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BRAF and *NRAS* Mutation Status and Response to Checkpoint Inhibition in Advanced Melanoma

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abstract

PURPOSE Little is known about the effect of specific gene mutations on efficacy of immune checkpoint inhibitors in patients with advanced melanoma.

MATERIALS AND METHODS All patients with advanced melanoma treated with first-line anti–PD-1 or ipilimumab-nivolumab between 2012 and 2021 in the nationwide Dutch Melanoma Treatment Registry were included in this cohort study. Objective response rate, progression-free survival (PFS), and overall survival (OS) were analyzed according to *BRAF* and *NRAS* status. A multivariable Cox model was used to analyze prognostic factors associated with PFS and OS.

RESULTS In total, 1764 patients received anti–PD-1 and 759 received ipilimumab-nivolumab. No significant differences in PFS were found in the anti–PD-1 cohort. In the ipilimumab-nivolumab cohort, median PFS was significantly higher for *BRAF*-mutant melanoma (9.9 months; 95% CI, 6.8 to 17.2) compared with *NRAS*-mutant (4.8 months; 95% CI, 3.0 to 7.5) and double wild-type (5.3 months; 95% CI, 3.6 to 7.1). In multivariable analysis, *BRAF*-mutant melanoma was significantly associated with a lower risk of progression or death in the ipilimumab-nivolumab cohort. Median OS was significantly higher for *BRAF*-mutant melanoma compared with *NRAS*-mutant and double wild-type melanoma for both immune checkpoint inhibitor regimens.

CONCLUSION Ipilimumab-nivolumab–treated patients with *BRAF*-mutant melanoma display improved PFS and OS compared with patients with *NRAS*-mutant and double wild-type melanoma. *BRAF* mutation status is a factor to consider while choosing between mono and dual checkpoint inhibition in advanced melanoma.

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INTRODUCTION

The emergence of molecular genetics led to more possibilities in defining genetic tumor profiles, and mutation analysis is becoming a common practice in a growing number of malignancies.[1] The ability to analyze and target tumor mutations has significantly altered the oncology treatment landscape over the last few decades. The most frequent driver mutation in melanoma is the BRAF mutation, being present in approximately 40%-50% of melanomas. The *BRAF* V600E mutation accounts for 70%-88% of all *BRAF* mutations.[2] Other frequently present driver mutations are mutations in the *NRAS* gene (15%-20%), *NF1* gene (10%-15%), and *KIT* gene (1%-2%).[3,4] Some of these mutations have proven to be targetable and led to the development of targeted therapies, such as BRAF inhibition. Tumor mutational burden (TMB) has been identified as an important predictor of immune checkpoint inhibitor (ICI) efficacy.[5,6] Melanoma was found to harbor the highest average mutation frequency of all cancer types,[7] which explains the relatively high response rate to ICIs.[8] However, little is known about the effect of the different driver mutations on the response rate to ICIs and survival in advanced melanoma.

Theoretically, patients with *BRAF* V600E–mutant melanomas could obtain less benefit from ICI because of their lower TMB compared with *NRAS*-mutant and double wild-type melanoma[2,9] and the supposed direct immunosuppressive effects of the *BRAF* V600E mutation.[10,11] However, large randomized studies have shown improved outcomes for *BRAF*-mutant melanoma.[12,13]

This study aims to investigate the influence of *BRAF* and *NRAS* mutations on objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) in patients with unresectable stage III and

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Anti–PD-1 is the preferred first-line treatment for patients with advanced melanoma. The choice between anti–PD-1 monotherapy and anti–PD-1 combined with a cytotoxic T-cell lymphocyte-4 inhibitor, such as ipilimumab-nivolumab, is less obvious. There are few known factors that identify patients who benefit from the more toxic ipilimumab-nivolumab regimen. This article describes the effects of *BRAF* and *NRAS* mutations on the outcomes of 2,523 patients with advanced melanoma treated with anti–PD-1 monotherapy or ipilimumab-nivolumab.

Knowledge Generated

This research shows that ipilimumab-nivolumab–treated patients with *BRAF*-mutant melanoma display a significantly improved progression-free survival and overall survival compared with patients with *NRAS*-mutant or double wild-type melanoma, whereas this difference was not seen in anti-PD1–treated patients.

Relevance

BRAF mutation status can be a factor to take into account while choosing between anti–PD-1 monotherapy and ipilimumab-nivolumab in advanced melanoma.

IV melanoma treated with ICIs (anti–PD-1 monotherapy and ipilimumab-nivolumab combination therapy).

MATERIALS AND METHODS

For this study, we used data from the Dutch Melanoma Treatment Registry (DMTR). The DMTR prospectively registers data of all patients with unresectable stage IIIc and IV melanoma in the Netherlands since 2012.[14] All patients with advanced cutaneous melanoma treated with either first-line anti–PD-1 antibody monotherapy or ipilimumab-nivolumab combination therapy registered in the DMTR between December 2012 and June 2021 were included in this study. Three patient groups were identified: patients with *BRAF*-mutant melanoma, patients with *NRAS*-mutant melanoma, and patients with *BRAF* and *NRAS* wild-type melanoma. Patients with both *BRAF*-mutant and *NRAS*-mutant melanoma and patients whose mutation status was not determined were excluded from this study. We further divided patients into two groups: patients with *BRAF* V600–mutant melanoma (consisting of *BRAF* p.V600E, p.V600K, p.V600R, p.V600D, and p.V600_K601delinsE mutations) versus patients with *BRAF* V600 wild-type melanoma (consisting of all other melanoma mutations). Baseline characteristics, objective response rate, and survival outcomes were compared between the different groups. Research using DMTR data was approved by the medical ethical committee and was not deemed subject to the Medical Research Involving Human Subjects Act in compliance with Dutch regulations. For this study, the data set cutoff date was August 3, 2021.

