Combination of TMB and CNA Stratifies Prognostic and Predictive Responses to Immunotherapy Across Metastatic Cancer

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Abstract

**Purpose:** Although tumor mutation burden (TMB) has been well known to predict the response to immune checkpoint inhibitors (ICI), lack of randomized clinical trial data has restricted its clinical application. This study aimed to explore the significance and feasibility of biomarker combination based on TMB and copy-number alteration (CNA) for the prognosis of each tumor and prediction for ICI therapy in metastatic pan-cancer milieu.

**Experimental Design:** Non-ICI-treated MSK pan-cancer cohort was used for prognosis analysis. Three independent immunotherapy cohorts, including non–small cell lung cancer (\(n = 240\)), skin cutaneous melanoma (\(n = 174\)), and mixed cancer (Dana-Farber, \(n = 98\)) patients from previous studies, were analyzed for efficacy of ICI therapy.

**Results:** TMB and CNA showed optimized combination for the prognosis of most metastatic cancer types, and patients with TMB\(^{\text{high}}\)-CNA\(^{\text{low}}\) showed better survival. The predictive analysis, both TMB and CNA were independent predictive factors for ICI therapy. Remarkably, when TMB and CNA were jointly analyzed, those with TMB\(^{\text{high}}\)-CNA\(^{\text{low}}\) showed favorable responses to ICI therapy. Meanwhile, TMB\(^{\text{high}}\)-CNA\(^{\text{low}}\) as a new biomarker showed better prediction for ICI efficacy compared with either TMB-high or CNA-low alone. Furthermore, analysis of the non-ICI-treated MSK pan-cancer cohort supported that the joint stratification of TMB and CNA can be used to categorize tumors into distinct sensitivity to ICI therapy across pan-tumors.

**Conclusions:** The combination of TMB and CNA can jointly stratify multiple metastatic tumors into groups with different prognosis and heterogeneous clinical responses to ICI treatment. Patients with TMB\(^{\text{high}}\)-CNA\(^{\text{low}}\) cancer can be an optimal subgroup for ICI therapy.

Introduction

Cancer immunotherapy has revolutionized treatment across various tumor types, with its main focus on the therapy involving immune checkpoint inhibitors (ICI) such as anti-PD-1 (programmed death 1), anti-PD-L1 (the ligand of PD-1), and anti-CTLA-4 (cytotoxic T lymphocyte antigen 4; refs. 1–3). Despite durable response and improved survival in a subset of patients, the objective response rate (ORR) was merely 20%–30% of the unselected patients with advanced stage diseases (4).

Substantial efforts have been put into identifying and developing effective predictive biomarkers, including IHC-based approaches such as PD-L1 protein expression and the presence of tumor-infiltrating lymphocytes (TIL), especially the CD8\(^{+}\) cytolytic T cells (5). Although higher response rates have been reported for patients with stronger PD-L1 expression, such quantified methods for immunotherapy response prediction are still imperfect, highlighting the critical need for developing more improved biomarkers (6).

Advances in high-throughput technologies have enabled unbiased approaches to biomarker discovery. DNA sequencing data provide a more comprehensive view of the genetic features of individuals that may help reveal mechanisms related to general gene dosage imbalance rather than the function of specific genes (7–9). Several recent studies have shown that tumor mutation burden (TMB) correlates with response to immunotherapy due to its reflection of overall neoantigen load (2, 10, 11). Furthermore, copy-number alteration (CNA) has recently been shown to correlate with gene signatures of immune evasion and worse survival in response to CTLA-4 blockade (12, 13). However, the prognostic role of these two biomarkers and their joint associations with clinical responses to checkpoint blockade immunotherapy has not yet been clarified across multiple cancer types.

