original reports

Targeted Therapy in Advanced Melanoma With Rare *BRAF* Mutations

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abstract

PURPOSE BRAF/MEK inhibition is a standard of care for patients with *BRAF* V600E/K–mutated metastatic melanoma. For patients with less frequent *BRAF* mutations, however, efficacy data are limited.

METHODS In the current study, 103 patients with metastatic melanoma with rare, activating non-V600E/K *BRAF* mutations that were treated with either a BRAF inhibitor (BRAFi), MEK inhibitor (MEKi), or the combination were included. *BRAF* mutation, patient and disease characteristics, response, and survival data were analyzed.

RESULTS Fifty-eight patient tumors (56%) harbored a non-E/K V600 mutation, 38 (37%) a non-V600 mutation, and seven had both V600E and a rare *BRAF* mutation (7%). The most frequent mutations were V600R (43%; 44 of 103), L597P/Q/R/S (15%; 15 of 103), and K601E (11%; 11 of 103). Most patients had stage IV disease and 42% had elevated lactate dehydrogenase at BRAFi/MEKi initiation. Most patients received combined BRAFi/MEKi (58%) or BRAFi monotherapy (37%). Of the 58 patients with V600 mutations, overall response rate to BRAFi monotherapy and combination BRAFi/MEKi was 27% (six of 22) and 56% (20 of 36), respectively, whereas median progression-free survival (PFS) was 3.7 months and 8.0 months, respectively ($P = .002$). Of the 38 patients with non-V600 mutations, overall response rate was 0% (zero of 15) to BRAFi, 40% (two of five) to MEKi, and 28% (five of 18) to combination treatment, with a median PFS of 1.8 months versus 3.7 months versus 3.3 months, respectively. Multivariable analyses revealed superior survival (PFS and overall survival) with combination over monotherapy in rare V600 and non-V600 mutated melanoma.

CONCLUSION Patients with rare *BRAF* mutations can respond to targeted therapy, however, efficacy seems to be lower compared with V600E mutated melanoma. Combination BRAFi/MEKi seems to be the best regimen for both V600 and non-V600 mutations. Yet interpretation should be done with care because of the heterogeneity of patients with small sample sizes for some of the reported mutations.

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INTRODUCTION

The discovery of BRAF V600E as a therapeutic target has been a milestone in the modern treatment of advanced melanoma. Approximately 35% to 50% of melanomas harbor an activating *BRAF* mutation that promotes tumor proliferation through the mitogen-activated protein kinase (MAPK)/ERK signaling pathway.[1,2] Of these, 70% to 80% account for the V600E mutation and another 10% to 20% for the less active V600K.[3-8] Genetic analyses revealed rarer *BRAF* mutations in 3.4% to 14% of melanomas[2,5,8-10]

The drug registration studies Combi-D (dabrafenib/trametinib)[4] and Columbus (encorafenib/binimetinib)[3] excluded patients with melanoma with mutations other than V600E/K, and the CoBRIM study (vemurafenib/cobimetinib)[11] relied on the Cobas test, which mainly detects V600E mutations. Here, combined BRAF inhibitor (BRAFi)/MEK inhibitor (MEKi) therapy produced an improvement in overall response rate (ORR) from 50% to 70%, prolongation of progression-free survival (PFS) from a median of 7 months to 12 months, and overall survival (OS) from 17 months to approximately 2 years compared with BRAFi monotherapy.[3,12,13] Results from the METRIC (ClinicalTrials.gov identifier: NCT01245062) study with trametinib monotherapy in patients with *BRAF* V600E/K–mutated melanoma show that patients with activating *BRAF* mutations respond to downstream MEK inhibition.[14] Hence, the use of MEKi with or without BRAFi is rational for melanomas with likely activating *BRAF* mutations, including those with rare V600 or non-V600 mutations; however, clinical experience in such patients is based on anecdotal case reports.

