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ORIGINAL RESEARCH

Molecular profiling of Asian patients with advanced melanoma receiving check-point inhibitor treatment

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Objective: Melanoma is major medical challenge and being able to monitor treatment response is critical. This study aimed to use molecular profiling of Asian patients with advanced melanoma who were receiving treatment with check-point inhibitors (CPIs) to identify novel biomarkers of tumor response.

Methods: Next-generation sequencing (NGS) was performed using tumor specimens collected from 178 Asian patients with metastatic melanoma receiving CPIs. The NGS data and clinical-pathological factors were analyzed for potential genetic biomarkers of tumor response to CPI treatment.

Results: The most common melanoma subtype was acral melanoma (40%), followed by cutaneous melanoma (32%), mucosal melanoma (26%), and others (2%). For calculation of treatment efficacy, 164 of the patients could be evaluated. The overall response rate was 45.7%, of which 41 cases exhibited complete responses (25.0%) and 34 showed partial responses (20.7%). There were no significant differences in tumor responses based on melanoma subtype ($P = 0.295$). Genetically, *NRAS* mutations, *TP53* mutations, and *NF2* deletions were significantly associated with resistance to CPIs ($P < 0.05$). In contrast, *MYC* and *RPS6KB1* amplifications were associated with responsiveness to CPIs ($P < 0.05$). Median progression-free survival (PFS) for patients treated with CPIs was 5.9 months (95% CI, 3.8-8.05 months). Univariate analysis identified *TP53* and *BRAF* mutations, *NF2* deletions, and *BIRC2* amplifications as poor prognostic factors for PFS ($P < 0.05$).

Conclusions: This study determined the integrated genomic profiles of Asian patients with metastatic melanoma receiving CPIs and identified candidate biomarkers that reflected treatment outcomes.

Key words: next-generation sequencing, melanoma, check-point inhibitor

INTRODUCTION

Melanoma is one of the leading causes of death among patients with skin cancer and is responsible for over 9000 deaths annually in the United States.[1] Complete surgical resection is considered the best option for cure of early stage melanoma; for patients with advanced melanoma, treatment outcomes remain poor with median overall survival (OS) being approximately 7.5 months.[2,3] Recently, technological advances in molecular and immunological sciences, such as the development of targeted therapeutic agents and immunotherapy, have lengthened survival times of patients with melanoma.

BRAF mutations are considered novel genetic alterations related to melanoma carcinogenesis and act through the mitogen activated protein (MAP) kinase and phosphatidylinositol 3′ kinase (PI3K) signaling pathway.[4,5] Such alterations have been found in 25%-70% of melanoma patients and vary according to the anatomic location of the melanoma and the ethnicity of the patient.[4,6,7] Novel agents that target *BRAF V600E* and the *MEK* pathway have been shown to provide significant survival benefits to patients with advanced melanoma harboring target mutations.[8-10] For example, advanced melanoma patients with a *BRAF V600* mutation treated with the *BRAF* inhibitor vemurafenib demonstrate longer OS than those treated with dacarbazine (median OS, 13.6 months versus 9.7 months, respectively).[9] In addition, a combination of the *BRAF* inhibitor dabrafenib and the *MEK* inhibitor trametinib shows greater improvement in survival compared to a *BRAF* inhibitor alone with 3-year progression free survival (PFS) being 22% versus 12%,

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respectively, and 3-year OS being 44% versus 32%, respectively.[10] Currently, evaluation of *BRAF* variants in patients has become a standard diagnostic tool to guide treatment strategies for advanced melanoma.[11]

Based on the improved survival of patients with metastatic melanoma treated with the cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) inhibitor ipilimumab,[12] immunotherapy has established itself as a new paradigm for anti-cancer treatment. Subsequently, programmed death 1 (PD-1) inhibitors, such as nivolumab and pembrolizumab, have been widely evaluated and have become the standard treatment for patients with melanoma.[13-17] However, there are currently no reliable biomarkers that can be used to predict immunotherapy responses in subsets of patients. Although expression of programmed cell death ligand 1 (PD-L1) and tumor mutation burden (TMB) are considered predictive biomarkers for immunotherapy response,[18] novel biomarkers for immunotherapy in melanoma are still needed.

Use of next-generation sequencing (NGS) can be a beneficial tool to guide treatment strategies in advanced melanoma and may also help screen for 'druggable' genetic variants for target agents, as well as aid in identifying new candidates for immunotherapy. However, the molecular characterization of Asian patients with melanoma receiving check-point inhibitors (CPIs) using NGS has not been reported. In the current study, we performed NGS analyses of Asian patients with advanced melanoma who were receiving CPIs to establish molecular profiles and identify novel biomarkers for CPI-treatment outcomes for this population of patients.