In this study, we first evaluated the prognostic role of TMB and CNA levels in non-ICI-treated patients across different tumor types. Then we analyzed three independent cohorts including non–small cell lung cancer (NSCLC), skin cutaneous melanoma (SKCM), and Dana-Farber ICI-treated patients from previous studies for efficacy of ICIs based on TMB and CNA. Moreover,
Study design and clinical cohorts

We combined TMB and CNA together to generate a model that can afford greater accuracy in the prediction of prognosis and immunotherapeutic benefits.

Materials and Methods

Study design and clinical cohorts

We collated data from previously published clinical cohorts of metastatic cancer. All of the patients included in this study were available for TMB and CNA profiling by DNA sequencing. The cohorts included for prognostic analysis had sufficient follow-up data for overall survival (OS), and the cohorts included for predictive analysis possessed at least one type of survival data (progression-free survival [PFS] or OS) and ORR for ICIs therapy. We analyzed the prognostic and predictive roles of TMB and CNA across these cohorts as illustrated in the flowchart (Supplementary Fig. S1).

MSK pan-cancer cohort. The MSK pan-cancer cohort contained 9,301 patients in 24 cancer types, who did not receive ICIs therapy. The clinical information and DNA sequencing data were available through the cBioPortal for cancer genomics at http://cbioportal.org/msk-impact (14). The following solid tumor types were selected: NSCLC (n = 1,557), SKCM (n = 350), bladder urothelial carcinoma (BLCA, n = 406), colon and rectal adenocarcinoma (COAD, n = 976), breast cancer (BRCA, n = 1,234), glioblastoma multiforme (GBM, n = 512), head and neck squamous cell carcinoma (HNSC, n = 283), and prostate adenocarcinoma (PRAD, n = 618). Altogether, samples of eight cancer types (N = 5,114) were included in the prognostic analysis.

MSK NSCLC ICI cohort. Clinical and mutational data for 240 patients with NSCLC were retrieved from the cBioPortal for cancer genomics at http://cbioportal.org/study?id = nsclc_pdl_msk_2018. All patients were treated with anti-PD-L1 monotherapy or in combination with anti-cytotoxic T-cell lymphocyte-4 (anti-CTLA-4) between April 2011 and January 2017 with the approval of Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review Board. Objective response was determined by investigator-assersted RECIST version 1.1; efficacy was defined as durable clinical benefit [DCB: complete response (CR)/partial response (PR) or stable disease (SD) that lasted 6 months] or no durable benefit (NDB: PD or SD that lasted ≤6 months; ref. 15). The PFS and ORR data were available for further analysis from patients in MSK NSCLC ICI cohort.

SKCM ICI cohort. Data from a total of 174 patients with metastatic melanoma were derived from the following studies: Van Allen and colleagues (n = 110) and Snyder and colleagues (n = 64; refs. 2, 16). All patients were treated with anti-CTLA-4 (ipilimumab) monotherapy. The OS and ORR data were available for further analysis from patients in SKCM ICI cohort. Tumors from formalin-fixed, paraffin-embedded (FFPE) blocks were obtained for whole-exome sequencing (WES).

Dana-Farber ICI cohort. Data from a total of 98 patients with mixed cancer from the Dana-Farber Cancer Institute with metastatic tumors were obtained from recently published article (17) and cBioPortal for cancer genomics at http://www.cbioportal.org/study?id = mixed_allen_2018. Among them, bladder cancer (n = 27), HNSC (n = 12), lung cancer (n = 57), sarcoma (n = 1), and anal cancer (n = 1) were included. Patients received treatment of anti-PD-1, anti-PD-L1, or a combination of anti-PD-L1 and anti-CTLA4. These studies were conducted in accordance with the Declaration of Helsinki and approved by the Dana-Farber Cancer Institute Institutional Review Board. Patients from Dana-Farber ICI cohort were available of OS, PFS, and ORR for further analysis. Clinicopathologic and molecular information of these cohorts are provided in Supplementary Table S1. This study was approved by the Institutional Ethical Review Boards of Nanfang Hospital. Patients included in these above cohorts had provided signed informed consent in accordance with their clinical study protocols.