Patient Characteristics

Baseline patient and tumor characteristics analyzed for all patients were age at diagnosis, sex, Eastern Cooperative Oncology Group Performance Status (ECOG PS), lactate dehydrogenase (LDH) levels, primary melanoma location, type of melanoma, Breslow thickness, ulceration, liver metastasis, brain metastasis, number of organ sites with metastases and stage according to the American Joint Committee on Cancer 8th edition,[15] and type of therapy.

Statistical Analysis

Baseline characteristics were analyzed using descriptive statistics. Pearson's chi-squared test was used to compare categorical variables, and the *t*-test for continuous variables. Median follow-up time was estimated from the date of the first visit using the reversed Kaplan-Meier method.[16] The objective response rate was calculated for the first treatment line. Response evaluation was determined by the treating physician and was based on the RECIST version 1.1.[17] The objective response rate was defined as the proportion of evaluable patients who were tumor-free or achieved a complete response or partial response. Patients were deemed not evaluable for ORR if they died from nonmelanoma-related cause before their first evaluation of response or did not have a response registered in the DMTR. Median PFS and OS were calculated using the Kaplan-Meier method. PFS was calculated from the start of systemic therapy until progression, death by any cause, or last moment of follow-up. OS was calculated from the start of systemic therapy until death by any cause or last moment of follow-up. Patients not reaching the end point were censored at the date of the last contact. A Cox proportional hazards model was used to perform a multivariate regression analysis to assess individual factors associated with PFS and OS. Factors that were significantly ($P < .01$) associated with PFS and OS in the univariable analysis were selected for the multivariable analysis. Comparisons were considered statistically significant for two-sided P values $< .05$. Data handling and statistical analyses were performed using R studio (version 4.0.2),[18] packages tidyverse,[19] tableone,[20] survival,[21] and survminer.[22]

RESULTS

In total, 2,547 first-line ICI-treated patients met the inclusion criteria. After excluding 24 patients with both *BRAF*-mutant

TABLE 1. Patient Characteristics

Characteristic	BRAF Mutation	NRAS Mutation	BRAF and NRAS Wild-Type	*P*
No.	1,002	713	808	
Age, years, No. (%)				
< 70	702 (70.1)	394 (55.3)	401 (49.6)	< .001
≥ 70	300 (29.9)	319 (44.7)	407 (50.4)	
Median age (IQR)	62.0 (52.0-71.0)	68.0 (59.0-75.0)	70.0 (60.0-77.0)	< .001
Sex, No. (%)				
Male	600 (59.9)	474 (66.5)	500 (61.9)	.041
Female	402 (40.1)	239 (33.5)	308 (38.1)	
ECOG PS, No. (%)				
0	618 (61.7)	351 (49.2)	393 (48.6)	< .001
1	307 (30.6)	262 (36.7)	296 (36.6)	
≥ 2	29 (2.9)	62 (8.7)	73 (9.0)	
Unknown	48 (4.8)	38 (5.3)	46 (5.7)	
Melanoma location, No. (%)				
Primary unknown	134 (13.4)	114 (16.0)	128 (15.8)	< .001
Head-neck	132 (13.2)	70 (9.8)	176 (21.8)	
Trunk	463 (46.2)	237 (33.2)	230 (28.5)	
Extremities	260 (25.9)	265 (37.2)	224 (27.7)	
Acral	11 (1.1)	22 (3.1)	40 (5.0)	
Unknown	2 (0.2)	5 (0.7)	10 (1.2)	
Melanoma type, No. (%)				
Superficial spreading	463 (46.2)	260 (36.5)	262 (32.4)	< .001
Nodular	189 (18.9)	181 (25.4)	159 (19.7)	
Acral lentiginous	5 (0.5)	10 (1.4)	42 (5.2)	
Lentigo maligna	11 (1.1)	6 (0.8)	37 (4.6)	
Desmoplastic	1 (0.1)	1 (0.1)	15 (1.9)	
Others	19 (1.9)	12 (1.7)	30 (3.7)	
Unknown	314 (31.3)	243 (34.1)	263 (32.5)	
Breslow thickness, mm, No. (%)				
< 1.01	113 (11.3)	54 (7.6)	70 (8.7)	< .001
1.01-2.00	229 (22.9)	154 (21.6)	129 (16.0)	
2.01-4.00	236 (23.6)	185 (25.9)	192 (23.8)	
> 4.00	196 (19.6)	142 (19.9)	196 (24.3)	
Unknown	228 (22.8)	178 (25.0)	221 (27.4)	
Median Breslow thickness (IQR)	2.3 (1.3-4.1)	2.6 (1.6-4.2)	3.0 (1.7-5.0)	< .001
Ulceration, No. (%)				
No	418 (41.7)	284 (39.8)	307 (38.0)	.330
Yes	270 (26.9)	197 (27.6)	228 (28.2)	
Unknown	314 (31.4)	232 (32.5)	273 (33.8)	
LDH levels, No. (%)				
Normal	728 (72.7)	444 (62.3)	526 (65.1)	< .001
250-500	207 (20.7)	182 (25.5)	198 (24.5)	
> 500	50 (5.0)	79 (11.1)	68 (8.4)	
Unknown	17 (1.7)	8 (1.1)	16 (1.9)	