Table 1. Baseline characteristics of study population

	Median (range) $N = 178$
Age at time of NGS (years)	61 (23-84)
Sex	
Male	88 (49.4)
Female	90 (50.6)
Subtype	
Acral	71 (39.9)
Cutaneous	57 (32.0)
Mucosal	46 (25.8)
Uveal	1 (0.6)
Unknown	3 (1.7)
LDH (U/L)	
Elevated	120 (67.4)
Normal	27 (15.2)
Missing	31 (17.4)
Performance status (ECOG)	
0	3 (1.7)
1	167 (93.8)
2	8 (4.5)
PD-L1 expression	
<1	42 (23.6)
>1	59 (33.1)
Missing	77 (43.3)
Immunotherapy ($N = 164$)	
Pembrolizumab	135 (82.3)
Nivolumab	28 (17.1)
Ipilimumab	1 (0.6)
Immunotherapy line ($N = 164$)	
1	137 (83.5)
≥2	27 (16.5)

ECOG, Eastern Cooperative Oncology Group; LDH, lactic dehydrogenase; NGS, next generation sequencing; PD-L1, programmed cell death ligand 1.

MATERIALS AND METHODS

Study design

Patients with advanced melanoma who were receiving CPIs at Samsung Medical Center between 2016 and 2019 were enrolled in the study. Before starting treatment with CPIs, all the patients were tested using the same NGS platform with the Oncomine Comprehensive Assay (OCA; Thermo Fisher Scientific, Waltham, MA; www.thermofisher.com), which is a commercial test consisting of 143 actionable genes. The medical record of each patient was evaluated for patient age at initiation of immunotherapy, sex, pathology, melanoma stage, serum lactic dehydrogenase (LDH) level, and immunohistochemistry (IHC) results for PD-L1. The expression of PD-L1 protein was quantitated using a combined positive score (CPS), which was calculated as the number of PD-L1-stained cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100. A tumor specimen with CPS ≥1 was considered positive for PD-L1 expression. Tumor response to CPI treatment was evaluated based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The study was approved by the Institutional Review Board of Samsung Medical Center and was conducted in accordance with the ethical principles of the Declaration of Helsinki and the Korea Good Clinical Practice guidelines.

NGS using a custom panel

NGS was performed on formalin-fixed, paraffin-embedded specimens using the extensively validated OCA v1 platform. The methods for DNA/RNA extraction and for sequencing/reporting/validation of the assay were carried out according to previously published reports.[19] As the genomic profiles of two of the patients were identified using extracted RNA, their genomic data were excluded from further analysis.

From all mutations reported by OCA, we used only the deleterious (PROVEAN prediction) and damaging (SIFT prediction) mutations[20] to identify functional genomic correlates of response to CPI treatment. However, for analysis of microsatellite instability (MSI)-associated genetic alterations, all unfiltered mutations were used as MSI-induced mutations are passenger mutations.

Statistical analysis

Patient characteristics were summarized using descriptive statistics. Response categories were assessed according to RECIST v1.1. Each nominal variable was compared using Fisher's exact test or the χ^2 test. PFS was defined as the time from starting CPI treatment to the documentation of disease progression or death. PFS was estimated using the Kaplan–Meier method combined with log-rank analysis.