Mutation data analysis

Data of the TMB in this study were directly obtained from previously published article and cBioPortal for cancer genomics, which were analyzed by MSK-IMPACT and WES.

MSK-IMPACT sequencing for TMB. MSK pan-cancer non-ICI-treated cohort (n = 9,301) and MSK NSCLC ICI-treated cohort (n = 240) were molecularly profiled by MSK-IMPACT sequencing, which was performed as described previously (14, 15). Germline and somatic single nucleotide variants and indels were identified using GATK Haplotype Caller and MuTect. Candidate mutations were filtered for various criteria, including recurrence in previously sequenced normals, total and allele-level coverage, and known systematic artifacts (18–20). Germline variants were eliminated through the use of patient-matched DNA extracted from blood. To normalize somatic TMB across panels of various sizes, the total number of mutations was divided by the coding region captured in each panel, which covered 0.98, 1.06, and 1.22 megabases (Mb) in the 341-, 410-, and 468-gene panels, respectively (15).

WES for TMB. SKCM ICI-treated cohort (n = 174) and Dana-Farber ICI-treated cohort (n = 98) were both profiled by WES,
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which was performed as described previously (13, 17). Somatic SNPs were identified by MuTect, with computational filtering of artifacts introduced by DNA oxidation during sequencing or FFPEx-based DNA extraction using a filter-based method that mutations with allelic fractions of less than 0.05 or coverage of ≤30× were excluded. Indelocator (http://www.broadinstitute.org/cancer/cga/indelocator) was applied to detect small indels. Annotation of the variants identified was performed using Oncotator (http://www.broadinstitute.org/cancer/cga/oncotator; ref. 21).

The cut-off value for high and low TMB of the three independent ICI-treated cohorts (MSK-NSCLC, SKCM, and Dana-Farber) was defined as the median TMB of each cohort, which was referred to the previous article with ICI-treated study (10). The cut-off value for high and low TMB of the MSK pan-cancer non-ICI cohort analyzed for OS was defined as the median TMB of each cancer type (22).

CNA analysis

MSK-IMPACT sequencing for CNA. Data of CNAs in the MSK pan-cancer non-ICI cohort and MSK NSCLC ICI cohort were analyzed from MSK-IMPACT sequencing, which were directly obtained from cBioPortal and the analytical process was described in the previously published articles (14, 15). A set of normal FFPE and blood control samples were used for reference diploid genome comparison. Normalized coverage values from tumor samples were divided by corresponding values in normal samples, and log-transformed to yield log ratios. Log-ratio coverage values were subsequently segmented by circular binary segmentation (23).

The fraction of copy number-altered genome was defined as the fraction of genome with log_{2} copy number gain > 0.2 or loss <-0.2 relative to the size of the genome with copy number profiled (15).

WES for CNA. Data of CNA in the SKCM ICI cohort and Dana-Farber ICI cohort were analyzed from WES, which were also obtained from cBioPortal and the analytical process was described in the previously published articles (13, 17). Copy ratios were calculated for each captured target by dividing the tumor coverage by the median coverage obtained in a set of reference normal samples. The resulting copy ratios were segmented using the circular binary segmentation algorithm similar to what was described above (24). Segments were considered amplified or deleted when the log_{2} copy ratio > 0.3 or <-0.3 for SKCM ICI cohort and log_{2} copy ratio > 0.5 or <-0.5 for Dana-Farber ICI cohort.

The cut-off value for high and low CNA of the three independent ICI-treated cohorts (MSK-NSCLC, SKCM, and Dana-Farber cohort) was defined as the lower tertile CNA of each cohort. The cut-off value for high and low CNA of the MSK pan-cancer non-ICI cohort analyzed for OS was defined as the lower tertile CNA of each cancer type (25).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.01 (GraphPad Software) and SPSS version 22.0 (IBM Corp.). Kaplan–Meier curve analysis of PFS and OS was compared using the log-rank test. The Cox proportional hazards model was applied for multivariate survival analysis. ORR and DCB in each cancer type (25).

