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Author Contributions

RMS, RS, LGTM performed the analyses. CHL, ANS, MDH, YYJ, DAB, SMK, EJJ provided clinical annotations. AZ, MFB, DBS, ML, JB established the assays and coordinated data collection. CHL, ANS, MDH, YYJ, RJM, GJR, CAB, SB, PR, HAA, JR, DFB,AAH,BHB, MHV, CMR, HIS, ALH, DGP, NL, RJW, VT ,PHG, CB, LMD,IKM, JEC, RY, NHS, RPD, LS, JDW, CEA, ZKS, WDT, MMT,NAR, SD, JB, PR, GYK, DBS contributed to sample acquisition and patient recruitment. LGTM, DS, NR and TAC supervised the study. LGTM, TAC, CHL, ANS, MDH,and RMS wrote the manuscript with contributions from all authors.

Data Availability

All data is publicly available at [http://www.cbioportal.org/study?id=tmb\\_mskcc\\_2018](http://www.cbioportal.org/study?id=tmb_mskcc_2018).

Competing Interests Statement

RMS, TAC and LGTM are inventors on a provisional patent application (62/569,053) filed by MSK, relating to the use of TMB in cancer immunotherapy.MDH, NAR and TAC are inventors on a PCT patent application (PCT/US2015/062208) filed by MSK, relating to the use of TMB in lung cancer immunotherapy.MSK and the inventors may receive a share of commercialization revenue from license agreements relating to these patent applications. CHL received research funding from Eisai, BMS, Exelixis, Pfizer, Calithera and consulting fees from Exelixis and Eisai. ANS has received research support from Bristol Myers Squibb, Immunocore, AstraZeneca, Xcovery and serves on the advisory board for Bristol Myers Squibb, Immunocore, Castle Biosciences; he also receives royalties from UpToDate. MDH receives research funding from Bristol-Myers Squibb; is paid consultant to Merck, Bristol-Myers Squibb, AstraZeneca, Genentech/Roche, Janssen, Nektar, Syndax, Mirati, and Shattuck Labs. YYJ received research funding from Boehringer Ingelheim, Bayer, Genentech/Roche, Bristol-Myers Squibb, Eli Lilly, Merck and served on advisory boards for Merck Serono, Bristol-Myers Squibb, Eli Lilly, Pfizer, Bayer, Imugene, Merck. SB currently works for Flatiron Health which is a for-profit company. RJM received research support from Pfizer, Genentech/Roche, Bristol Myers Squibb and Eisai and consulting fees from Pfizer, Genentech/Roche, Merck, Incyte, Novartis, Eisai and Exelixis. MHV received commercial research grants from Bristol-Myers Squibb and Genentech/Roche; honoraria from Novartis; travel/accommodation from Eisai, Novartis and Takeda; consultant/advisory board member for- Alexion Pharmaceuticals, Bayer, Calithera Biosciences, Corvus Pharmaceuticals, Exelixis, Eisai, GlaxoSmithKline, Natera; Novartis and Pfizer.

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# Tumor mutational load predicts survival after immunotherapy across multiple cancer types

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## Abstract

Immune checkpoint inhibitor (ICI) treatments benefit some patients with metastatic cancers, but predictive biomarkers are needed. Findings in select cancer types suggest that tumor mutational burden (TMB) may predict clinical response to ICI. To examine this association more broadly, we analyzed the clinical and genomic data of 1662 advanced cancer patients treated with ICI, and 5371 non-ICI treated patients, whose tumors underwent targeted next-generation sequencing (MSK-IMPACT). Among all patients, higher somatic TMB (highest 20% in each histology) was associated with better OS (HR 0.52;  $p=1.6 \times 10^{-6}$ ). For most cancer histologies, an association between higher TMB and improved survival was observed. The TMB cutpoints associated with improved survival varied markedly between cancer types. These data indicate that TMB is associated with improved survival in patients receiving ICI across a wide variety of cancer types, but that there may not be one universal definition of high TMB.