(Continued on following page)

TABLE 1. Patient Characteristics (Continued)

Characteristic	BRAF Mutation	NRAS Mutation	BRAF and NRAS Wild-Type	*P*
Liver metastases, No. (%)				
No	968 (78.4)	646 (73.3)	736 (70.4)	< .001
Yes	258 (20.9)	223 (25.3)	296 (28.3)	
Unknown	9 (0.7)	12 (1.4)	14 (1.3)	
Brain metastases, No. (%)				
No	761 (75.9)	529 (74.2)	604 (74.8)	.121
Yes, asymptomatic	152 (15.2)	93 (13.0)	105 (13.0)	
Yes, symptomatic	70 (7.0)	74 (10.4)	76 (9.4)	
Unknown	19 (1.9)	17 (2.4)	23 (2.8)	
Organ sites, No. (%)				
< 3	599 (59.8)	412 (57.8)	461 (57.1)	.578
≥ 3	398 (39.7)	298 (41.8)	340 (42.1)	
Unknown	5 (0.5)	3 (0.4)	7 (0.9)	
AJCC stage (eighth edition), No. (%)				
IIIc unresectable	92 (9.2)	79 (11.1)	105 (13.0)	.132
IV-M1a	104 (10.4)	62 (8.7)	60 (7.4)	
IV-M1b	159 (15.9)	112 (15.7)	106 (13.1)	
IV-M1c	423 (42.2)	292 (41.0)	353 (43.7)	
IV-M1d	222 (22.2)	167 (23.4)	181 (22.4)	
Unknown	2 (0.2)	1 (0.1)	3 (0.4)	
BRAF mutation, No. (%)				
V600	714 (71.3)	0 (0.0)	0 (0.0)	NA
Treatment type, No. (%)				
Anti-PD1	699 (69.8)	498 (69.8)	567 (70.2)	.981
Ipilimumab-nivolumab	303 (30.2)	215 (30.2)	241 (29.8)	

NOTE. Comparison of baseline characteristics stratified by mutation: *BRAF* mutation, *NRAS* mutations, and *BRAF* and *NRAS* wild-type.

Abbreviations: AJCC, American Joint Committee on Cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase.

and *NRAS*-mutant melanoma, 1764 patients with cutaneous melanoma treated with anti–PD-1 and 759 treated with ipilimumab-nivolumab were included. The median follow-up was 30.2 months for anti–PD-1-treated patients and 21.6 months for ipilimumab-nivolumab–treated patients.

In 87% of patients, information regarding the genetic tests used to demonstrate *BRAF* and *NRAS* mutations was available. Next-generation sequencing was most often used (60% of patients), followed by Sequenom MassARRAY analysis, Sanger sequencing, and High-Resolution Melting. The *BRAF* V600E mutation was mostly the common mutation in the *BRAF* V600 group (82%). Of the 1764 first-line anti–PD-1-treated patients, 699 had *BRAF*-mutant melanoma, 498 had *NRAS*-mutant melanoma, and 567 had double wild-type melanoma. Of the 759 ipilimumab-nivolumab–treated patients, 303 had *BRAF*-mutant melanoma, 215 had *NRAS*-mutant melanoma, and 241 had double wild-type melanoma.

Patient Characteristics

ICI-treated patients with a *BRAF* mutation were younger and more often female and had better ECOG PS compared with *NRAS*-mutant and wild-type patients. The primary melanomas in the *BRAF* group were more often located on the trunk, were more frequently of the superficial spreading subtype, and had lower Breslow thickness. LDH levels in the *BRAF*-mutant group were also lower than in the *NRAS* and double wild-type groups. Baseline characteristics stratified by genetic mutation are shown in Table 1. Baseline characteristics for the anti–PD-1 and ipilimumab-nivolumab groups separately and different genetic subgroups are shown in the Data Supplement. Patients with *BRAF*-mutant melanoma generally appeared to have more favorable disease characteristics (eg, lower ECOG PS, lower LDH, and no symptomatic brain metastases) in the anti-PD1 monotherapy cohort and the combinational treatment cohort. The anti–PD-1-treated cohort appeared to