The cut-off value for high and low TMB of the three independent ICI-treated cohorts (MSK-NSCLC, SKCM, and Dana-Farber) was defined as the median TMB of each cohort, which was referred to the previous article with ICI-treated study (10). The cut-off value for high and low TMB of the MSK pan-cancer non-ICI cohort analyzed for OS was defined as the median TMB of each cancer type (22).

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The cut-off value for high and low CNA of the three independent ICI-treated cohorts (MSK-NSCLC, SKCM, and Dana-Farber cohort) was defined as the lower tertile CNA of each cohort. The cut-off value for high and low CNA of the MSK pan-cancer non-ICI cohort analyzed for OS was defined as the lower tertile CNA of each cancer type (25).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.01 (GraphPad Software) and SPSS version 22.0 (IBM Corp.). Kaplan–Meier curve analysis of PFS and OS was compared using the log-rank test. The Cox proportional hazards model was applied for multivariate survival analysis. ORR and DCB in different subgroups based on TMB and CNA were analyzed by Pearson χ² test. The correlation between TMB and CNA in different cancer types was analyzed by Pearson correlation test. All reported P values were two-tailed and for all analyses, P ≤ 0.05 was considered statistically significant.

Results

Prognostic role of TMB and CNA alone or in combination across different types of advanced tumors

We first assessed the prognostic value of each genomic biomarker separately across different cancer types. As was illustrated in Fig. 1, we selected non-ICI–treated patients from the MSK pan-cancer cohort, and divided them into high and low TMB groups using median TMB as the cut-off value. In total, patients with lower TMB had longer OS in some cancer types including NSCLC (P < 0.001), COAD (P = 0.012), PRAD (P = 0.012), and HNSC (P = 0.006). However, BLCA (P = 0.006) exhibited an association that was opposite (higher TMB correlated with improved OS), which indicated the existence of intertumor heterogeneity of TMB as a prognostic biomarker (Fig. 1A). Then, patients were classified into high and low CNA groups with the lower tertile of CNA levels as the cut-off value. Similarly, lower CNA levels were associated with longer OS in some cancer types including NSCLC (P = 0.001), BLCA (P = 0.002), BRCA (P = 0.004), GBM (P = 0.013), and PRAD (P < 0.001; Fig. 1B), which was consistent with the previously published data (25, 26).

To demonstrate whether TMB and CNA served as independent prognostic biomarkers or interacted with each other, we did multivariate analysis of clinical and genetic features and prognosis with OS in eight cancer types of MSK pan-cancer ICI–treated cohort, and we found that TMB and CNA were independent as prognostic factors in the NSCLC cohort (TMB: hazard ratio (HR), 0.72; P = 0.002 and CNA: HR, 0.78; P = 0.034; respectively), BLCA cohort (TMB: HR, 2.16; P = 0.002 and CNA: HR, 0.38; P = 0.01; respectively), and prostate adenocarcinoma cohort (TMB: HR, 0.61; P = 0.043 and CNA: HR, 0.22; P < 0.001; respectively; Supplementary Table S2).