This international retrospective study assessed the therapeutic response to treatment with BRAFi or MEKi monotherapy, or the combination, in patients with metastatic melanoma harboring either activating

ASSOCIATED CONTENT

Appendix

Data Supplement

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

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BRAF mutations other than class I and II V600E/K or kinase-impaired class III mutations and translocations leading to MAPK pathway activation via upstream mechanisms.[14] The aim of the current study was to share the therapeutic experience of BRAFi/MEKi in patients with these rare melanomas to give treating physicians a foundation on which to make informed discussions about possible targeted therapy options.

METHODS

Retrospective data from patients with advanced melanoma who were treated with BRAFi, MEKi, or the combination between November 2009 and April 2018 and who harbored *BRAF* mutations other than V600E/K or translocations that are known or likely to activate the MAPK pathway were collected for this study.[5,14-16] *BRAF* sequencing was performed locally as clinical routine in the respective cancer center and hence the methods used varied (Appendix, online only). Data were extracted from medical records from 20 participating institutions from four countries (Germany, Australia, the Netherlands, and the United States) and Novartis (three patients from Combi-MB study). The data cutoff date was September 27, 2018. The history of three patients—V600M, V600G, and D594G—had been published, in part, previously.[17-19] Retrospective analyses of patient data were approved by the ethics committee of the University Hospital Heidelberg (S-454/2015).

Statistical Analysis

Patients were grouped for V600 and non-V600 *BRAF* mutations and for the treatment regimen used. Univariable analyses to compare V600 with non-V600 groups consisted of Fisher's exact test for qualitative data and the Wilcoxon rank-sum test for quantitative data. Melanoma stages were determined according to the American Joint Committee on Cancer classification (eight edition).[20] PFS and OS were measured from BRAFi/MEKi initiation to the date of first progression or death, respectively, or censored at the date of last contact when patients were still on therapy. The Kaplan-Meier method was used for their estimation, and differences between groups were assessed using the log-rank test. Lactate dehydrogenase (LDH) at treatment start was dichotomized as normal or elevated according to the institutional upper limit of normal. This variable was missing for nine patients and multiple imputation was used for multivariable analyses. Multivariable logistic regression was used to model response to treatment, including the most relevant factors—*BRAF* genotype, therapy regimen, age, sex, and LDH. Multivariable Cox proportional hazards regression served to model PFS and OS. As the proportional hazards assumption was violated for the factor *BRAF* genotype, separate analyses were performed for V600 and non-V600 patients, including the following factors: therapy regimen, age, sex, and LDH. To investigate the effect of therapy in this observational study, additional Cox proportional hazards regression analysis with inverse probability of treatment weighting (IPTW), including the covariables age, sex, and LDH, was performed to account for the observational nature of the data (Appendix Tables A1 and A2, online only).[21] Two analyses were performed for non-V600 patients, one for each comparison of mono- and combination therapy. A P value of $\leq .05$ was considered statistically significant. All computations were performed using R (The R Foundation for Statistical Computing, version 3.4.3) with packages survival, survminer, prodlim, mice, and glm.

RESULTS

In total, 103 patients with advanced melanoma and rare activating *BRAF* mutations or translocations were identified. A cohort of seven patients with a V600E/K plus another rare *BRAF* mutation (Appendix Table A3, online only) were excluded from statistical analyses, as the cohort was small and suspicion is high that the V600E/K mutation had a greater influence on the outcome of these patients. Median age for the full cohort was 62 years and 74% were male. At the time of targeted therapy commencement, 96% of patients had stage IV melanoma (M1c, 45%; M1d, 29%) and 42% had elevated LDH. More than one half of patients (n = 60; 58%) were treated with combined BRAFi/MEKi, 38 (37%) with BRAFi monotherapy, and five (5%) with MEKi monotherapy. The latter group included one patient who was treated with trametinib plus the multikinase inhibitor sorafenib. A majority of patients (76%) were treatment naïve. Those with prior treatment had received immunotherapies with either ipilimumab and/or programmed death-1 antibodies (20 of 26; 77%) or chemotherapy with mostly dacarbazine (11 of 26; 42%). Several factors, such as the physician's assessment of possible therapeutic benefit and area-specific availability and drug approval status at the time of therapy, influenced whether BRAFi or MEKi monotherapy or the combination were administered. As time progressed, more patients received the BRAFi/MEKi combination.[3,12,13]

Assessed patients (n = 96) consisted of two groups: *BRAF* mutations in codon 600 (V600; n = 58; 56%; class I mutation) and *BRAF* mutations or translocations affecting other codons nearby (non-V600; n = 38; 37%; class II and III mutations; Table 1), with similar characteristics concerning age, sex, elevated LDH, and line of treatment (Table 1).

