BRAF mutation predicts survival after immunotherapy across multiple cancer types

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Abstract

Recently studies in selected tumors suggested that BRAF mutation may be associated with survival benefit from immune checkpoint inhibitor (ICI) therapy. To broadly investigate this association at a pan-cancer level, we analyzed two independent ICI treatment cohorts (MSKCC: n = 1630, and Dana-Farber: n = 249). BRAF-mutant patients exhibit better overall survival in the MSKCC cohort (Hazard ratio [HR] = 0.55, 95% confidence interval [CI]: 0.43-0.72; P <.001) and the result is validated by the Dana-Farber cohort (HR = 0.68, 95% CI: 0.46-0.99; P = .045). A multivariate analysis adjusting tumor mutational burden, mismatch repair status, cancer type, age and sex confirmed the results (adjusted HR = 0.58, 95% CI = 0.43-0.78; P < .001). Immunogenomic features analysis of TCGA dataset indicated that patients may respond to immunotherapy in various mechanisms. This finding substantially improves the therapeutic prospects for a sizeable fraction of patients who benefit from immunotherapy.

Full Text

BRAF Mutation Predicts Survival After Immunotherapy Across Multiple Cancer Types

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Abstract

Recent studies in selected tumors have suggested that BRAF mutation may be associated with survival benefit from immune checkpoint inhibitor (ICI) therapy. To broadly investigate this association at a pan-cancer level, we analyzed two independent ICI treatment cohorts (MSKCC: n = 1,630, and Dana-Farber: n = 249). BRAF-mutant patients exhibited better overall survival in the MSKCC cohort (Hazard ratio [HR] = 0.55, 95% confidence interval [CI]: 0.43–0.72; P < .001), and this result was validated in the Dana-Farber cohort (HR = 0.68, 95% CI: 0.46–0.99; P = .045). Multivariate analysis adjusting for tumor mutational burden, mismatch repair status, cancer type, age, and sex confirmed these findings (adjusted HR = 0.58, 95% CI = 0.43–0.78; P < .001). Immunogenomic feature analysis of the TCGA dataset indicated that patients may respond to immunotherapy through various mechanisms. This finding substantially improves the therapeutic prospects for a sizeable fraction of patients who benefit from immunotherapy.

Immune checkpoint inhibitor therapy has revolutionized the treatment of advanced cancer; however, durable benefit is limited to a minority of patients. Previous studies have suggested that PD-L1 immunohistochemistry, deficient mismatch repair (dMMR), and tumor mutational burden (TMB) may correlate with clinical response. BRAF mutation is associated with poor prognosis and chemotherapy resistance in most cancers, for which new therapeutic options are urgently needed. Recently, a phase III trial reported that BRAF-mutant melanoma patients who received first-line ICI treatment showed better overall survival than wild-type patients. Additionally, retrospective studies have reported potential benefits from ICI treatment in BRAF-mutant glioblastoma and non-small cell lung cancer patients. However, it remains unclear whether BRAF mutation is associated with ICI treatment outcomes across diverse human cancers. In this pan-cancer study, we identified that BRAF-mutant tumors are

associated with better outcomes after ICI treatment and present a small number of tumor neoantigens. Our study also indicates that patients with and without BRAF mutation may respond to immunotherapy through different mechanisms.

Methods

All clinical and mutational data, including TMB scores, were obtained from the cBioPortal database (https://www.cioportal.org) on May 25, 2020. The accession IDs for the two ICI treatment cohorts are TMB_{MSKCC}*{2018} and MIXED*{ALLEN}_{2018}. Immunogenomic data and MMR status were obtained from published analyses of the TCGA PanCancer Atlas and MSKCC-IMPACT cohorts, respectively. All cases without BRAF mutation status or cancer type information were excluded. Kaplan–Meier survival analysis with a two-sided log-rank test was employed to estimate the relationship between BRAF mutation and overall survival (OS). Multivariate Cox regression analysis was performed to determine the contribution of mutation to OS. A two-sided Mann–Whitney U test was used to examine differences between groups. Statistical analysis was conducted using R v3.6.1. All analyses used deidentified public-use data. This study was deemed exempt from institutional review board approval, and patient informed consent was waived.

Results

After excluding 8,518 cases without BRAF mutation status or cancer type information, we obtained 38,142 patients across 30 cancer types from a curated set of non-redundant studies in cBioPortal. In total, 1,292 patients harbored BRAF V600E mutations, 858 patients had other BRAF mutations, and the overall mutation frequency was 5.6% (Fig. 1). The three cancer types with the highest BRAF mutation frequencies were thyroid cancer (53.9%), melanoma (45.0%), and colorectal cancer (13.6%).