We next considered whether the combination of these two genomic biomarkers would distinguish diverse prognosis among different cancer types in the same cohort. Our Pearson correlation test identified that TMB and CNA showed weak correlation in most cancer types (NSCLC: r = 0.21, P < 0.01; BRCA: r = 0.08, P = 0.004; COAD: r = -0.19, P < 0.001; and PRAD: r = 0.08, P = 0.004; Supplementary Fig. S2A). Lacking of correlation with each other, plus independent prognostic merits, suggests that TMB and CNA might act as a complementary prognostic biomarker for each other. Patients were subsequently stratified into four groups by TMB and CNA. Notably, the combination of TMB and CNA was significantly associated with OS in almost all the cancer types. Patients with TMB^{low} and CNA^{low} showed favorable survival, whereas those with TMB^{high} and CNA^{high} had the worst survival in most cancer types, including NSCLC (P < 0.001; Fig. 2A), COAD (P = 0.020; Fig. 2D), BRCA (P = 0.029; Fig. 2E), GBM (P = 0.011; Fig. 2F), PRAD (P < 0.001; Fig. 2G), and HNSC (P = 0.016; Fig. 2H). However, patients with BLCA with TMB^{high} and CNA^{low} showed favorable OS and TMB^{low}/CNA^{high} patients showed poorer OS (P < 0.001; Fig. 2C). These results indicated that the combination of TMB and CNA may serve as a better prognostic biomarker than each single biomarker in most cancer types, and those with
The TMB\textsuperscript{low}–CNA\textsuperscript{low} subgroup demonstrated a better survival in most metastatic cancer types.

**TMB and CNA were both independent predictive factors for ICI therapy**

We then analyzed the predictive values of TMB and CNA for response to ICI treatment in three ICI-treated cohorts from previous studies and open source database. For patients with SKCM (n = 174) receiving anti-CTLA-4 treatment (13), those with lower CNA levels had longer OS (P = 0.001), yet TMB level was not associated with treatment outcome (P = 0.272; Supplementary Fig. S3A). For patients with NSCLC (n = 240) receiving anti-PD-1/L1 treatment (15), higher TMB was associated with longer PFS (P = 0.009), while lower CNA showed only marginal statistical difference compared with higher CNA (P = 0.052; Supplementary Fig. S3B). As for the third cohort, consisting of 98 mixed-tumor patients (Dana-Farber ICI cohort) receiving anti-PD-1/L1 monotherapy or combination with anti-CTLA4 (17), both TMB and CNA were associated with better OS (TMB: HR, 0.68; P = 0.008 and CNA: HR, 1.47; P = 0.014; respectively) and Dana-Farber ICI cohort (TMB: HR, 0.38; P = 0.040 and CNA: HR, 3.77; P = 0.023; respectively). To further explore whether the choice of specific cut-off point may influence the outcome, we did a sensitivity analysis.

To investigate whether TMB and CNA served as independent predictive biomarkers or interacted with each other for ICI therapy, we did univariate and multivariate analyses of these three cohorts. Table 1 lists the relationships identified between the clinical and biological variables with treatment outcome. After multivariate adjustment of these variables, TMB and CNA were identified as independent predictive factors in the MSK NSCLC ICI cohort (TMB: HR, 0.68; P = 0.025 and CNA: P = 0.004; Supplementary Fig. S3C) and only TMB was associated with superior PFS (P < 0.001; Supplementary Fig. S3D).
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Figure 2.
Kaplan–Meier curves of OS according to TMB combined with CNAs in patients across eight tumor types from the non-ICI-treated MSK-IMPACT cohort. Non–small cell lung cancer (NSCLC, n = 1,557; A); skin cutaneous melanoma (SKCM, n = 283; B); bladder urothelial carcinoma (BLCA, n = 406; C); colon and rectal adenocarcinoma (COAD, n = 976; D); breast cancer (BRCA, n = 1,234; E); glioblastoma multiforme (GBM, n = 512; F); prostate adenocarcinoma (PRAD, n = 618; G); and head and neck squamous cell carcinoma (HNSC, n = 283; H). We calculated P values using the log-rank test.

Smoker (yes vs. no) 1.17 (0.50–2.60) 0.53 (0.28–0.96) 0.040
Gender (male vs. female) 0.53 (0.28–1.03) 0.69 (0.49–1.03) 0.38 (0.35–0.96) 0.032