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In recent years, immune checkpoint inhibitors (ICI) have revolutionized the treatment of patients with advanced stage cancers. These agents include antibodies that target CTLA4 or PD-1/PD-L1.<sup>1</sup> Durable benefit, however, is limited to a minority of patients. Recently, several large phase 3 trials have reported negative results both in unselected patients and some selected groups, highlighting the clinical need to identify better predictive biomarkers.<sup>2–5</sup> Early reports have suggested that PD-L1 immunohistochemistry, T cell infiltration levels, T-cell receptor clonality, gene expression signatures, and peripheral blood markers may correlate with clinical response.<sup>6</sup> Additionally, an association between high mutational load and clinical benefit was observed in small cohorts of melanoma patients treated with CTLA4 blockade,<sup>7,8</sup> and non-small cell lung cancer (NSCLC), melanoma, and bladder cancer patients treated with PD-1/PD-L1 inhibitors.<sup>9–11</sup> However, it is unclear whether TMB is robustly predictive of clinical benefit across diverse human cancers, or outside of these specific clinical trial populations.

In prior studies, mutation load was determined using whole exome sequencing, which is not widely utilized in routine clinical care. Currently, the majority of precision oncology platforms utilize next-generation sequencing of targeted gene panels. At Memorial Sloan Kettering Cancer Center, as part of clinical care, patients undergo genomic profiling using the FDA authorized Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay.<sup>12</sup> This test is performed in a CLIA environment and identifies somatic

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exonic mutations in a pre-defined subset of 468 cancer-related genes (earlier versions included 341 or 410 genes), using both tumor-derived and matched germline normal DNA.

We examined the association between non-synonymous somatic TMB, as measured by MSK-IMPACT, and overall survival (OS) after treatment with ICI. The cohort included 1662 patients whose tumors were profiled by next generation sequencing and who received at least one dose of ICI therapy, representing a variety of cancer types with sufficient number of patients for analysis (Supp-Fig 1). Patients who received atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab, pembrolizumab, or tremelimumab as monotherapy or in combination were included in the study. The vast majority of patients (1446, 94% of tumors excluding glioma) had stage IV or metastatic disease. A small number of patients had locally recurrent disease (n=10), or were melanoma patients with regionally advanced unresectable disease (stage III, n= 989 (Supp Table 1). In total, 146 received anti-CTLA4, 1447 received anti-PD1 or PD-L1, and 189 received both. A large number of patients had cancers for which ICI is FDA-approved, including 350 NSCLCs, 321 melanomas, 151 renal cell carcinomas (RCC), 214 bladder cancers and 138 head and neck squamous cell cancers (Supp-Table-2). To calculate TMB, the total number of somatic non-synonymous mutations was normalized to the total number of megabases sequenced. OS was measured from the date of first ICI treatment to time of death or last follow-up. The median followup was 19 months (range 0–80, with 830 [50%] patients alive and censored at last followup.

We defined TMB subgroups by percentiles within each histology. We took this approach because the median and range of mutational load has been shown to vary across tumor types<sup>13</sup>; therefore, a universal cutoff for “high TMB” would be enriched for tumor types with higher mutation load. Across the entire cohort, stratifying tumors by TMB decile within histology revealed that a higher number of mutations was associated with improved OS. This significant association, stratified by histology, was seen across a variety of cutpoints chosen to define the high TMB group (ranging from top 10–50%; Fig-1 A, Supp. Fig 3–4). A clear trend toward decreasing hazard ratio (HR) of death with increasing TMB cutoff was observed across cancer types demonstrating increasing benefit from ICI with higher TMB (Fig-1 B, Supp-Fig-3).<sup>14</sup>

To confirm that these results were present across multiple cancer types, we performed two additional analyses. First, a multivariable analysis across the entire cohort using Cox proportional-hazards regression demonstrated that the tumor mutation burden was significantly associated with OS both as a continuous variable (HR=0.985,  $p=3.4\times 10^{-7}$ ) and with a binary cutoff (top 20% of each histology, HR 0.61  $p=1.3\times 10^{-7}$ ), adjusting for cancer type, age, drug class of ICI, and year of ICI start (Table-1). Furthermore, this association remained significant with removal of melanoma and NSCLC patients from the cohort (Supp Table 2), indicating that this effect was not solely driven by these histologies.