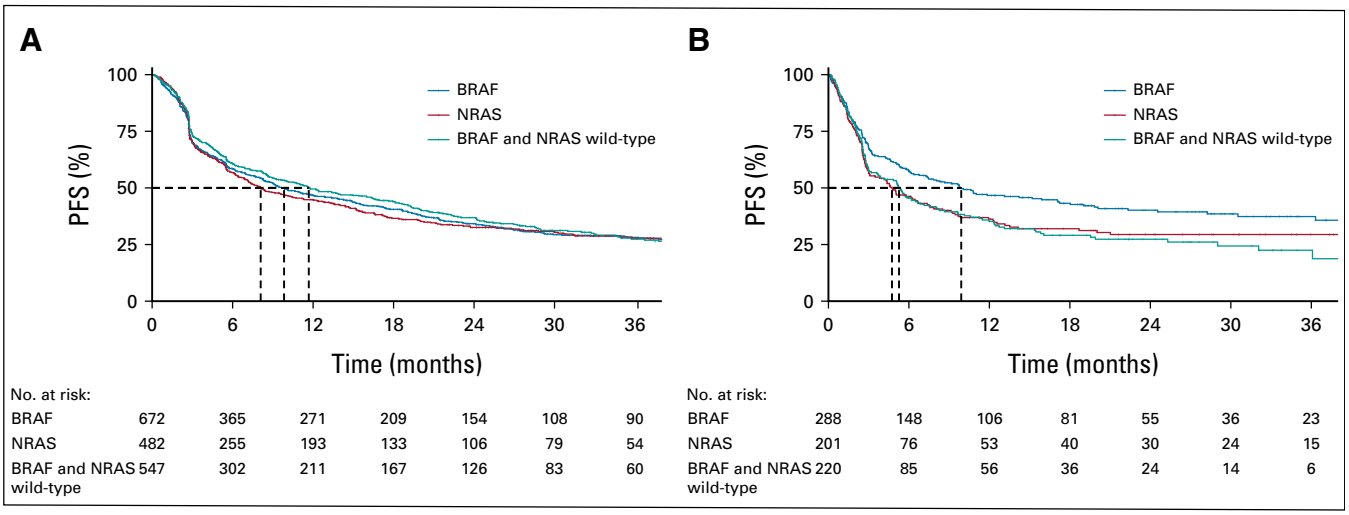

FIG 1. Kaplan-Meier estimate of PFS of the (A) anti–PD-1 and (B) ipilimumab-nivolumab-treated cohort: BRAF, NRAS, and double wild-type. PD-1, programmed cell death-1; PFS, progression-free survival.

have more favorable disease characteristics than the ipilimumab-nivolumab cohort.

ORR

First-line objective response rates are shown in the Data Supplement. Patients with *BRAF*-mutant melanoma and *NRAS*-mutant melanoma treated with first-line anti–PD-1 therapy reached an ORR of 55% and 53%, respectively. For double wild-type melanoma, the ORR was 56%. In the ipilimumab-nivolumab–treated cohort, the ORR was 59% for *BRAF*-mutant melanoma, 48% for *NRAS*-mutant melanoma, and 45% for double wild-type melanoma.

PFS

No significant differences in median PFS were found in anti–PD-1-treated patients between patients with double wild-type melanoma (11.7 months; 95% CI, 9.1 to 16.5) and patients with *BRAF*-mutant melanoma (9.8 months; 95% CI, 8.3 to 12.9; $P = .91$), patients with double wild-type and *NRAS*-mutated melanoma (8.1 months; 95% CI, 6.8 to 11.3; $P = .35$), or patients with *BRAF*-mutant and *NRAS*-mutant melanoma ($P = .59$; Fig 1A). When treated with ipilimumab-nivolumab, median PFS was significantly higher for *BRAF*-mutant melanoma compared with *NRAS*-mutant melanoma ($P = .016$) and compared with double wild-type melanoma ($P = .0032$). The median PFS for *BRAF*-mutant melanoma was 9.9 months (95% CI, 6.8 to 17.2), that for *NRAS*-mutant melanoma was 4.8 months (95% CI, 3.0 to 7.5), and that for double wild-type melanoma was 5.3 months (95% CI, 3.6 to 7.1). No significant difference was found between *NRAS* and double wild-type melanoma ($P = .78$; Fig 1B). For anti–PD-1-treated patients, median PFS was not significantly different ($P = .62$) between *BRAF* V600–mutant (median PFS 9.6 months; 95% CI, 8.1 to 13.8) and *BRAF* V600 wild-type melanoma (median PFS 10.1 months; 95% CI, 8.4 to 12.3; Data Supplement)]. In the ipilimumab-nivolumab cohort, median PFS was significantly longer for *BRAF* V600–mutant melanoma (median PFS 10.1 months; 95% CI, 7.4 to 18.0) compared with *BRAF* V600 wild-type melanoma (median PFS 5.2 months; 95% CI, 4.4 to 6.3; $P = .0057$; Data Supplement). Univariable analysis in the anti–PD-1 cohort showed no significant association in PFS between *BRAF*-mutant melanoma and *NRAS*-mutant melanoma (HR, 1.01; 95% CI, 0.88 to 1.17; $P = .889$) or *BRAF* and double wild-type melanoma (HR, 0.94; 95% CI, 0.82 to 1.08; $P = .386$). Univariable analysis in the ipilimumab-nivolumab cohort did show a significantly higher risk for progression or death for *NRAS*-mutant melanoma (HR, 1.33; 95% CI, 1.06 to 1.68; $P = .014$) and double wild-type melanoma (HR, 1.39; 95% CI, 1.11 to 1.73; $P = .004$) compared with *BRAF*-mutant melanoma. In the multivariable analysis, the presence of a *BRAF* mutation was not significantly associated with a difference in risk of progression or death in the anti–PD-1 cohort. Higher age, higher ECOG score, elevated LDH, liver metastases, symptomatic brain metastases, and ≥ 3 organ sites with metastasis were associated with lower PFS in this cohort (Fig 2A). In the multivariable analysis of the ipilimumab-nivolumab cohort, the presence of a *BRAF* mutation was significantly association with a lower risk of progression or death compared with both *NRAS* and double wild-type melanoma. Higher ECOG score, LDH $>$ 500 U/l, and symptomatic brain metastases were associated with a higher hazard of progression (Fig 2B).