Table 1. Univariate and multivariable Cox regression analysis of predictive factors in three cohorts treated with ICI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td><strong>MSK NSCLC ICI (PFS, n = 240)</strong></td>
<td></td>
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<tr>
<td>TMB (≥median vs. &lt;median)</td>
<td>0.73 (0.55–0.97)</td>
<td><strong>0.030</strong></td>
</tr>
<tr>
<td>CNA (lower tertile vs. &lt;lower tertile)</td>
<td>1.29 (1.00–1.61)</td>
<td>0.034</td>
</tr>
<tr>
<td>Smoker (yes vs. no)</td>
<td>1.00 (0.48–1.69)</td>
<td>0.69</td>
</tr>
<tr>
<td>Gender (male vs. female)</td>
<td>1.09 (0.83–1.44)</td>
<td>0.544</td>
</tr>
<tr>
<td>Age (≥65 vs. &lt;65)</td>
<td>1.27 (0.91–1.59)</td>
<td>0.207</td>
</tr>
<tr>
<td><strong>SKCM ICI (OS, n = 174)</strong></td>
<td></td>
<td></td>
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<tr>
<td>TMB (≥median vs. &lt;median)</td>
<td>1.22 (0.85–1.75)</td>
<td>0.037</td>
</tr>
<tr>
<td>CNA (lower tertile vs. &lt;lower tertile)</td>
<td>2.06 (1.34–3.15)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Gender (male vs. female)</td>
<td>0.86 (0.59–1.25)</td>
<td>0.420</td>
</tr>
<tr>
<td>Age (≥65 vs. &lt;65)</td>
<td>0.95 (0.66–1.37)</td>
<td>0.801</td>
</tr>
<tr>
<td><strong>Dana-Farber ICI (OS, n = 98)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMB (≥median vs. &lt;median)</td>
<td>0.49 (0.26–0.93)</td>
<td><strong>0.028</strong></td>
</tr>
<tr>
<td>CNA (lower tertile vs. &lt;lower tertile)</td>
<td>3.07 (1.42–7.56)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Gender (male vs. female)</td>
<td>0.53 (0.28–1.03)</td>
<td>0.059</td>
</tr>
<tr>
<td>Smoker (yes vs. no)</td>
<td>1.17 (0.50–2.73)</td>
<td>0.716</td>
</tr>
</tbody>
</table>

NOTE: The cut-off point of TMB and CNA in each cohort was calculated independently; bold numerical values were defined as significant difference. Abbreviation: CI, confidence interval.
OS (not reached vs. 12.63 vs. 15.53 vs. 11.15 months; \(P = 0.006\); Fig. 3D) and PFS (16.32 vs. 3.39 vs. 5.59 vs. 2.80 months; \(P = 0.0001\); Fig. 3C) as well as increased proportion of ORR (81.3% vs. 11.8% vs. 40.6% vs. 18.2%; \(P < 0.001\); Fig. 4D) and DCB (93.8% vs. 35.3% vs. 56.3% vs. 24.2%; \(P < 0.001\); Fig. 4E). These findings suggested that the combination of TMB and CNA may act as a promising biomarker to improve the predictive power of ICI therapy in metastatic cancer.

**TMB**\(^{\text{high}}\)CNA\(^{\text{low}}\) as a new biomarker showed better predictive superiority at ICI therapy than high TMB or low CNA alone

Because the biomarker of TMB\(^{\text{high}}\)CNA\(^{\text{low}}\) can clearly discriminate responsive patients from nonresponders in ICI-treated cohorts, we then sought to compare the predictive superiority of TMB\(^{\text{high}}\)CNA\(^{\text{low}}\) as a new merit in a whole with the existing classical biomarkers, such as high TMB or low CNA alone. Because all of them may indicate better treatment efficacy, we aimed at evaluating the optimized signature to select a superior solution from the already superior ones. We thus classified all the patients into two groups based on anti-CTLA4 therapy (\(n = 174\), SKCM ICI cohort) or anti-PD-1/L1 therapy (\(n = 338\), MSK NSCLC ICI and Dana-Farber ICI cohort). There was significantly prolonged PFS (\(P = 0.039\); Fig. 5A) and increased ORR (\(P = 0.017\); Fig. 5B) in patients with TMB\(^{\text{high}}\)CNA\(^{\text{low}}\) than those only with a high TMB or low CNA underwent anti-PD1/L1 treatment. Similarly, patients with TMB\(^{\text{high}}\)CNA\(^{\text{low}}\) SKCM showed prolonged OS and increased ORR compared with those only with a high TMB or low CNA underwent anti-CTLA4 treatment, although with a marginal statistical difference (OS: \(P = 0.052\); Fig. 5C and ORR: \(P = 0.070\); Fig. 5D). Taken together, these results supported that TMB\(^{\text{high}}\)CNA\(^{\text{low}}\) as a new biomarker was better at selecting the most sensitive immunotherapy patients, compared with TMB\(^{\text{high}}\) or CNA\(^{\text{low}}\) alone, because higher TMB has been demonstrated a robust association with better OS, implying the achievement of our goal of selecting a superior biomarker from the already superior ones.