We also performed a stratified analysis within each cancer type, by selecting the higher mutation load quintile (top 20%) in each histology as the TMB-high group. Using this approach, we observed a similar association of longer OS with higher TMB (top 20% within each histology) across multiple cancer types (Fig 2, Sup-Fig-5). Although the effect for some individual cancers did not reach statistical significance, possibly due to smaller sample

size, the numerical trend of better OS ( $HR < 1$ ) was observed in nearly all cancer types, with glioma the clearest exception. Taken together, these data indicate that the association between TMB and improved survival after ICI is likely to be present in the majority of cancer histologies.

Consistent with varying distributions of TMB across histologies, the TMB cutoff associated with the top 20% of each cancer type varied markedly (Fig 2). Importantly, this suggests that there is not likely to be a universal number defining high TMB that is predictive of clinical benefit to ICI across all cancer types, and that the optimal cutpoint is likely to vary for different cancers.

A similar numerical trend was observed for longer OS with TMB measured as a continuous variable, across many histologies, concordant with the number of patients in the subgroup (Supp-Fig-6). Consistent with the differences in OS, we also observed similar associations between TMB and rates of clinical benefit to ICI, or progression free survival, in patients with cancer types for which response data was available – NSCLC, melanoma, esophagogastric, head and neck, and renal cell cancer (Supp-Fig 7–8).<sup>151617</sup>

To investigate the possibility that the observed survival differences among patients with higher TMB tumors could simply be attributable to a general prognostic benefit of high mutational load, unrelated to ICI, we analyzed the outcomes of 5371 patients with metastatic cancers who did not receive ICI, and whose tumors were sequenced with MSK-IMPACT. In these patients, there was no association between higher TMB and improved OS ( $HR$  1.12,  $p=0.11$ ). This lack of prognostic benefit was also observed within each histology (Supp-Fig-5, 9).

Of note, the TMB cutpoint for the top 20% of colorectal cancer patients was high (52.2/MB), potentially consistent with many MSI-high colorectal tumors receiving ICI treatment. To evaluate the possibility that the ICI-treated cohort of patients might be enriched for patients with higher TMB – if, for example, clinicians were more likely to triage higher TMB patients to ICI therapy – we repeated the survival analyses, instead calculating the top 20% of TMB among all (both ICI and non-ICI treated) patients. The TMB cutpoints in other cancer types were not changed with this calculation, and the associations with survival in each cancer type remained very similar, in both the ICI and non-ICI treated cohorts (Supp Figs 10 and 11).

Distinct from the other cancer types, there was no association between higher TMB and improved survival in patients with glioma; in fact, the trend was toward poorer survival. Although there have been case reports of dramatic responses to ICI in patients with glioblastoma associated with childhood biallelic mismatch repair deficiency<sup>18</sup>, mismatch repair is very rare in GBM, and higher TMB in many glioma patients may reflect prior exposure to the alkylating agent temozolomide, which can promote the expansion of less immunogenic subclonal mutations.<sup>19</sup> Alternatively, anti-tumor immune responses in the CNS may be distinct and less dependent on TMB.

As would be expected in a large multi-cancer analysis of tumors sequenced as part of clinical care, the patients included were heterogeneous, with some having been heavily pre-

treated whereas others were treated with a variety of combination therapies. The timing of MSK-IMPACT testing relative to ICI start was also variable. Nevertheless, the finding of a significant association with OS in a heterogeneous cohort underscores the robustness of TMB as a predictive biomarker, suggesting it is likely to be clinically meaningful.

TMB, as measured by targeted NGS panels such as MSK-IMPACT, has been previously shown to have a high correlation with total mutational burden as measured by WES.<sup>20–24</sup> MSK-IMPACT offers the advantage of matched normal germline sequencing for each patient, permitting precise identification of true somatic mutations.