OS

Patients with *BRAF*-mutant melanoma had significantly better OS than patients with *NRAS*-mutant and double wild-type melanoma. For anti–PD-1-treated patients, median OS was significantly better for patients with *BRAF*-mutated

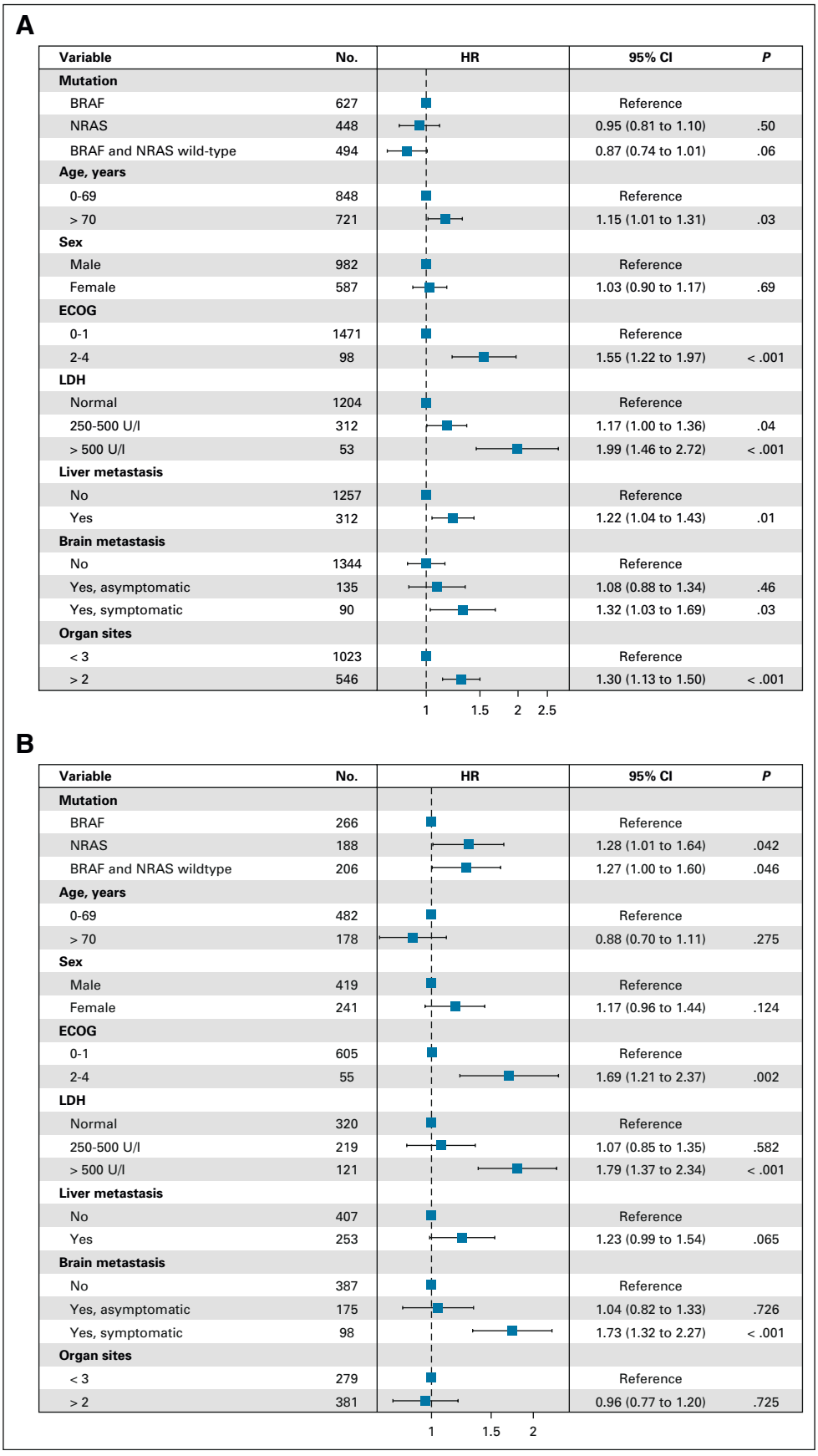

FIG 2. (A) Multivariable Cox proportional HR for PFS of the anti–PD-1-treated cohort: BRAF, NRAS, and double wild-type. (B) Multivariable Cox proportional HR for PFS of the ipilimumab-nivolumab-treated cohort: BRAF, NRAS, and double wild-type. ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; LDH, lactate dehydrogenase; PD-1, programmed cell death-1; PFS, progression-free survival.