Melanoma Characteristics

The majority of included melanomas were of cutaneous origin (non-V600, 89%; V600, 81%), with nodular melanomas and superficially spreading melanomas the most frequent subtypes. Although not significant, the V600 group contained more melanomas with unknown primary

TABLE 1. Patient Characteristics for V600 and Non-V600 Mutations

BRAF Genotype	All (N = 96)	Non-V600 (n = 38; 40%)	V600 (n = 58; 60%)	*P*
Median age, years (range)	61.7 (19.4-90.0)	59.8 (26.1-90.0)	62.4 (19.4-81.6)	.92
Sex				.77
Female	25 (26)	11 (29)	14 (24)	
Male	71 (74)	27 (71)	44 (76)	
LDH				.95
Elevated	41 (43)	17 (45)	24 (41)	
Normal	47 (49)	18 (47)	29 (50)	
Missing	8 (8)	3 (8)	5 (9)	
BRAF first line				.46
No	23 (24)	11 (29)	12 (21)	
Yes	73 (76)	27 (71)	46 (79)	
Type of melanoma				.14
Cutaneous	81 (84)	34 (89)	47 (81)	
Mucosal	1 (1)	1 (3)	0	
Unknown primary	14 (15)	3 (8)	11 (19)	
Melanoma subtype (for cutaneous)				.31
NM	30 (37)	10 (29)	20 (43)	
SSM	24 (30)	13 (38)	11 (23)	
ALM	1 (1)	1 (3)	0	
LMM	1 (1)	1 (3)	0	
Other	4 (5)	1 (3)	3 (6)	
Missing	21 (26)	8 (24)	13 (28)	
Tumor thickness (for cutaneous), mm (range)	2.4 (0.23-19)	1.95 (0.23-9)	2.5 (0.25-19)	.17
Ulceration (for cutaneous)				.35
Yes	27 (33)	13 (44)	14 (38)	
No	33 (41)	15 (38)	18 (30)	
Missing	21 (26)	6 (18)	15 (32)	

NOTE. Values are presented as No. (%) unless otherwise indicated.

Abbreviations: ALM, acral lentiginous melanoma; LDH, lactate dehydrogenase; LMM, lentigo maligna melanoma; NM, nodular melanoma; SSM, superficially spreading melanoma.

compared with the non-V600 group (19% *v* 8%; *P* = .14). Median Breslow thickness was slightly higher in V600-mutated melanomas (2.5 mm *v* 1.95 mm; *P* = .17; Table 1).

BRAF Mutations

V600 mutations included V600R (44 of 58; 76%), V600D (five of 58; 9%), V600M (two of 58; 3%), V600_K601 (four of 58; 7%), and unique mutations (V600G, V600L, and V600_S602delinsDT). Non-V600 mutations included mainly class II kinase-activating mutations L597P/Q/R/S (15 of 38; 39%), K601E (11 of 38; 29%), G469R/S/A (five of 38; 13%), and unique mutations, including kinase-impaired class III mutations leading to MAPK pathway activation via upstream coalterations (A598V, 1596_1597insTAC, T599_V600insT, D594G, and G593D). Subgroup demographics for *BRAF* mutations with more than 10 patients are provided in Appendix Table A4, online only.

Therapeutic Response

Of 96 patients, 56% received BRAFi/MEKi combination treatment, 39% BRAFi monotherapy, and 5% MEKi monotherapy (Table 2). Most patients received the targeted treatment in the first-line setting, with the exception of patients with MEKi monotherapy (Appendix Table A5, online only).