While TMB measured in exome sequencing is highly correlated with measurements in targeted sequencing, it is important to note that numerical cutpoints may differ across platforms. Additionally, we note that TMB cutoffs for individual histologies may not represent the ideal values for use clinically, and are shown primarily to demonstrate that a relationship exists between TMB and survival for each histology. We chose a top 20<sup>th</sup> percentile cutoff for TMB in order to dichotomize our data, and this does not imply any clinical significance to this threshold.

The variable threshold of TMB across histologies can likely be attributed to distinct tumor microenvironments as well as the numerous other factors shown to independently predict response to ICI including clonality, immune infiltration, immune cell exclusion, HLA genotype and alterations, expression levels of checkpoint molecules, as well as others.<sup>19,25–28</sup> Our data overall suggest that TMB is associated with increasing OS in a dose-dependent fashion. The pan-cancer nature of this biomarker likely reflects fundamental mechanisms by which ICI functions. Our data are also consistent with the hypothesis that higher mutation load is associated with a higher number of tumor neoantigens presented on MHC molecules that facilitate immune recognition as foreign and the development of an anti-tumor immune response.<sup>29,30</sup>

This finding is in line with the observation that patients with hypermutated tumors as a result of defective mismatch repair have high response rates to pembrolizumab, a finding that had led to the FDA's tissue/site-agnostic approval of this agent for microsatellite instability-high or mismatch repair deficient tumors<sup>31</sup>. Further elucidation of appropriate mutational load cutoffs with integration of relevant clinical variables within each cancer type will be necessary, likely in the context of prospective clinical studies, to allow for implementation of TMB as a predictive biomarker.

Our study addresses several fundamentally important questions in immuno-oncology. Mutational load can predict survival across diverse types of human cancers and is relevant in patients treated with either anti-CTLA4 or anti-PD1 therapies. Second, previous studies on the association between mutational load and survival after ICI had examined small cohorts and therefore, the effects of TMB on clinical benefit could not be quantified in a precise manner. This study presents genomic data from the largest cohort of patients treated with ICI to date and demonstrates the continuous association between higher TMB and superior OS. Capturing as little as 3% of the coding exome using targeted panels such as MSK IMPACT appears to provide a sufficient estimation of total tumor mutational load to confer predictive

value for patients in whom ICI treatment is being considered. Finally, the mutational number defining TMB high appears to vary across cancer types, and there is unlikely to be a universal number that defines the likelihood of benefit from ICI across all histologies.

Given the potential toxicities of immunotherapy, the highly variable response to ICI, as well as the significant economic cost of these agents, there is an urgent need for biomarkers that can predict for immunotherapy response. Future studies that integrate other genomic or pathologic biomarkers may allow for the development of an even more optimized predictive test to inform clinical decisions on the use of ICI.

## Online Methods

### Patient Selection

After receiving institutional review board approval from the Memorial Sloan Kettering Cancer Center, institutional pharmacy records were used to identify patients who received at least one dose of immunotherapy (atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab, pembrolizumab, or tremelimumab) and then cross-referenced with patients who had MSK-IMPACT testing done in the context of routine clinical care. Cancer types with greater than 35 patients on initial collection were selected for further analysis in the cohort. The majority of patients who received MSK-IMPACT testing on tumor tissue are enrolled on an institutional IRB-approved research protocol (NCT01775072) with the remaining patients receiving testing as part of routine clinical care; all patients provided informed consent permitting return of results from sequencing analyses and broader characterization of banked specimens for research. Details of tissue processing and next generation sequencing and analysis have been previously described.<sup>11</sup> Importantly, concurrent sequencing of germline DNA from peripheral blood is performed for all samples to identify somatic tumor mutations. Patients enrolled on ongoing clinical trials for which publication of outcomes data was prohibited were removed as well as a small proportion of patients with localized disease treated in the neoadjuvant setting (n=9) or who had localized disease. Other preceding or concurrent non-ICI treatments were not recorded or accounted for in the analysis. The timing of tissue pathology on which MSK-IMPACT was performed relative to ICI administration is also heterogeneous with a small portion of patients with testing after ICI administration.