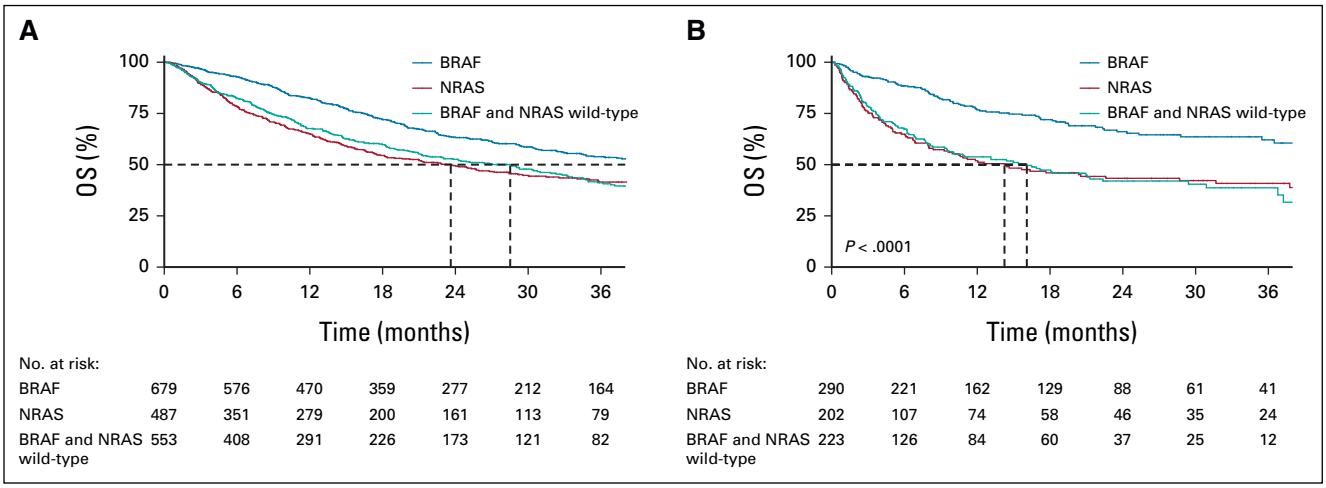

FIG 3. Kaplan-Meier estimate of OS: (A) anti–PD-1 and (B) ipilimumab-nivolumab-treated cohort: BRAF, NRAS, and double wild-type. OS, overall survival; PD-1, programmed cell death-1.

melanoma (42.5 months; 95% CI, 34.8 to 51.4) compared with *NRAS* (23.6 months; 95% CI, 17.8 to 30.0; $P < .0001$) and double wild-type (28.5 months; 95% CI, 21.3 to 33.2; $P < .0001$). No significant difference was found between patients with *NRAS* and double wild-type melanoma ($P = .66$; Fig 3A). Similar differences were seen in the ipilimumab-nivolumab–treated group. In the ipilimumab-nivolumab cohort, median OS was not reached (95% CI, 39.1 to NR) for *BRAF*-mutated melanomas versus 14.2 (95% CI, 9.3 to 31.7) months in *NRAS*-mutated melanomas ($P < .0001$) and 16.1 (95% CI, 9.6 to 29.4) months in double wild-type melanomas ($P < .0001$). Median OS between *NRAS* and double wild-type melanomas was not significantly different ($P = .83$; Fig 3B). Patients with *BRAF* V600–mutant melanoma had significantly better OS than patients with *BRAF* V600 wild-type melanoma in both treatment cohorts. In the anti–PD-1 cohort, the median OS was 42.5 months (95% CI, 36.3 to 51.4) for *BRAF* V600–mutant melanoma versus 26.3 months (95% CI, 22.5 to 30.5) for *BRAF* V600 wild-type melanoma ($P < .0001$). In ipilimumab-nivolumab–treated patients, median OS was not reached (95% CI, 36.5 to NR) in patients with *BRAF* V600–mutant melanoma and 16.9 months (95% CI, 12.0 to 29.4) in patients with *BRAF* V600 wild-type melanoma ($P < .0001$). Univariable analysis showed a higher risk of death for *NRAS*-mutant and double wild-type melanoma compared with *BRAF*-mutant melanoma in both treatment cohorts. In multivariable analysis, *BRAF*-mutant melanoma remained associated with a lower hazard of death in both the anti–PD-1-treated cohort and the ipilimumab-nivolumab-treated cohort (Figs 4A and 4B).

Subsequent Treatment Lines

In our cohort, 448 patients with *BRAF*-mutant melanoma who progressed in their first treatment line received a second treatment line. Of this group, 184 patients (45%) received another ICI and 404 (90%) received BRAF-MEK or BRAF inhibition in any subsequent treatment line. Of the 142 patients with *NRAS*-mutant melanoma who had progression and received a second treatment line, 120 (85%) received a different type of ICI in any following treatment line, with only one patient receiving BRAF-MEK inhibition. In the double wild-type group, 163 patients had progression and received a subsequent treatment line. Of this group, 131 patients (80%) received a different type of ICI and 17 patients (10%) received BRAF and/or MEK inhibitors in a subsequent treatment line.