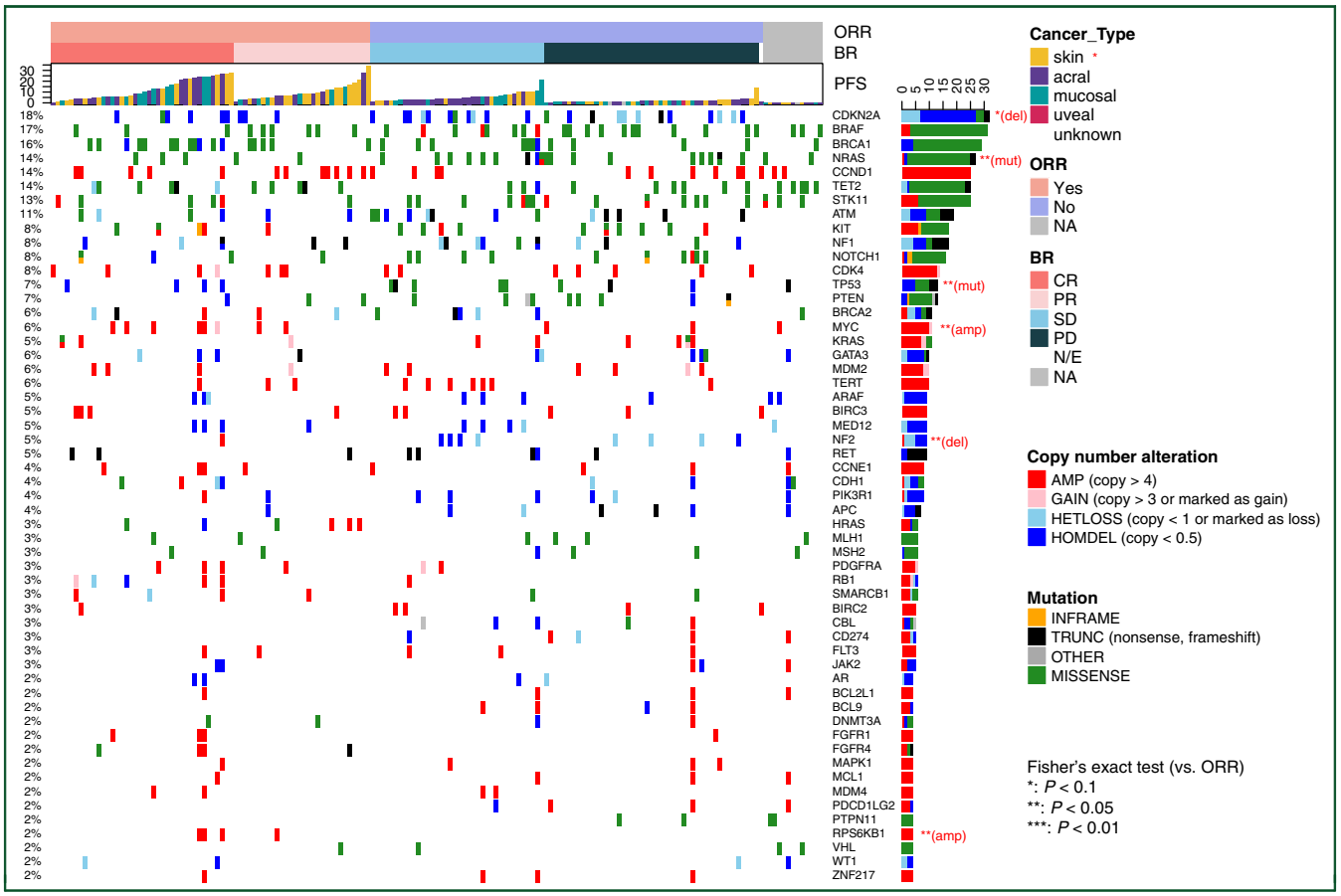

Figure 1. Somatic gene mutation and copy number alteration profiles of 178 Asian patients with melanoma.
Each row represents the genetic alterations and each column represents a single patient. Genetic alterations are indicated in different colors according to type. BR, best response; CR, complete response; NA, not alplicable; N/E, not evaluated; ORR, overall response rate; PFS, progression-free survival; PD, progressive disease; PR, partial response; SD, stable disease.

Two-sided null hypotheses of no difference were rejected if *P* values were <0.05 or if the 95% confidence interval (CI) of risk point estimates was excluded. Cox proportional hazards regression modeling was employed in univariate analysis to identify significant and independent prognostic factors for various clinical parameters and molecular aberrations for survival. The analyses to evaluate the associations between genetic alterations and responses to CPIs were performed using R language 3.5 (Foundation for Statistical Computing, Vienna, Austria), while the other analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 19.0 (SPSS Inc., Chicago, IL).

RESULTS

Study population

From May 2017 to September 2019, tumor specimens from a total of 188 patients at Samsung Medical Center who had pathologically confirmed advanced melanoma were analyzed by NGS. Of the 188 samples, 10 were excluded from subsequent analysis due to insufficient amounts of tumor sample. The clinical features of the remaining 178 patients are presented in Table 1. The median patient age was 61 years (range, 23-84 years). There were 90 females (50.6%) and 88 males (49.4%). The LDH levels before immunotherapy exceeded the upper normal level (UNL) in 120 of the 178 patients (67.4%). The most common melanoma subtype in the study population was acral (39.9%), followed by cutaneous (32.0%), mucosal (25.8%), and uveal (0.6%). The status of PD-L1 expression was available for 101 of the patients, for which 59 (58.4%) had PD-L1 CPS positivity in their tumors.

Genetic alterations

Of the 178 tumor samples analyzed by NGS, 106 (59.6%) had at least one genomic alteration (Figure 1). The most commonly detected mutations were in *BRAF* (16.3%), followed by *NRAS* (14.6%), *KIT* (6.2%), *TP53* (6.2%), *PTEN* (5.6%), and *NF1* (5.1%). There were no detectable somatic mutations in 72 of the patients (40.2%). Gene copy number variations were observed in 98 patients (55.1%), with the most common variations being *CDKN2A* loss (14.6%) and *CCND1* amplification (14.0%). There were different distributions of genomic alterations based on melanoma subtype (Figure 2). For example, *KIT* mutations were observed in 11 of 121 patients with non-cutaneous melanoma (acral and mucosal subtypes), but *KIT* mutations were not observed in any of the 57 patients with cutaneous melanoma. *BRAF* mutations were frequently observed in patients with acral and cutaneous subtypes of melanoma compared with those with mucosal subtype.

