivariable model. These included age, smoking, ethnicity, and primary tissue of origin, in addition to TMB tier. Intermediate TMB resulted in a decreased survival as compared with low TMB [HR = 1.29; 95% confidence interval (CI), 1.07–1.54; multivariate, $P < 0.01$; **Table 1**]. As TMB increased to high and very high levels, the HR returned to baseline (multivariate P for high and very high TMB as compared with low TMB = 0.90 and 0.15, respectively; with very high TMB trending towards protective effect). This finding was robust to a bootstrap resampling study of internal validation with 1,000 iterations. Comparing intermediate TMB to high/very-high TMB found that intermediate TMB fared significantly worse, with HR 1.53 (95% CI, 1.08–2.18; $P = 0.018$). Survival curves demonstrate that intermediate-range TMB survival curve is significantly worse than other tiers, whereas low and grouped high TMB survival curves remain indistinguishable from one another (**Fig. 1A**). Median overall survival for the patients with low, intermediate, and high/very high TMB was 238, 174, and 237 weeks ($P < 0.0001$), respectively.

TMB was subsequently studied as a continuous variable using log-transformed TMB in quadratic univariate and multivariate models, to decrease potential artifact from subjective tiering of TMB. These analyses demonstrated an increased risk of mortality with intermediate range increases in TMB, and then decreased risk of mortality with the higher ranges of TMB. Indeed, visual representation of these models (**Fig. 1B** and **C**; Supplementary Table S4) plotting predicted HRs against TMB finds inverse parabolic "U-shaped" curves suggesting that as TMB increases the HR for death initially rises as well, but that at higher TMB this effect is reversed. This parabolic relationship is

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Table 1. Patient demographics ($N = 1{,}526$ patients; includes 111 patients treated with immunotherapy).

Variable	Group	Patients all TMB, *N* (%)	Immunotherapy treated, *N* (%)	TMB level, *N* (%)[a]				*P*[b]
				Low	Intermediate	High	Very high	
Overall	Patients	1,526	111 (7%)	1,034 (68%)	337 (25%)	62 (4%)	53 (3%)	
Age, y	<60	892 (58%)	66 (7%)	649 (73%)	198 (22%)	23 (3%)	22 (2%)	$\mathbf{1.21 \times 10^{-7}}$
	≥60	634 (42%)	45 (7%)	385 (61%)	179 (28%)	39 (6%)	31 (5%)	
Sex	Women	775 (51%)	59 (8%)	523 (67%)	208 (27%)	29 (4%)	15 (2%)	0.74
	Men	751 (49%)	52 (7%)	511 (68%)	169 (23%)	33 (4%)	38 (5%)	
Ethnicity	African-American	55 (4%)	2 (4%)	33 (60%)	20 (36%)	2 (4%)	0 (0%)	0.36
	Asian	147 (10%)	10 (7%)	115 (78%)	28 (19%)	3 (2%)	1 (1%)	2.4×10^{-3}
	Hispanic	207 (14%)	13 (6%)	157 (76%)	44 (21%)	5 (2%)	1 (0%)	3.1×10^{-3}
	Other	65 (4%)	5 (8%)	46 (71%)	17 (26%)	0 (0%)	2 (3%)	0.48
	NHW	1,052 (69%)	81 (8%)	683 (65%)	268 (25%)	52 (5%)	49 (5%)	$\mathbf{5.0 \times 10^{-5}}$
Smoking history	No	915 (60%)	67 (7%)	670 (73%)	198 (22%)	24 (3%)	23 (3%)	$\mathbf{4.5 \times 10^{-9}}$
	Yes	611 (40%)	44 (7%)	364 (60%)	179 (29%)	38 (6%)	30 (5%)	
Type of cancer	Brain	160 (10%)	10 (6%)	129 (81%)	27 (17%)	3 (2%)	1 (1%)	$\mathbf{1.3 \times 10^{-4}}$
	Breast	158 (10%)	22 (14%)	106 (67%)	48 (30%)	4 (3%)	0 (0%)	0.76
	Colon/rectum	148 (10%)	7 (5%)	93 (63%)	47 (32%)	3 (2%)	5 (3%)	0.29
	Hematologic	205 (13%)	4 (2%)	173 (84%)	26 (13%)	5 (2%)	1 (0%)	$\mathbf{3.9 \times 10^{-8}}$
	Lung	171 (11%)	11 (6%)	89 (52%)	69 (40%)	9 (5%)	4 (2%)	$\mathbf{2.3 \times 10^{-5}}$
	Cutaneous	98 (6%)	8 (8%)	23 (23%)	25 (26%)	21 (21%)	29 (30%)	$\mathbf{2.2 \times 10^{-16}}$
	Other	586 (38%)	49 (8%)	421 (72%)	135 (23%)	17 (3%)	13 (2%)	$\mathbf{3.0 \times 10^{-3}}$