The joint relationship of TMB and CNA can be applied to a wide range of tumor types across genomic databases

To explore the generalizability of our study and the utility of our stratification model across different tumor types, the relationship between TMB and CNA was further explored in the same non-ICI MSK pan-cancer cohort (9,301 patients across 24 tumor types). We attempted to get an insight into the potential efficacy of TMB/CNA in the recognition of the inherent sensitivity for ICI therapy of each cancer type, and it was the tendency presented in the analysis that we mainly focused on. The results turned out to be in accordance with clinical praxis. Levels of TMB and CNA across different tumor types were presented in Fig. 6A and B. We selected a single cut-off point for TMB (four mutations/Mb,
A cut-off value was chosen as the median over the 24 tumor types) and CNA (0.066, cut-off value was defined as the lower tertile over the 24 tumor types) to make different cancer types comparable, which was referred to the recently published article (27). Patients were stratified by TMB and CNA as previously described. To our expectations, the tendency about proportion of TMBhighCNAlow subgroup presented here, which was identified as an effective predictive biomarker of ICI therapy in our analysis, showed significant enrichment in patients with tumors that were generally more responsive to ICIs, such as BLCA, non-Hodgkin lymphoma, lung squamous cell carcinoma (LUSC), and lung adenocarcinoma. Similarly, the tendency about proportion of the TMBlowCNAhigh subgroup, which was identified to have decreased sensitivity to ICI therapy in our analysis, revealed predominant enrichment trend in patients with tumors that were generally more prone to be resistant to ICIs, such as small-cell lung cancer (SCLC), sarcoma, gastrointestinal stromal tumor, and testicular germ cell tumors (Fig. 6C). However, there still existed outliers, for example, SKCM, which was known to have good response to immune checkpoints blockade therapy, had nonetheless lower proportion of TMBhighCNAlow subgroup, indicating these exploratory results await verification in larger, independent studies. Ultimately, our data demonstrated that joint stratification of TMB and CNA can be used to categorize tumors into discrete subgroups that exhibit distinct prognosis and heterogeneous sensitivity to ICIs among multiple cancer types.

**Discussion**

Although several studies have shown that either TMB or CNA correlate with clinical response to checkpoint blockade immunotherapy and patient survival in specific cancer types (2, 9), lacking of data demonstrating the utility of certain biomarkers from randomized controlled trials may restrict their application. Whether the combination of these two genomic biomarkers will produce synergic and complementary effects in prognosis
indicating and immunotherapy efficacy prediction has not yet been clarified across cancer types. In this study, we had identified that patients with TMB\textsuperscript{low}CNA\textsuperscript{low} subtypes showed favorable survival, whereas those with TMB\textsuperscript{high}CNA\textsuperscript{high} had the worst survival in most cancer types. More importantly, we had demonstrated that patients with TMB\textsuperscript{high}CNA\textsuperscript{low} subtypes showed favorable response to ICI therapy, while those with TMB\textsuperscript{low}CNA\textsuperscript{high} represented resistance to ICI therapy. These findings first proposed that the combination of TMB and CNA can jointly stratify patients with metastatic cancer into groups with different prognosis and clinical responses to ICI treatment.