DISCUSSION

To our knowledge, this is the largest study to report the influence of *BRAF* and *NRAS* mutation status on the response to first-line checkpoint inhibitors. We found a significantly better PFS in the ipilimumab-nivolumab cohort for patients with *BRAF*-mutant melanoma. For patients with advanced melanoma, anti–PD-1-based treatment undisputedly is the preferred first-line treatment. The choice between mono and dual therapies is less obvious, with only a few known factors that identify patients who particularly benefit from the more toxic ipilimumab and nivolumab regimen.

Our finding of improved PFS in *BRAF* V600–mutant patients is consistent with data from the CheckMate 067 trial in which patients with advanced melanoma were randomly assigned between nivolumab and ipilimumab combination therapy, nivolumab monotherapy, or ipilimumab.[12] In the CheckMate 067 trial, the median PFS in the ipilimumab-nivolumab combination group was higher (16.8 months) for *BRAF* V600–mutant than for *BRAF* V600 wild-type patients (11.2 months), whereas the median PFS for nivolumab-treated patients was similar or even lower (5.6 months) in the *BRAF*-mutant group compared with the *BRAF* wild-type group (8.2 months). Median OS was longer for ipilimumab-nivolumab–treated and nivolumab monotherapy–treated

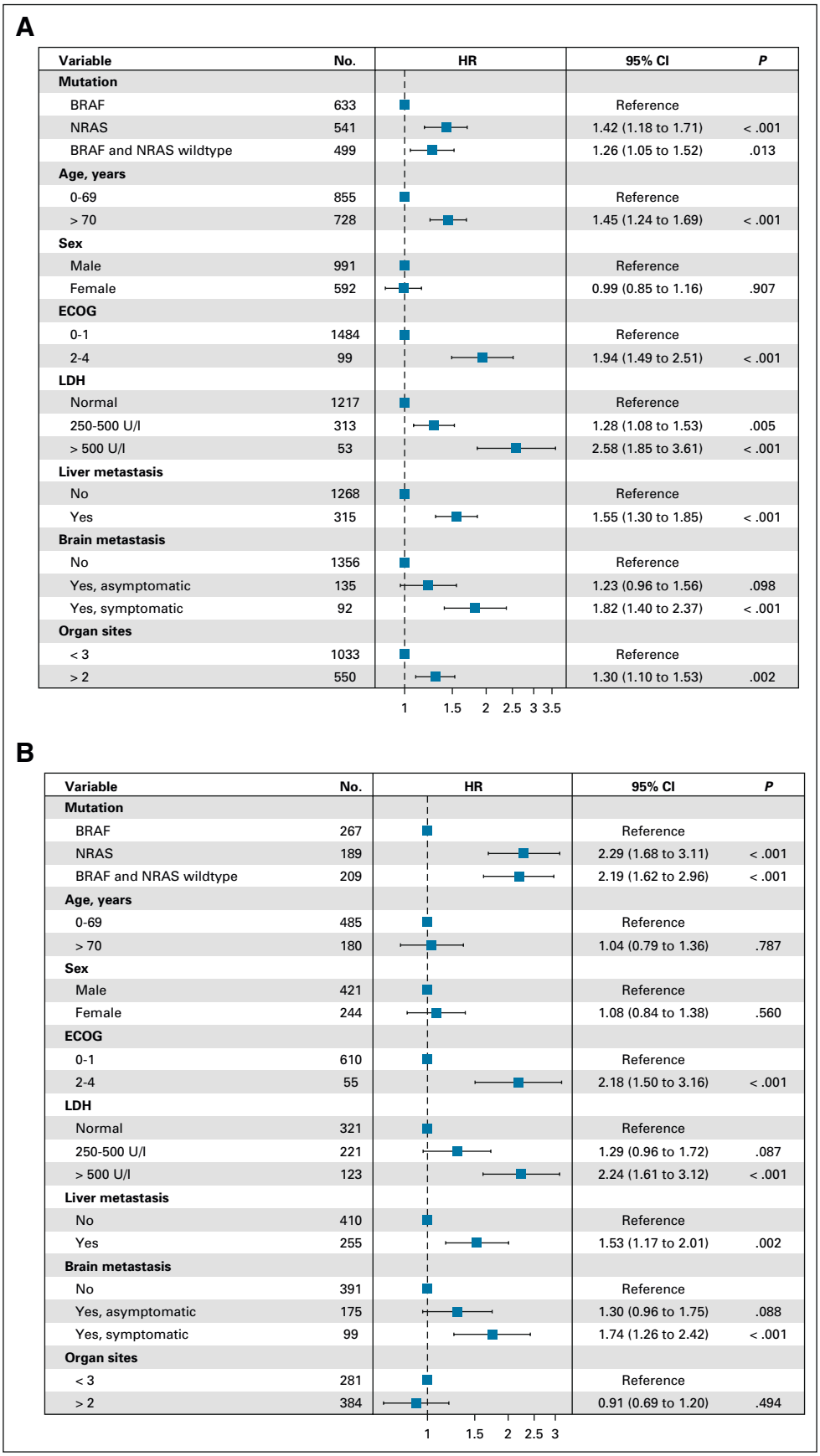

FIG 4. (A) Multivariable Cox proportional HR for OS of the anti–PD-1-treated cohort: BRAF, NRAS, and double wild-type. (B) Multivariable Cox proportional HR for OS of the ipilimumab-nivolumab-treated cohort: BRAF, NRAS, and double wild-type. ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PD-1, programmed cell death-1.