	Group	Low TMB	Intermediate TMB	High TMB	Very high TMB
Median OS (weeks) by Cox (95% CI)	From biopsy[c]	155 (137–184)	101 (83–131)	151 (105–NA)	384 (155–NA)
	From advanced disease	239 (209–284)	174 (136–190)	192 (151–NA)	350 (209–NA)

Abbreviation: NA, not applicable.
[a]Low TMB defined as ≤5/Mb, intermediate TMB defined as >5 and ≤20/Mb, high TMB defined as >20 and ≤50/Mb, and very high TMB defined as >50 mutations/Mb.
[b]Probability calculated from Kruskal–Wallis as appropriate with aggregates used as reference for variables with >2 categories; significant values with Bonferroni corrected α are bolded. *P* values for weeks followed represent distribution of time followed between the TMB levels.
[c]Patients missing date of biopsy were omitted.

preserved when correcting for other significant ($P = 0.004$) variables in backwards stepwise Cox proportional hazards regression. These findings also correlate well with the ranges studied in tiered TMB analysis, as the HR returns to that of the low TMB within the high-range TMB.

We separately studied the relationship between tumor mutational burden and microsatellite instability (MSI), using the tiered TMB approach and MSI status (stable, ambiguous, or high) in the 767 patients who had a known MSI status from sequencing. We found that all high MSI patients had high or very-high TMB (20% of high TMB and 25% of very-high TMB had high MSI; $P < 0.001$, Fisher exact test).

To assess the effect of patients receiving biopsies for NGS at variable times in their treatment course, we performed sensitivity analyses on all above tiered and continuous TMB regressions using survival from date of biopsy. We additionally performed a sensitivity analysis replicating all studies adding the 111 sequenced patients who had received checkpoint blockade immunotherapy (total $N = 1526$). None of these resulted in changes to the conclusions or effect size of the study (Supplementary Tables S1, S3, and S5; Supplementary Figs. S2–S4).

To evaluate for possible confounding from primary cancer type, we studied the interaction between this and TMB tier, finding it to be nonsignificant ($P = 0.299$, likelihood ratio test). Furthermore, to evaluate the potential of confounding from CNS and hematologic malignancies (both of which had a preponderance towards low TMB), we performed a sensitivity analysis removing these two primary sites from the cohort. We found that (as compared with low TMB), intermediate TMB still had decreased survival (HR = 1.32; 95% CI, 1.09–1.60), and that high and very high TMB patients did not have a statistically significant difference in survival compared with low TMB (HR = 1.11; 95% CI, 0.73–1.70 and HR = 0.60; 95% CI, 0.34–1.08, respectively). These suggest that our findings are robust from hypothetical confounding from these two primary sites.

Discussion

Our results demonstrate that TMB correlates with survival in a range-dependent manner, such that intermediate-range TMB is associated with increased risk of death whereas higher-range TMB gradually confers decreased risk, ultimately associating with a protective effect. These effects are tissue agnostic (**Fig. 1C**) similar to several other predictors of immune response (9). However, this pattern (inverted U with highest hazard of death in intermediate TMB) can also be visualized for individual histologies in our dataset (**Fig. 1C**; Supplementary Figs. S2B and S3B; but statistical analysis of these individual histologies is limited by small patient numbers). Previously, another study in resected localized non–small cell lung cancer documented the correlation between a high nonsynonymous TMB and favorable disease-free and overall lung cancer survival, similar our findings, albeit singularly in this primary disease site (10). In our study, the modest change in HR (**Fig. 1B**; **Table 1**) for intermediate-range TMB when correcting for known confounders suggests that TMB is an independent deleterious prognostic indicator in the advanced cancer setting.

This study was limited by the low number of patients with high or very high TMB (7% of patients; $N = 115$), and also by the lower

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Table 2. Distribution of TMB across cohort (excluding patients treated with immunotherapy; $N = 1{,}415$ patients).