### Mutational Load Assessment and Statistical Analysis

The total number of somatic mutations identified was normalized to the exonic coverage of the respective MSK-IMPACT panel in megabases. Mutations in driver oncogenes were not excluded from the analysis. Overall survival analysis on ICI patients was performed from the date of first infusion of any ICI. For patients who received multiple courses of ICI, the first treatment was used for analysis. Patients were censored at the date of last attended appointment at MSK if death was not recorded in the electronic medical record.

For analysis of patients who did not receive ICI, all patients for whom MSK-IMPACT data was available across all histologies were included. Overall survival analysis was performed from the date of first infusional chemotherapy.



Survival analysis was performed using Kaplan Meier with log-rank p values reported. Multivariable analysis was performed using Cox proportional hazard regression with inclusion of variables significant on univariate regression including normalized TMB, cancer type, age, ICI drug class, and year of ICI administration. Year of ICI administration was included in order to avoid any possible differences in patients treated in the early years of MSK-IMPACT testing being available.

For each histology, we subsequently identified cases in the top 20% percentile of TMB and determined the log-rank p-value for difference in OS and the direction of the effect with a HR determined from a coxph model. Additional analyses were performed with the TMB cutoff ranging from 10 to 50%, as well as with the TMB cutoff instead defined among all patients (both ICI-treated and non-ICI-treated).