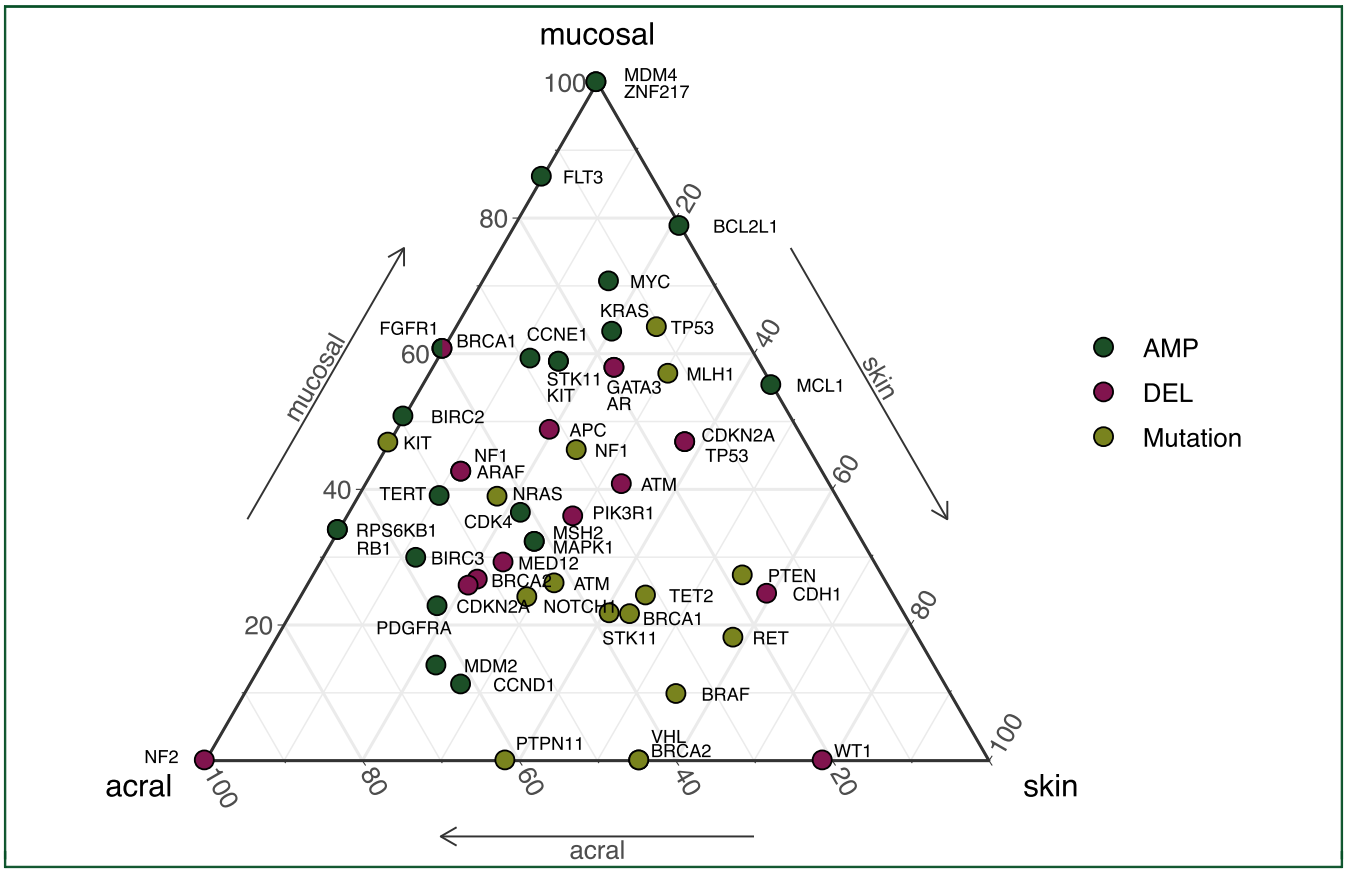

Figure 2. Ternary diagram of genetic alterations according to melanoma subtype: mucosal, acral, and cutaneous.
Genetic alterations with an allele frequency >3% were selected for clear visualization. The size of each circle is relative to the number of patients with that particular genetic alteration. PD-L1, programmed cell death ligand 1; PFS, progression-free survival.