ORR of rare *BRAF*-mutated melanomas to MAPK pathway inhibition was 34% (33 of 96) and was higher in V600 than non-V600 melanomas (45% *v* 18%; *P* = .009; Table 2). Response rates to BRAFi/MEKi combinations were 56% (20 of 36) in V600 and 28% (five of 18) in non-V600 melanomas (*P* = .082). Non-V600 mutations showed no response with BRAFi monotherapy, but only with (additional) MEKi (MEKi, 40% [two of five]; BRAFi/MEKi combination, 28% [five of eight]; *P* = .026). In V600, the response rate was also higher with combination treatment compared with BRAFi monotherapy (56% [20 of 36] *v* 27%

TABLE 2. Response Rates and Median PFS/OS on the Basis of the *BRAF* Genotype Per Group

BRAF Genotype	Treatment	No.	ORR, No. (%)	DCR, No. (%)	Median PFS, Months (95% CI)	Median OS, Months (95% CI)
Non-E/K V600	Total	58	26 (45)	42 (72)	6.0 (4.1 to 8.1)	12.3 (8.9 to 17.4)
	BRAFi	22	6 (27)	12 (55)	3.7 (1.9 to 7.3)	7.3 (5.2 to 12.5)
	MEKi	0	—	—	—	—
	BRAFi/MEKi	36	20 (56)	30 (83)	8.0 (5.1 to 15.0)	17.3 (12.3 to NA)
Non-V600	Total	38	7 (18)	18 (47)	2.6 (1.8 to 7.8)	7.8 (5.7 to 20.9)
	BRAFi	15	0	6 (40)	1.8 (1.4 to 29.2)	7.6 (5.7 to 29.2)
	MEKi	5	2 (40)	3 (60)	3.7 (1.8 to NA)	5.9 (5.2 to NA)
	BRAFi+MEKi	18	5 (28)	9 (50)	3.3 (2.2 to NA)	11.3 (3.8 to NA)
V600E/K + other	Total	7	3 (43)	4 (57)	2.0 (1.5 to NA)	NA (20.3 to NA)
	BRAFi	1	0	1	NA, PFS > 1.1	NA, PFS > 1.1
	MEKi	0	—	—	—	—
	BRAFi+MEKi	6	3 (50)	3 (50)	2.0 (1.5 to NA)	NA (20.3 to NA)

NOTE. Not available (NA) is given when the upper limit of the 95% CI for the median cannot be determined. The confidence interval is defined by drawing a horizontal line at 0.5 on the plot of the confidence bands of the survival curve.

Abbreviations: DCR, disease control rate; OS, overall survival; ORR, overall response rate; PFS, progression-free survival.

[six of 22]; $P = .056$), with superior disease control rate (DCR, 83% [30 of 36] *v* 55% [12 of 22]; $P = .032$). In non-V600 melanoma, DCR did not differ significantly between combined treatment and the respective monotherapies (Table 2; P = .74). Again, DCR was higher in patients with V600 mutation compared with non-V600 ($P = .018$).

In multivariable analysis, response rate was significantly dependent on the *BRAF* genotype and treatment regimen, with a better response in V600-mutated melanomas ($P = .006$) and with BRAFi/MEKi combination treatment ($P = .005$) compared with BRAFi monotherapy. Response rates for the different *BRAF* genotypes are provided in Appendix Table A3. Of note, for many unique *BRAF* genotypes no responses were observed.

Patient Survival

Although median PFS was generally lower in patients with non-V600 melanoma, both groups demonstrated no significant difference in overall PFS and OS, likely because of small patient numbers. Median PFS was 6.0 months for V600 and 2.6 months for non-V600 patients with melanoma ($P = .63$). Median OS was 12.3 months for V600 and 7.8 months for non-V600 patients with melanoma ($P = .74$; Table 2 and Appendix Fig A1, online only). However, as combination treatment in V600E/K-mutated melanoma leads to better survival compared with monotherapy, different treatment groups were evaluated separately. In the combination therapy group, median PFS/OS was numerically higher in V600-mutated melanomas (Table 2 and Figs 1A and 1C). There was no difference between the groups treated with BRAFi monotherapy (Figs 1B and 1D). As expected, BRAFi/MEKi combination was superior to BRAFi alone for rare V600 mutations with a median PFS of 8 months versus 3.7 months and OS of 17.3 months versus 7.3 months ($P \leq .05$; Figs 2A and 2C). In the non-V600 group, no difference was observed (Figs 2B and 2D). However, median PFS tended to be greater for BRAFi/MEKi combination and MEKi monotherapy compared with BRAFi monotherapy (Table 2).