Variable	Group	Patients all TMB, *N* (%)	TMB Level, *N* (%)[a]				*P*[b]
			Low	Intermediate	High	Very high	
Overall	Patients	1,415	960 (68%)	348 (25%)	58 (4%)	49 (3%)	
Age, y	<60	826 (58%)	599 (73%)	184 (22%)	22 (3%)	21 (3%)	$\mathbf{5.1 \times 10^{-6}}$
	≥60	589 (42%)	361 (61%)	164 (28%)	36 (6%)	28 (5%)	
Sex	Women	716 (51%)	485 (68%)	188 (26%)	29 (4%)	14 (2%)	0.71
	Men	699 (49%)	475 (68%)	160 (23%)	29 (4%)	35 (5%)	
Ethnicity	African-American	53 (4%)	33 (62%)	18 (34%)	2 (4%)	0 (0%)	0.55
	Asian	137 (10%)	106 (77%)	27 (20%)	3 (2%)	1 (1%)	7.1×10^{-3}
	Hispanic	194 (14%)	147 (76%)	41 (21%)	5 (3%)	1 (1%)	5.2×10^{-3}
	Other	60 (4%)	42 (70%)	16 (27%)	0 (0%)	2 (3%)	0.59
	NHW	971 (69%)	632 (65%)	246 (25%)	48 (5%)	45 (5%)	$\mathbf{1.6 \times 10^{-4}}$
Smoking history	No	848 (60%)	625 (74%)	180 (21%)	21 (2%)	22 (3%)	$\mathbf{1.8 \times 10^{-9}}$
	Yes	567 (40%)	335 (59%)	168 (30%)	37 (7%)	27 (5%)	
Type of cancer	Brain	150 (11%)	120 (80%)	26 (17%)	3 (2%)	1 (1%)	$\mathbf{4.4 \times 10^{-4}}$
	Breast	136 (10%)	93 (68%)	39 (29%)	4 (3%)	0 (0%)	0.59
	Colon/rectum	141 (10%)	90 (64%)	44 (31%)	3 (2%)	4 (3%)	0.44
	Hematologic	201 (14%)	170 (85%)	26 (13%)	4 (2%)	1 (0%)	$\mathbf{3.0 \times 10^{-8}}$
	Lung	160 (11%)	82 (51%)	65 (41%)	9 (6%)	4 (3%)	$\mathbf{1.3 \times 10^{-5}}$
	Cutaneous	90 (6%)	22 (24%)	23 (26%)	18 (20%)	27 (30%)	$\mathbf{2.2 \times 10^{-16}}$
	Other	537 (38%)	383 (71%)	125 (23%)	17 (3%)	12 (2%)	0.01

	Group	Low TMB	Intermediate TMB	High TMB	Very high TMB
Median OS (weeks) by Cox (95% CI)	From biopsy[c]	157 (137–188)	97 (79–130)	151 (105–NA)	384 (166–NA)
	From advanced disease	238 (211–306)	174 (136–190)	195 (125–NA)	350 (209–NA)

Abbreviation: NA, not applicable.

[a]Low TMB defined as ≤5/Mb, intermediate TMB defined as >5 and ≤20/Mb, high TMB defined as >20 and ≤50/Mb, and very high TMB defined as >50 mutations/Mb.

[b]Probability calculated from Kruskal–Wallis as appropriate with aggregates used as reference for variables with >2 categories; significant values with Bonferroni corrected α are bolded. *P* values for weeks followed represent distribution of time followed between the TMB levels.

[c]Patients missing date of biopsy were omitted.

diversity of cancers represented at higher ranges of TMB (with many of these patients having relatively favorable cutaneous primary tumors). However, multivariate analysis studying the interaction between TMB and primary tissue found this to be nonsignificant ($P = 0.299$). Given the heterogeneity of clinical practice in precision oncology and the diversity of patients with advanced malignancies undergoing genomic sequencing, the single-institution nature of this study is a major limitation. In addition, although TMB is not calculated on the basis of known driver mutations, there may be differences in the number of actionable alterations across the TMB groups. However, it is reassuring that recent publications on high TMB in TCGA (11–14), in more restricted patient groups (such as melanoma, endometrial cancer or ovarian cancer) had similar findings to ours (although these reports did not eliminate immunotherapy-treated patients). Nonetheless, there remains a need for future studies to replicate our findings on intermediate-range TMB in an external cohort. Future studies should also evaluate for interactions between other therapies (e.g., chemotherapy, targeted agents, surgery, and radiation) and TMB, particularly as the genomic instability associated with high/very high TMB could sensitize tumors to DNA-damaging therapies.