Immunotherapy

During follow-up (median duration 16.9 months), 164 of the 178 patients received CPIs as immunotherapy. This included 135 patients (82.3%) that received pembrolizumab, 28 patients (17.1%) that received nivolumab, and one patient (0.6%) that received ipilimumab. The majority of patients ($n = 137$) received CPIs as a first-line therapy and 27 patients received CPIs as a second-line treatment (Table 1). Among the 164 patients receiving CPIs, 41 exhibited complete response, 34 had partial responses, 48 had stable disease, and 41 experienced disease progression (Table 2). The overall response rate (ORR) was 45.7% and disease control rate (DCR) was 70.7%. There was no significant difference in ORR to CPI treatment among the melanoma subtypes. In patients demonstrating tumor response to CPIs, *MYC* and *RPS6KB1* amplification were observed more frequently compared with those without tumor response. Meanwhile, *NRAS* mutations and *TP53* and *NF2* deletions were significantly associated with resistance to CPIs ($P < 0.05$). The median PFS was 5.9 months (95% CI, 3.8-8.1). According to univariate analysis, *TP53* mutations, *BRAF* mutations, *NF2* deletions, and *BIRC2* amplifications were poor prognostic indicators for PFS ($P < 0.05$), while old age (≥65 years) and *BRCA1* mutations were associated with longer PFS ($P < 0.05$; Figure 3A).

Multivariate analysis confirmed that *TP53* mutations, *BRAF* mutations, and *NF2* deletions were independent poor prognostic factors for PFS ($P < 0.05$), while old age (≥65 years) and *BRCA1* mutations were independent prognostic factor for longer PFS (Figure 3B).

Table 2. Response to check-point inhibitors (CPIs) according to melanoma subtypes

	Overall $N = 164$	Acral $n = 65$	Mucosal $n = 43$	Cutaneous $n = 53$	Others[a] $n = 3$	*P* value
Response to CPIs						
Complete response	41	17	11	13	0	
Partial response	34	11	7	16	0	
Stable disease	41	21	11	9	0	
Progressive disease	48	16	14	15	3	
Overall response rate	45.7%	43.1%	41.9%	54.7%	0%	0.320
Disease control rate	70.7%	75.4%	67.4%	71.7%	0%	0.085

[a] Others: 1 uveal, and 2 miscellaneous.

Exploratory analysis: genetic mutation associated to microsatellite instability and TMB

Since the current study utilized panel sequencing (OCA v1) to reveal clinically relevant genomic alterations in tumor samples, it was not able to directly assess microsatellite