We next used the MSKCC ICI treatment cohort to investigate the association between BRAF mutation and overall survival. Kaplan–Meier survival analysis was performed on 1,630 patients with follow-up data, including ten types of advanced cancer as previously described. As shown in Fig. 2a, BRAF-mutant patients exhibited significantly better overall survival than wild-type patients (Hazard ratio [HR] = 0.55, 95% confidence interval [CI]: 0.43–0.72; $P < .001$). This result was validated in an independent ICI treatment cohort from the Dana-Farber Cancer Institute comprising 249 patients with seven cancer types (HR = 0.68, 95% CI: 0.46–0.99; P = .045; Fig. 2b). To adjust for potential bias, we selected 1,078 cases with complete data including BRAF mutation, TMB score, MMR status, cancer type, age, and sex from the MSKCC cohort and performed multivariate Cox regression analysis. After adjusting for these factors, the association between BRAF mutation and improved overall survival after ICI treatment remained statistically significant (adjusted HR = 0.58, 95% CI = 0.43–0.78; $P < .001$). The clinical characteristics of patients in the two cohorts are shown in Supplementary Table 1 and Supplementary Table 2 .

We further employed TCGA immunogenomic data (n = 7,251) to investigate potential mechanisms that could explain the immunotherapy efficacy in BRAF-mutant patients. Although an increased number of tumor neoantigens has been linked to better response during immunotherapy, patients without BRAF mutation exhibited a significantly higher number of neoantigens ($P < 0.001$, Fig. 3a) compared to the BRAF-mutant group. We then compared intratumor heterogeneity (ITH) between groups, as previous studies have reported that increased ITH is associated with lower efficacy of immunotherapy across multiple cancer types. As shown in Fig. 3b, BRAF-mutant patients exhibited significantly lower ITH than wild-type patients ($P < .001$). We also compared six immune subtypes to systematically evaluate the immune landscape of cancer. The results showed that the C3 (inflammatory) subtype was enriched in the BRAF-mutant group (55.4% versus 21.4%), whereas the C4 (lymphocyte-depleted) subtype was relatively more frequent in the wild-type group (13.3% versus 6.1%, Fig. 3c).

Discussion

Our study is the first to report that BRAF-mutant patients who received ICI treatment demonstrated better overall survival than wild-type patients at a pan-cancer level. This finding addresses the current limitation that clinical response to ICI treatment is restricted to a minority of unselected patients. Higher TMB scores and dMMR, which have been identified as positive predictive markers, define approximately 20% of patients in the MSKCC ICI treatment cohort. Our findings will incorporate BRAF-mutant patients and substantially improve therapeutic prospects for a sizeable fraction of patients (28.7%). Nevertheless, we still need to identify better biomarkers to define the likelihood of benefit from immunotherapy.

Patients with higher TMB or dMMR harbor a greater number of tumor neoantigens presented on MHC molecules, and ICI treatment promotes an anti-tumor immune response that recognizes these neoantigens as foreign. Our study found that BRAF-mutant tumors present a small number of tumor neoantigens; however, these patients show better clinical outcomes after ICI treatment. This result suggests that mechanisms of immunotherapy response in BRAF-mutant tumors may not be entirely determined by tumor neoantigen presentation. Lower ITH is one mechanism that can improve immunotherapy efficacy. Additionally, immune landscape analysis revealed that the C3 subtype, characterized by a balanced immune response, comprises more than half of BRAF-mutant tumors. In contrast, the low lymphocyte infiltration subtype (C4) accounted for a small fraction in the BRAF-mutant group—less than half of that in the wild-type group. Lower ITH and immune equilibrium, as featured in our study, partially explain the favorable response of BRAF-mutant patients after ICI treatment. Therefore, the relationship between BRAF and the tumor immune microenvironment, as previously reported, deserves further analysis.

Our study has several limitations. First, the findings were primarily based on

the MSKCC and Dana-Farber ICI treatment cohorts, which did not include all cancer types with frequently mutated BRAF (e.g., thyroid cancer). Therefore, BRAF mutation still has great potential to identify additional patients who respond to immunotherapy across various cancer types. Second, our bioinformatics analysis of potential mechanisms underlying BRAF-mutant immunotherapy can inspire prospective studies but requires validation in clinical trials. Additionally, the specific mechanisms of immunotherapy response in tumors with different driver mutations or various microenvironments are not fully understood and warrant further investigation.