patients with a *BRAF* mutation. Except for the PFS in patients treated with anti–PD-1 monotherapy, we observed shorter PFS and OS than those reported by Larkin et al, presumably caused by patient selection in the CheckMate 067 trial, which excluded patients with active brain metastases and ECOG PS $>$ 1. In the IMMUNED study,[23] which randomly assigned between adjuvant nivolumab plus ipilimumab, nivolumab monotherapy, or placebo in patients with resected stage IV melanoma, the *BRAF*-mutant patients especially appeared to benefit from combination immunotherapy. Although we cannot rule out that the difference in PFS in our study could partially be explained by better baseline characteristics of the ipilimumab-nivolumab–treated *BRAF*-mutant patients, these more favorable characteristics for *BRAF*-mutant melanoma were seen in the anti–PD-1 monotherapy cohort and in the combinational treatment cohort. Moreover, similar findings in the randomized controlled trials suggest a mechanistic link. Of note, although *BRAF*-mutant patients in the CheckMate 238 study seemed to derive more benefit from adjuvant ipilimumab than *BRAF* wild-type patients, this difference was not seen for nivolumab-treated patients.[24] The presence of *BRAF* V600E mutations in melanoma has been associated with a higher recruitment of regulatory T cells during tumorigenesis.[25] Studies have described anti–cytotoxic T-cell lymphocyte-4 antibodies such as ipilimumab to induce depletion of regulatory T cells.[26,27] However, a study by Sharma et al[28] showed that this is not the case. It remains to be elucidated whether the improved outcomes for *BRAF*-mutant melanoma are a specific effect of ipilimumab or a broader immune-checkpoint blockade effect.

Several other smaller studies, including $<$ 200 patients per mutational subgroup, have retrospectively investigated the influence of genetic mutation status on the response to checkpoint inhibitors, producing conflicting results. Byeon et al[29] found *BRAF* mutations to be a poor prognostic factor for PFS and *NRAS* mutations to be associated with resistance to ICIs. In a study by Johnson et al,[30] *NRAS*-mutant patients had superior outcomes compared with the other cohorts in terms of response rate, PFS, and OS. In contrast to these results, Guida et al[31] reported no impact of *NRAS* mutations on the outcomes of ICI-treated patients and Rose et al[32] reported a nonsignificant trend toward shorter PFS in *BRAF* V600E/K–mutated and *NRAS*-mutated patients compared with wild-type patients.

Patients with *BRAF*-mutant melanoma displayed significantly higher OS in both the anti–PD-1 monotherapy and ipilimumab-nivolumab regimen. The longer OS that we found in *BRAF*-mutant melanoma is presumably the result of the subsequent BRAF/MEK inhibition treatment option in the *BRAF* group.[33] The limitation of using the Kaplan-Meier method for survival is the inability to correct for subsequent treatment options in the analysis. We previously showed that patients with acral melanomas, which most often are *BRAF* wild-type, have a worse PFS and OS when treated with checkpoint inhibitors than patients with cutaneous melanoma.[34] Differential activity between anti-PD1 and combination ICI for acral melanoma could not be demonstrated in that analysis, so it is unclear if the unbalance of this histologic subtype within the different mutation groups could influence our current findings. This study has some limitations. First, the observational nature of the DMTR might have introduced bias such as indication bias. Clinical characteristics were worse for the ipilimumab-nivolumab cohort. The treating physician might have been likely to provide more aggressive treatment to *NRAS* or double wild-type patients with poor prognostic features. Second, because of our study's retrospective nature, we cannot rule out residual confounding as a potential explanation for the observed associations. Unfortunately, we lacked data on TMB and information on the presence of other mutations than *BRAF* or *NRAS*. This would have allowed us to make a more accurate statement about the relationship between *BRAF* and *NRAS* mutations, the presence of other mutations, and tumor mutational burden in relation to response to ICIs. Population-based studies are generally more prone to missing data than clinical trials.

A strength of our study is the large number of patients who were included. The data in the DMTR are registered by independent data managers, who are trained annually. To further ensure the quality of the data, patients' data are checked by their treating physicians. The online registry in which patients are registered also warns data managers when data are inconsistent or missing values. Earlier studies have demonstrated the high quality of this registry.[14]

In conclusion, to our knowledge, in the largest cohort study to date, we show that *BRAF* mutational status is associated with differential survival upon treatment with ICIs. The observed improved PFS for patients with *BRAF*-mutant melanoma compared with *NRAS*-mutant and double wild-type patients treated with ipilimumab and nivolumab is intriguing and confirms observations in clinical trial subgroups. Although BRAF/NRAS mutation status alone is not sufficient to choose the optimal type of ICI, our data suggest that *BRAF* mutation status is a factor to take into account when choosing first-line checkpoint inhibitor treatment.

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