Multivariate Cox proportional hazards regression analysis demonstrated that patients with V600 who received combination treatment had superior PFS compared with BRAFi monotherapy (hazard ratio [HR], 0.42; 95% CI, 0.22 to 0.80; $P = .008$; age: $P = .11$; sex: $P = .06$; LDH: $P = .79$; line of treatment: $P = .55$; Table A1). Similarly, for non-V600-patients combination treatment had superior PFS compared with BRAFi monotherapy (HR, 0.28; 95% CI, 0.09 to 0.82; $P = .02$) and MEKi monotherapy (HR, 0.26; 95% CI, 0.07 to 0.98; $P = .047$; age: $P = .17$; sex: $P = .88$; LDH: $P = .003$; line of treatment: $P = .83$; Table A2). IPTW analysis—used to account for the observational nature of the data—qualitatively resulted in similar estimates for therapy effect for both patient cohorts.

As for OS in patients with V600, those with combination treatment had a superior outcome (HR, 0.21; 95% CI, 0.10 to 0.45; $P < .001$) compared with BRAFi monotherapy (age: $P = .37$; sex: $P = .29$; LDH: $P = .04$; line of treatment: $P = .019$; Table A1). Non-V600 patients receiving combination treatment also had superior OS compared with BRAFi monotherapy (HR, 0.21; 95% CI, 0.06 to 0.72; $P = .014$) and MEKi monotherapy (HR, 0.25; 95% CI, 0.05 to 1.12; $P = .07$; age: $P = .018$; sex: $P = .73$; LDH: $P = .011$; line of treatment: $P = .36$; Table A2). IPTW analysis resulted in similar estimates for therapy effect for V600 patients; however, for non-V600 patients, combination therapy was superior to BRAFi monotherapy but not to MEKi therapy.

The most common non-V600E/K mutation was the V600R mutation (44 of 58; 76%; Data Supplement). Results from