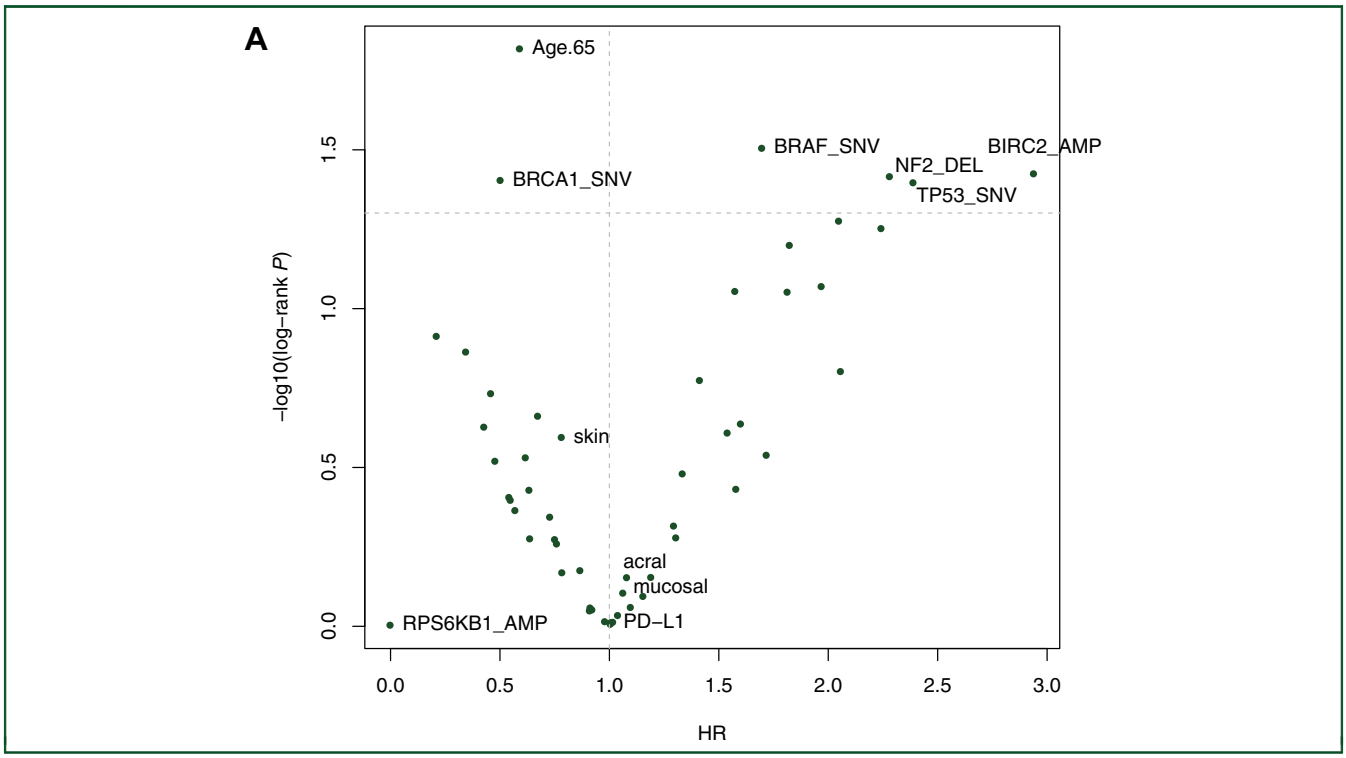

Figure 3. Cox survival analysis for progression-free survival in patients receiving immunotherapy.
(A) Univariate analysis, (B) multivariate analysis.

stability. Instead, we investigated the association between treatment responses and MSI-associated genetic alterations. Defective mismatch repair (MMR) genes are the cause of MSI, resulting in hypermutation of tumors. Therefore, alterations in MMR genes and the total number of mutations per sample were compared with responses to CPIs. *MSH2* genetic alterations were found in 6% of total study population and *MLH1* genetic alterations were found in 5% (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2020.100002). These mutations were not associated with response to CPI nor PFS (Table 3 and Figure 4A).

However, additional analysis revealed that the patients who possessed $\geq$10 mutations showed prolonged PFS after CPI treatment compared with the patients with $<$10 mutations ($P = 0.051$) (Figure 4B).

DISCUSSION

In the current study, we evaluated the different molecular profiles of 178 Asian patients receiving treatment with CPIs according to their melanoma subtype. There were no significant differences in ORR for CPIs among the patients with different subtypes of melanoma. In the patients that achieved tumor responses to CPI treatment, *MYC* and *RPS6KB1* amplifications were more frequently observed. Meanwhile, *NRAS* mutations, *TP53* mutations, and *NF2* deletions were significantly associated with resistance to CPIs ($P < 0.05$). These findings suggest that specific molecular alterations may influence treatment outcomes in patients receiving CPIs. To the best of our knowledge, this is the largest integrated genomic study to date that aimed to identify novel biomarkers of tumor response to CPIs in Asian patients with advanced melanoma.

We found that *BRAF* and *NRAS* mutations were present in 16.3% and 14.6% of the 178 melanoma patients, respectively. Previous studies have reported that *BRAF* mutations are generally detectable in 40%-50% of melanoma patients and *NRAS* mutations are present in approximately 20% of melanoma patients.[4,21] However, unlike Western patients, Asian patients with melanoma have relatively low incidences of *BRAF* mutations (14%-26%) and *NRAS* mutation (7%-10%).[22-24] Our current findings are consistent with these previous reports and suggest that *BRAF* and *MEK* inhibitors may have different efficacies between Western and Eastern patients with melanoma.

The ORR for CPI treatments of 45.7% and median PFS of 5.9 months observed in our study were consistent with those of Japanese melanoma patients treated with immunotherapy (43%).[23,25] A similar result was also reported in Western patients with acral or mucosal subtype melanoma (ORR, 23%-32%; PFS, 4.0 months).[26] Immunotherapy for acral or mucosal subtypes of melanoma is usually considered less effective than that for cutaneous melanoma as the lower mutational burden typically observed in acral/mucosal subtypes is associated with lower immunotherapy efficacy. The findings from our study support this theory in that PFS of patients with acral melanoma (5.0 months) and mucosal melanoma (5.9 months) was shorter than that of patients with cutaneous melanoma (9.3 months).

PD-L1 expression is a novel predictor of response to CPIs. However, not all patients with PD-L1 expression show a