Response data for individual histologies was obtained from published analyses of clinical outcome in the cohorts of patients with NSCLC or esophagogastric cancer patients.<sup>15,16</sup> For patients with head and neck cancer, radiology records were reviewed manually to determine evidence of progression or tumor response. In these tumor types, clinical benefit was defined as any partial/complete response, or evidence of stable disease for 6 months. For renal cell carcinoma, time to next treatment was recorded manually for all patients. Statistical analysis was performed in R using the survival package. Graph-Pad Prism was used for basic analysis and generating graphs. Additional information can be found in the Life Sciences Reporting Summary.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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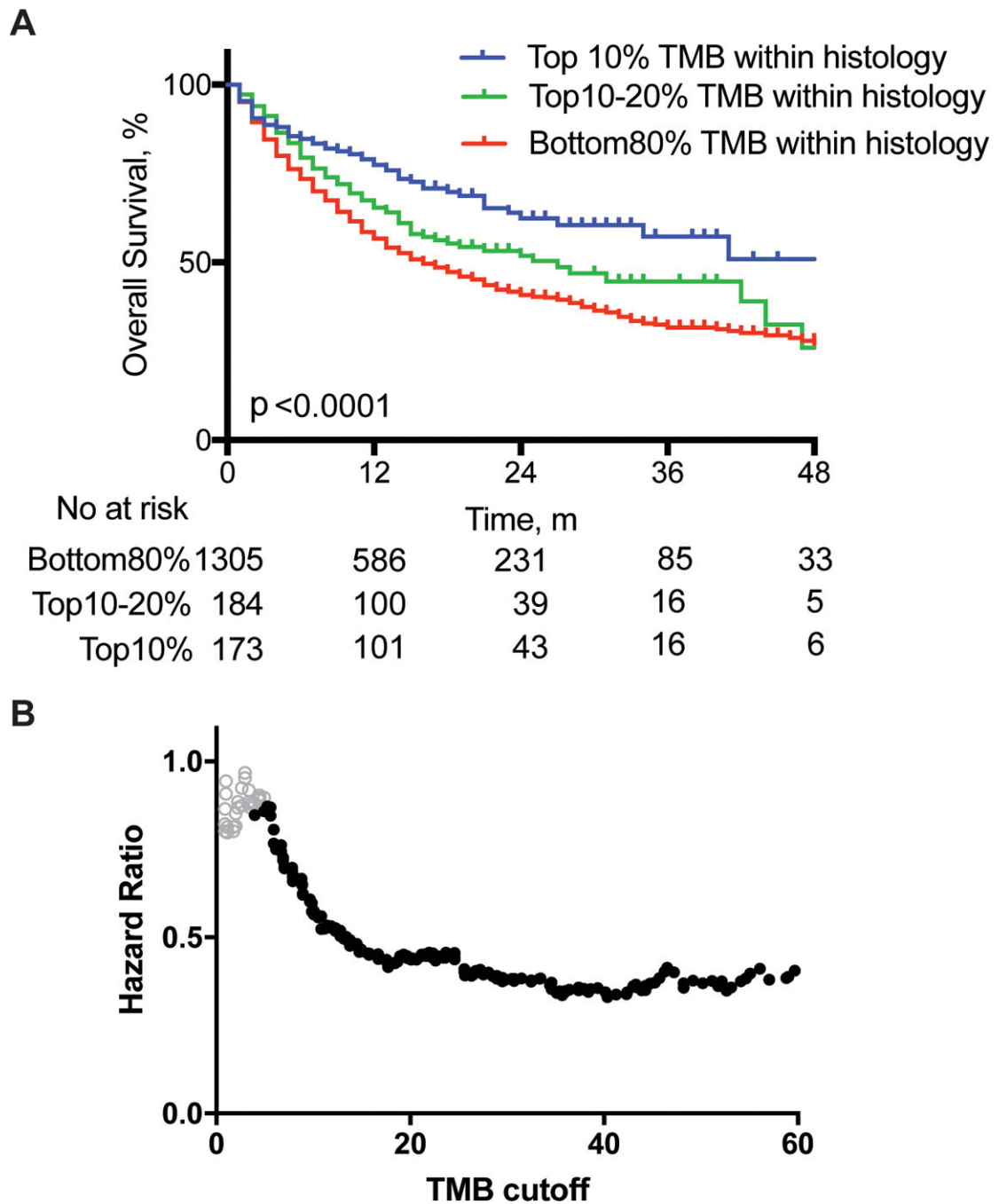
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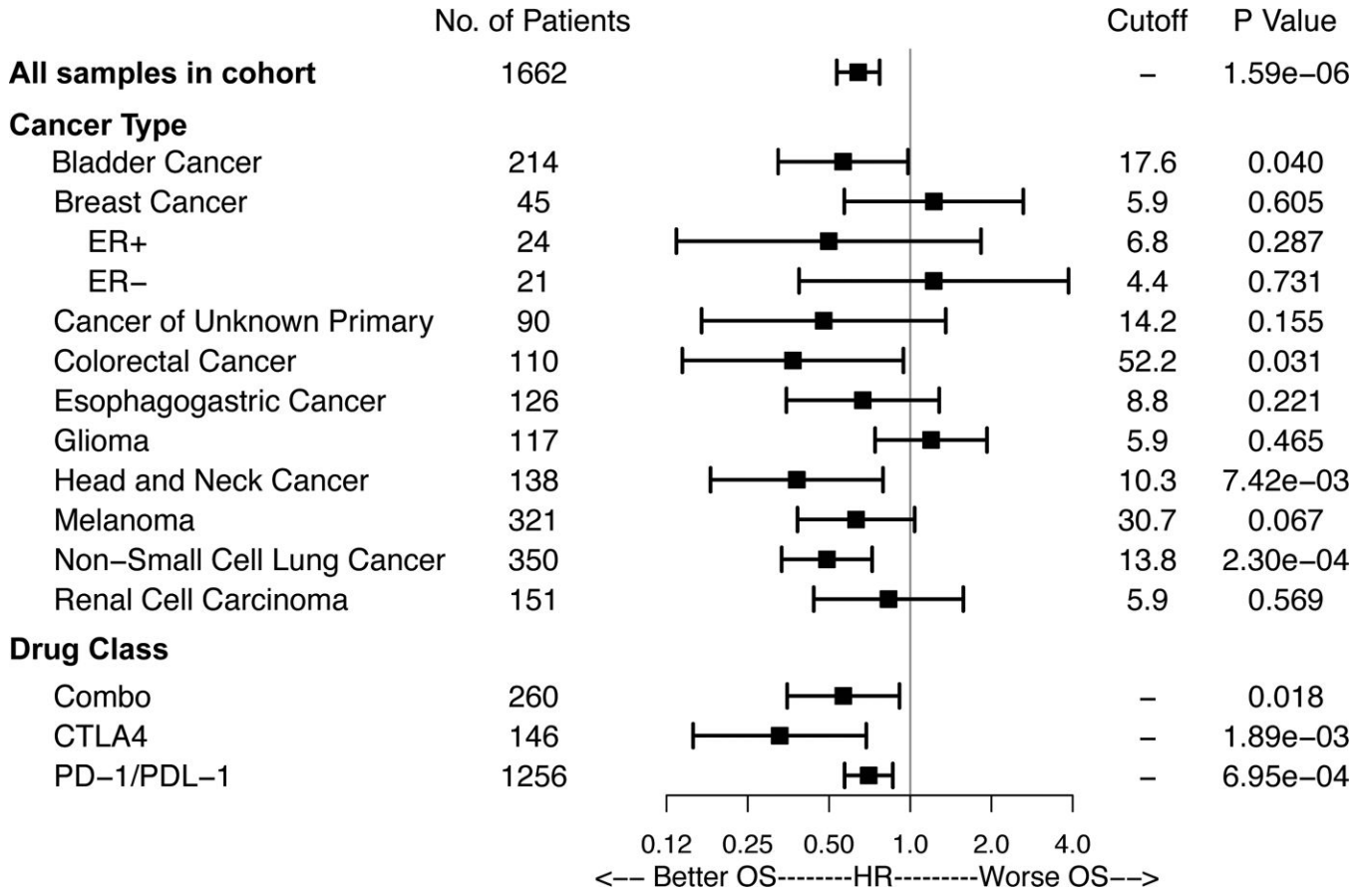
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**Figure 1: Effect of Mutational Load on Overall Survival after ICI Treatment.**