The underlying biology accounting for the U-shaped risk of death is an area of active investigation, possibly mediated by endogenous immune mechanisms via increased neoantigen production (1) or reduced cell viability via genetic instability (3). The immunologic explanation is especially compelling: previous data demonstrate that in select cancers tumor immune cell infiltration is associated with improved prognosis only in the presence of high TMB (14), and clinical studies in immunotherapy show better responses to checkpoint inhibitor therapy with higher TMB (2, 3), regardless if TMB was calculated from sequencing of tissue or cell-free liquid biopsy (4), suggesting that TMB is a promising addition to other markers of immunotherapy response prediction. Presumably, the relationship between higher TMB and immunotherapy response is the result of more robust activation of cytotoxic T-lymphocytes due to mutanome-generated neo-antigens, permitting eradication of the malignant cells; similarly, we hypothesize that higher TMB, even in the absence of immunotherapy, elicits an innate immune response that attenuates the risk of death. Of interest in this regard, Andor and colleagues (15) demonstrated that copy-number alterations affecting either <25% or >75% of a tumor's genome predicted reduced risk of mortality and that risk of death also decreased when >4 clones (reflecting greater intratumor heterogeneity) coexisted in a malignancy. An intriguing alternative hypothesis for our parabolic relationship is that increasing TMB from low to intermediate levels would decrease survival initially because of the presence of multiple oncogenic drivers (the "mutator phenotype"; ref. 16), and that this effect would reach a maximum at an intermediate-range TMB, after which survival would increase with TMB.

Taken together, our data demonstrate a dynamic interplay between the advantages and disadvantages of genomic instability. We revealed a

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Table 3. Univariate and multivariate analyses of survival from locally advanced or metastatic disease (excluding patients treated with immunotherapy; $N = 1{,}415$ patients).[a]

Variable	Group	Patients, *N* (%)	Median survival (weeks)	HR OS (95% CI)	*P*[a] univariate
Age, y	<60	826 (58%)	250	Reference Group	
	≥60	589 (42%)	170	1.57 (1.33–1.84)	**3.83×10^{-8}**
Sex	Women	716 (51%)	189	Reference Group	
	Men	699 (49%)	172	1.07 (0.91–1.25)	0.41
Ethnicity	African-American	53 (4%)	257	1.03 (0.67–1.60)	**5.1×10^{-3}**
	Asian	137 (10%)	170	1.31 (1.02–1.68)	
	Hispanic	194 (14%)	213	0.95 (0.75–1.21)	
	Other	60 (4%)	92	1.80 (1.26–2.58)	
	NHW	971 (69%)	212	Reference Group	
Smoking history	No	848 (60%)	234	Reference Group	
	Yes	567 (40%)	187	1.22 (1.05–1.43)	0.01
Tumor type	Brain[b]	150 (11%)	697	0.61 (0.46–0.80)	**1.65×10^{-8}**
	Breast	136 (10%)	214	0.86 (0.67–1.11)	
	Colon/rectum	141 (10%)	174	0.91 (0.69–1.19)	
	Hematologic	201 (14%)	707	0.48 (0.37–0.63)	
	Lung	160 (11%)	146	1.15 (0.89–1.48)	
	Cutaneous	90 (6%)	535	0.59 (0.40–0.86)	
	Other	537 (38%)	177	Reference Group	
TMB level	Low (≤5 mutations/Mb)	960 (68%)	238	Reference Group	
	Intermediate (≥6 and <20 mutations/Mb)	348 (25%)	174	1.44 (1.21–1.71)	**1.8×10^{-4}**
	High (≥20 and <50 mutations/Mb)	58 (4%)	195	1.12 (0.75–1.67)	
	Very high (≥50 mutations/Mb)	49 (3%)	350	0.73 (0.43–1.25)	

Variable	Group	HR (95% CI)	*P*[a] multivariate	Bootstrap[c] *P* multivariate
Age, y	<60		Reference group	
	≥60	1.54 (1.30–1.84)	**9.42×10^{-7}**	**6.1×10^{-4}**
Smoking history	No		Reference group	
	Yes	1.15 (0.98–1.36)	0.09	0.20
Ethnicity	African-American	1.06 (0.70–1.60)	0.78	0.49
	Asian	1.30 (1.00–1.67)	0.05	0.15
	Hispanic	1.11 (0.87–1.42)	0.41	0.43
	Other	1.71 (1.19–2.47)	**3.9×10^{-3}**	**0.05**
	NHW		Reference group	
Tumor type	Brain	0.61 (0.46–0.81)	**7.3×10^{-4}**	**0.02**
	Breast	0.93 (0.72–1.20)	0.58	0.45
	Colon/rectum	0.94 (0.71–1.25)	0.66	0.48
	Hematologic	0.49 (0.37–0.64)	**2.2×10^{-7}**	**2.2×10^{-4}**
	Lung	0.97 (0.75–1.27)	0.84	0.49
	Cutaneous	0.76 (0.50–1.15)	0.19	0.29
	Other		Reference group	
TMB level	Low (≤5 mutations/Mb)		Reference group	
	Intermediate (≥6 and <20 mutations/Mb)	1.29 (1.07–1.54)	**5.4×10^{-3}**	**0.05**
	High (≥20 and <50 mutations/Mb)	0.98 (0.63–1.50)	0.90	0.49
	Very high (≥50 mutations/Mb)	0.65 (0.36–1.35)	0.15	0.25