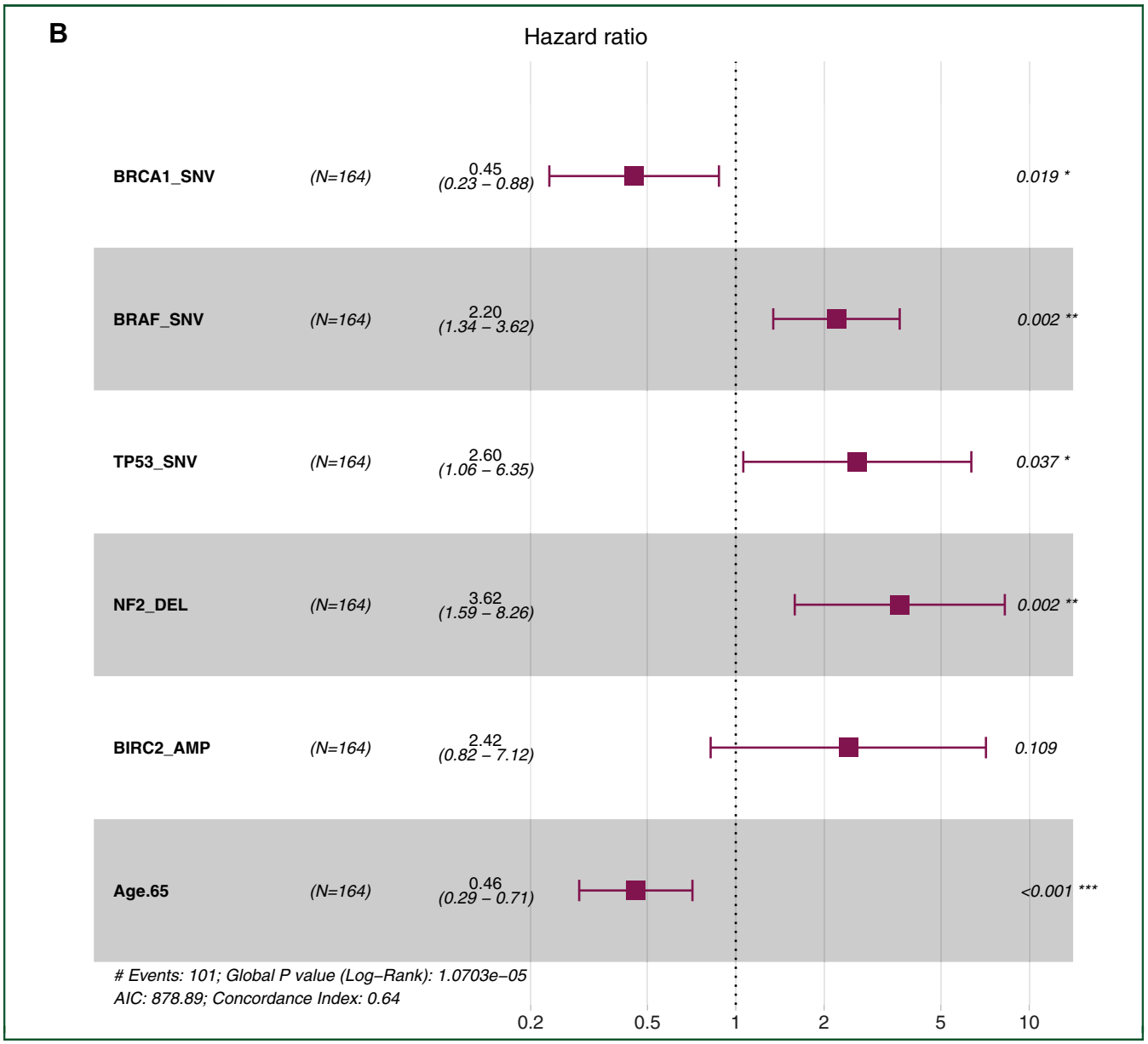

Figure 3. **(Continued)**

Table 3. Statistical significance between responses to check-point inhibitors and microsatellite instability-associated genetic alterations

	Odd ratio	*P* value
Total number of mutations	1.38	0.46
MSH2 alterations	1.25	1
MLH1 alterations	2.56	0.30
MSH2/MLH1 alterations	0.58	0.33

response to CPIs.[15,27,28] Some studies have suggested that genomic alterations may affect the efficacy of immunotherapy.[29-32] *TP53* mutations are associated with impaired T-cell immunity and can lead to decreased responses to immunotherapeutic agents.[33] A previous study reported that a *TP53* mutation was associated with worse outcomes for anti-CTLA-4 treatment of patients with metastatic melanoma.[34] These investigators explained that the down regulation of FS-7-associated surface antigen (FAS) induced by the *TP53* mutation impedes cytotoxic T-cell activity and results in less effective anti-CTLA-4 activity. Our analysis of *TP53* mutations resulted in findings similar to the previous report. In the current study, *NRAS* mutations were also significantly associated with resistance to CPIs ($P < 0.05$). However, this finding was discordant with those of a previous study in which Johnson et al.[32] reported that *NRAS* mutations were associated with a better response to immunotherapy. However, the number of patients with *NRAS* mutations analyzed in that study was small. Regardless, they suggested that *NRAS* may affect the response to immunotherapy through a link to higher expression of PD-L1.[32] Until now, most studies on the relationship between genomic characterization and immunotherapy