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Data Availability

All clinical and mutational data are publicly available at https://www.cioportal.org. The accession IDs for the two ICI treatment cohorts are TMB_{MSKCC}*{2018} and MIXED*{ALLEN}_{2018}. The immunogenomic data are publicly available in the published analyses of the TCGA PanCancer Atlas. MMR status is publicly available in the published analyses of the MSKCC-IMPACT cohort.

Code Availability

The Kaplan–Meier survival analysis, multivariate Cox regression analysis, and Mann–Whitney U test were performed using R v3.6.1. All codes from this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no competing interests.

Figure Legends

Figure 1 [Figure 1: see original paper]. Prevalence of BRAF mutation in 38,142 patients across 30 cancer types. GIST: gastrointestinal stromal tumor; CNS: central nervous system.

Figure 2 [Figure 2: see original paper]. Kaplan–Meier survival analysis of BRAF mutation and overall survival after immunotherapy. Patients were obtained from the MSKCC ICI treatment cohort and Dana-Farber ICI treatment cohort. ICI: immune checkpoint inhibitor; HR: hazard ratio; Mut: mutation; WT: wild type.

Figure 3 [Figure 3: see original paper]. Immunogenomic differences between patients with and without BRAF mutation. Patients were obtained from the TCGA PanCancer Atlas.

Supplementary Table 1 . Patients' Clinical Characteristics in MIXED_{ALLEN}_{2018} Cohort

Characteristic	Wild type (n=168)	BRAF mut (n=81)	P value
Age*, mean ± SD	61.8 ± 13.0	56.1 ± 17.0	
Sex, n (%)			
Female	63 (37.50)	32 (39.51)	
Male	105 (62.50)	49 (60.49)	<0.001
Cancer Type, n (%)			
Melanoma	74 (44.05)	77 (95.06)	
Non-Small Cell Lung Cancer	53 (31.55)	3 (3.70)	
Bladder Cancer	26 (15.48)	1 (1.23)	

Characteristic	Wild type (n=168)	BRAF mut (n=81)	P value
Head and Neck Cancer	12 (7.14)	0 (0.00)	
Anal Cancer	1 (0.60)	0 (0.00)	
Small Cell Lung Cancer	1 (0.60)	0 (0.00)	
Soft Tissue Sarcoma	1 (0.60)	0 (0.00)	
Drug Type, n (%)			<0.001
anti-CTLA-4	72 (42.86)	73 (90.12)	
anti-PD-1/PD-L1	90 (53.57)	4 (4.94)	
Combo	6 (3.57)	4 (4.94)	

*Data on age were missing for 66 patients in the wild type group and 31 patients in the BRAF-mutant group. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 20. Differences in age were analyzed with t-test. Differences in sex were analyzed with Chi-square test. Differences in cancer type and drug type were analyzed with Fisher' s exact test.

Supplementary Table 2 . Patients' Clinical Characteristics in TMB_{MSKCC}_{2018} Cohort

Characteristic	Wild type (n=1461)	BRAF mut (n=169)	P value
Age, mean ± SD	61.6 ± 13.4	60.5 ± 15.3	
Sex, n (%)			
Female	549 (37.58)	62 (36.69)	
Male	912 (62.42)	107 (63.31)	<0.001
Cancer Type, n (%)			
Melanoma	199 (13.62)	118 (69.82)	
Non-Small Cell Lung Cancer	311 (21.29)	24 (14.20)	
Colorectal Cancer	97 (6.64)	13 (7.69)	
Bladder Cancer	206 (14.10)	6 (3.55)	
Cancer of Unknown Primary	82 (5.61)	4 (2.37)	
Head and Neck Cancer	136 (9.31)	2 (1.18)	
Esophagogastric Cancer	122 (8.35)	1 (0.59)	
Glioma	114 (7.80)	1 (0.59)	
Renal Cell Carcinoma	150 (10.27)	0 (0.00)	
Breast Cancer	43 (2.94)	0 (0.00)	
Skin Cancer, Non-Melanoma	1 (0.07)	0 (0.00)	
Drug Type, n (%)			<0.001
anti-CTLA4	70 (4.79)	29 (17.16)	
anti-PD-1/PD-L1	1193 (81.66)	85 (50.30)	
Combo	198 (13.55)	55 (32.54)	

*Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 20. Differences in age were analyzed with t-test. Differences in sex and drug type were analyzed with Chi-square test. Differences in cancer type were analyzed with Fisher' s exact test.

Note: Figure translations are in progress. See original paper for figures.