Low TMB was a favorable prognostic factor, yet predictive of unfavorable predictive efficacy of ICI treatment, which might be caused by the activation of oncogenic pathways. The mechanisms underlying the association between high TMB and benefits from immunotherapy are not entirely clear. A leading hypothesis suggests that the biological mechanism lies in the formation of neoantigens, tumor-specific nonself peptides resulting from somatic nonsynonymous mutations (28). In contrast, tumors with low levels of CNA showed decreased expression levels of cell cycle and cell proliferation markers and elevated expression levels of markers for cytotoxic immune cell infiltrates (13, 29); which on one hand restrained tumor proliferation and indicated better prognosis, and on the other hand, inflammatory immune microenvironment plays a positive role on the efficacy of cancer immunotherapy; this may uncover the reasons for our findings that low CNA was both a favorable prognostic factor and a predictive factor of better efficacy of ICI treatment. Furthermore, it was also worth noting that, in our subgroup analysis of Fig. 3, the survival of TMB\textsuperscript{high}CNA\textsuperscript{low} patients was significantly longer than those of TMB\textsuperscript{low}CNA\textsuperscript{low} patients among all these three ICI-treated cohorts, which indicated that when low CNA combined with high TMB, together this stratification schema demonstrated stronger prediction power for immunotherapy efficacy among patients. Thus the combination of CNA and TMB holds great promise by having demonstrated increased value for prognosis indication and efficacy prediction.

In contrast with a previous report showing that CNAs are more abundant in tumors with a high mutation burden (13), we found weak correlation between TMB and CNA levels in six of eight tumor types. The difference between previous study and our investigation may result from the tumor staging that The Cancer
Genome Atlas cancers are almost early stage whereas MSK cohort are metastatic cancers. This lack of correlation, combined with the finding that both TMB and CNA remained independent predictive factors for ICI treatment, suggested the potential for greater predictive value when these biomarkers are used together in the immunotherapy setting. In addition, CNA was reported to be closely correlated with homologous recombination deficiency (HRD), which was seen primarily in high-grade serous ovarian cancers, triple-negative breast cancer, and some other cancers (30), and HRD is now being routinely assessed in many clinical trials, similar to TMB. We thus further assessed whether HRD displayed the same orthogonal correlation with TMB as CNA. We found that the existence of HRD, with an algorithm of specific homologous recombination mutations from previous article (31), was significantly correlated with high TMB (MSK NSCLC ICI: \( P < 0.001 \); Dana-Farber ICI: \( P < 0.001 \); Supplementary Fig. S6). Taking this step further, we did univariate and multivariable cox regression analysis with HRD included in these two ICI-treated cohorts, and found that, TMB and CNA were still two independent predictive...
factors while HRD could be replaced (Supplementary Table S3), indicating TMB and CNA function as effective predictive factors for ICI treatment, which were independent of HRD.

We went on to investigate the joint stratification of TMB and CNA across various types of cancers. On the basis of our hypothesis, patients with BLCA, LUSC, and lung adenocarcinoma, which ranked high in TMB and low in CNA among patients with cancer with enrichment of $\text{TMB}^{\text{high}}\text{CNA}^{\text{low}}$, should respond well to immunotherapy. Results from clinical trials of these cancer types did yield inspiring response rates and various types of ICIs have been approved by the FDA for BLCA and NSCLC (32). Another immunotherapy, which might be a dominant factor leading to patients’ unfavorable prognosis and decreasing responses to immunotherapy.

In conclusion, this study provided evidence that TMB and CNA represent both prognostic and predictive factors for outcomes. Furthermore, the combination of TMB and CNA can jointly stratify metastatic cancers into groups with different prognosis and heterogeneous clinical responses to ICI treatments. These factors might guide immunotherapeutic decisions for patients with metastatic cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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