A. Kaplan-Meier curves for patients with tumors falling into the depicted deciles of TMB within each histology. Overall survival is from the first dose of ICI. Two-sided log-rank p value indicated for all patients, with univariate Cox regression hazard ratio of 0.76 (95% CI 0.62–0.94) and 0.52 (95% CI 0.42–0.64) for the 10–20% and Top10% groups, respectively, compared to Bottom80% group. m, months. B. Cox regression hazard ratios for overall survival on 1662 patients, at depicted cutoffs of TMB across all cancer subtypes. Solid black circles represent hazard ratios with p-values  $< 0.05$  (two-sided log rank p value).



**Figure 2: Effect of Non-synonymous Mutational Load on Overall Survival after ICI Treatment by Cancer Subtype and Drug Class.**

Forest plot for all patients in the identified cohort or individual cancer subtypes. Indicated are the number of patients and hazard ratio comparing overall survival after ICI in patients in the highest 20<sup>th</sup> percentile TMB within each histology. Horizontal lines represent the 95% confidence interval. The cutoff defining top 20% of normalized mutational burden from MSK-IMPACT for each cancer type is shown, as well as the two-sided log-rank p value for the comparison of high and low mutational burden survival curves. All cancer types in analysis are displayed.

**Table 1:**  
**Multivariable analysis of factors associated with overall survival.**

	HR	95% CI	P value
Normalized Mutation Count			
Continuous	0.985	0.979–0.991	$3.4 \times 10^{-7}$
Binary (Top 20% of each histology)	0.61	0.508–0.733	$1.3 \times 10^{-7}$
Cancer Type			
Melanoma (reference)			
NSCLC	2.08	1.61–2.68	$1.9 \times 10^{-8}$
Not Melanoma/NSCLC	1.52	1.21–1.92	$3.7 \times 10^{-4}$
Age	0.995	0.990–1.004	0.07
Drug Class			
PD-1/PD-L1 (reference)			
CTLA4	1.18	0.846–1.66	0.32
Combo	0.67	0.534–0.844	$6.6 \times 10^{-4}$
Year of ICI start			$2.3 \times 10^{-8}$

ICI-Immune Checkpoint Inhibitor

Cox proportional hazards multivariable analysis of overall survival in 1662 patients treated with ICI demonstrating the hazard ratios for individual covariates.