Note: All survival data are calculated from the time of advanced disease; patients with local disease only were not included in the analysis unless they had brain tumors or hematologic malignancies that were considered advanced disease at diagnosis.

[a]Bolded *P* values represent $P \leq 0.05$, or equivalent significance with Bonferroni correction for multiple hypotheses as appropriate in multivariate analysis. Results demonstrated that tiered TMB confers an increased risk of death with intermediate-range TMB, which returns to baseline risk at higher levels, even trending toward a protective effect at "high" and "very high" TMB tier (Supplementary Table S1 is a similar analysis that includes immunotherapy-treated patients). *P* value for multilevel factor variables in univariable analysis derived from likelihood ratio test.

[b]Brain tumors included 83 high-grade tumors, 70 grade III or less, and 7 nonglial tumors.

[c]Bootstrapped *P* values were generated using random resampling to create 1,000 computer-generated datasets.

novel parabolic correlation between TMB and survival, where patients with intermediate-range TMB had decreased survival whereas patients with low and very high TMB had similar mortality. As this analysis was performed in patients who had not received immunotherapy, TMB appears to have a prognostic relationship with survival independent of immunotherapy or systemic therapy type. Further investigation into the prognostic capability of TMB and mechanisms underlying this relationship are deserved.

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Disclosure of Potential Conflicts of Interest

P. Riviere reports personal fees from Peptide Logic LLC (consulting) outside the submitted work. A.M. Goodman reports personal fees from Seattle Genetics (consulting) and EUSA Pharma (consulting) outside the submitted work. D. Fabrizio reports other from Foundation Medicine (employee and stock holder) outside the submitted work, as well as a patent for "Methods for calculating tumor mutational burden" pending. A.B. Sharabi reports personal fees from AstraZeneca, grants from Varian Medical Systems, grants from Pfizer, and personal fees from Jounce Therapeutics outside the submitted work, and is a scientific founder with equity interest in Toragen, Inc. outside the submitted work. S. Kato reports other from Foundation Medicine (consulting) and Roche (speaker) outside the submitted work, as well as consulting for Foundation Medicine (speaker's fee: Roche; research grant: ACT Genomics, Sysmex, Konica Minolta, OmniSeq). R. Kurzrock reports stock and other equity interests in IDbyDNA, CureMatch, Inc., and Soluventis; consulting or advisory role for Gaido, LOXO, X-Biotech, Actuate Therapeutics, Roche, NeoMed, Soluventis, Pfizer, and Merck; speaker's fee from Roche; research funding for Incyte, Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, Guardant Health, Grifols, Konica Minolta, DeBiopharm, Boerhringer Ingelheim, and OmniSeq (all institutional); and serving as a board member for CureMatch, Inc., and CureMetrix, Inc. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH (P. Riviere).

Authors' Contributions

P. Riviere: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing-original draft, writing-review and editing. **A.M. Goodman:** Conceptualization, resources, data curation, supervision, investigation, methodology, writing-original draft, writing-review and editing. **R. Okamura:** Data curation, investigation, methodology, writing-review and editing. **D.A. Barkauskas:** Software, formal analysis, supervision, methodology, writing-review and editing. **T.J. Whitchurch:** Data curation, investigation, writing-review and editing. **S. Lee:** Resources, data curation, writing-review and editing. **N. Khalid:** Data curation, writing-review and editing. **R. Collier:** Data curation, writing-review and editing. **M. Marebiona:** Data curation, writing-review and editing. **G.M. Frampton:** Conceptualization, resources, writing-review and editing. **D. Fabrizio:** Resources, methodology, writing-review and editing. **A.B. Sharabi:** Conceptualization, writing-original draft, writing-review and editing. **S. Kato:** Conceptualization, resources, data curation, supervision, methodology, writing-review and editing. **R. Kurzrock:** Conceptualization, resources, supervision, validation, investigation, methodology, writing-original draft, writing-review and editing.

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Molecular Cancer Therapeutics

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