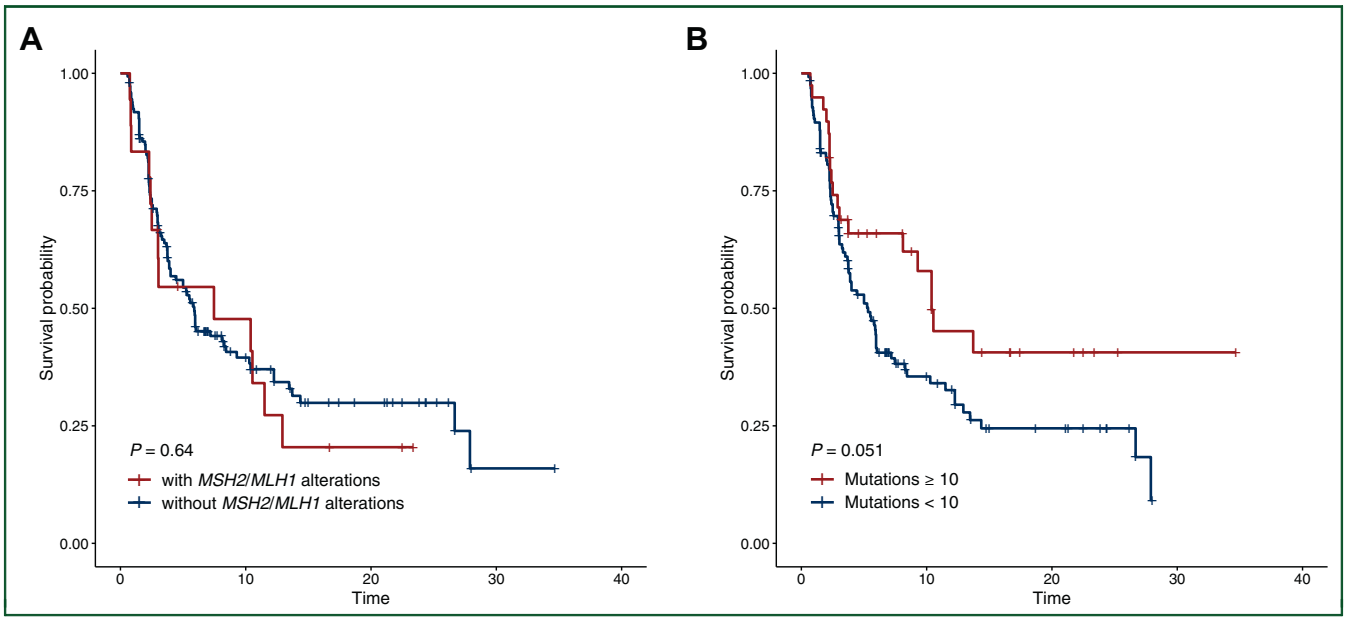

Figure 4. Kaplan–Meier estimates of progression free survival after check point inhibitor (CPI) treatment in melanoma patients.
(A) A plot showing survival differences between patients with *MSH2* or *MLH1* alterations and others. (B) A plot showing survival differences between patients with ≥10 mutations and others. The *x*-axis represents progression free survival (months) after CPI.

efficacy have been conducted retrospectively. Additional prospective trials with large sample sizes are needed to clarify the relationships.

In breast and ovarian cancers, *BRCA1* mutations are associated with PD-1 or PD-L1 expression, high mutation burden, and tumor-infiltrating lymphocytes.[35-37] A pre-clinical study using a *BRCA1*-deficient breast cancer mouse model demonstrated that a combination of CPIs and chemotherapy augmented antitumor activity.[35] Similar findings were observed in a study that evaluated a combination of CPIs and a poly-ADP ribose polymerase (PARP) inhibitor using a *BRCA1*-deficient murine ovarian cancer model.[38] While these results have not been confirmed clinically,[39,40] a clinical trial using CPIs in *BRCA1/2*-mutated breast cancer (NCT03025035) is currently being conducted.

In the current study, we also compared MSI-associated genetic alterations and the total number of mutation to CPI treatment outcomes. Although *MSH2* and *MLH1* alterations were not correlated with either overall response or PFS, we could not conclude that the genomic alterations in MMR genes are not associated with responses to CPI in melanoma patients since mutational status in other MMR genes such as *MSH6* or *PMS2* was not available in this study. Additional analysis found that a higher number of total mutations (10 or more) was associated with prolonged PFS. High TMB has been found in responders to CPIs in various tumors,[18] and our result implies that high TMB could be a potential prognostic marker for CPI treatment in melanoma as well.

Our current study had several limitations. First, it was a retrospective study, and clinically heterogeneous populations are subject to potential biases. Second, the study included a relatively small number of patients, making it difficult to draw definite conclusions regarding genomic biomarkers. Third, only Asian patients with melanoma were analyzed in the study, and differences in genomic profiles and clinical features exist between Western and Eastern patients with melanoma. Therefore, our findings must be interpreted with a level of caution. Nevertheless, our study revealed the integrated genomic profile of Asian patients with metastatic melanoma and identified candidate biomarkers for treatment outcomes of CPIs. Overall, our findings might provide useful information when deciding to include CPIs as a therapeutic intervention for patients with advanced melanoma.

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DISCLOSURE

None to declare.

ETHICS

The study was approved by the Institutional Review Board of Samsung Medical Center.

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