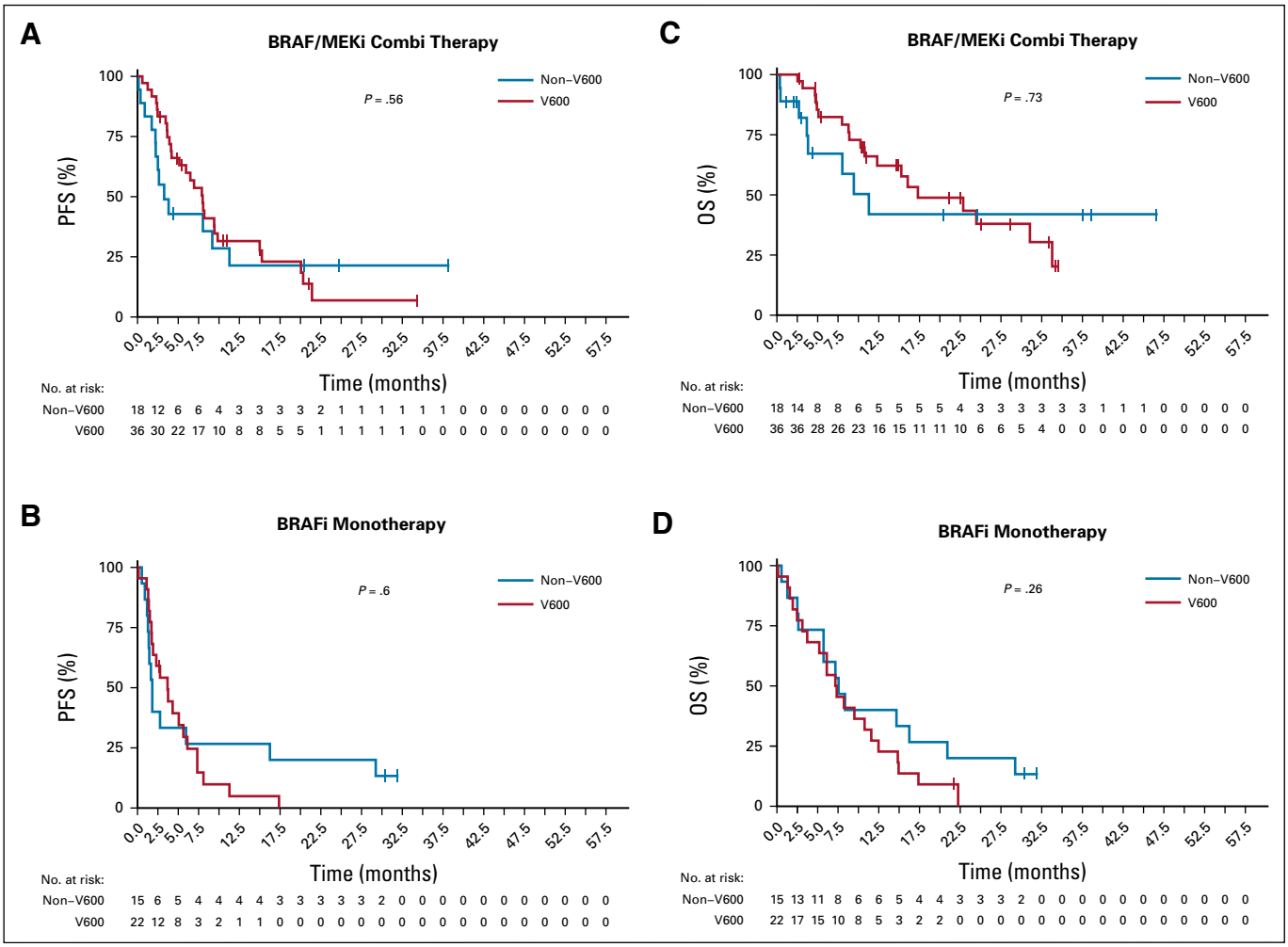

FIG 1. Kaplan-Meier estimates of (A and B) progression-free survival (PFS) and (C and D) overall survival (OS) for the different therapies BRAF inhibitor (BRAFi)/MEK inhibitor (MEKi) combination (A and C) and BRAFi monotherapy (B and D) in rare V600 (red line) and non-V600 mutations (blue line). In the combination therapy group, median PFS/OS was numerically higher in V600-mutated melanomas (A and C). There was no difference between the groups treated with BRAFi monotherapy (B and D).

this cohort resembled the overall V600 group with a significant advantage of the combination therapy for PFS (median, 8.0 months *v* 3.8 months; $P = .002$) and OS (median, 22.9 months *v* 7.3 months; $P = .002$; Appendix Fig A2, online only). For non-V600 patients with melanoma, the two most frequent mutations were L597P/Q/R/S (15 of 38; 40%) and K601E (11 of 38; 29%). In these, no significant differences were detected between the different treatment groups, comparable to the full non-V600 cohort (PFS: L597 [$P = .81$]; K601E [$P = .004$]; OS: L597 [$P = .57$]; K601E [$P = .36$]; Appendix Fig A3, online only). Of note, of three patients with G469R/S/A mutations who were treated with BRAFi/MEKi, two responded and all three showed controlled disease.

However, patients with the longest PFS (> 22 months) had V600R-, K601E-, or L597P/S -mutated melanoma (Fig 3). PFS and OS data for all and single rare BRAF mutations are provided in Appendix Table A3. In general, none of these single cases achieved durable PFS.

DISCUSSION

BRAFi/MEKi combinations have shown a high ORR and significantly prolonged PFS and OS for V600E and, to a lesser extent, V600K-mutated melanomas.[3,4,11,22,23] As patients with melanoma harboring less frequent *BRAF* mutations have been excluded from the controlled clinical registration trials, there are no evidence-based data available on response and survival benefit with BRAFi/MEKi for these patients.

The most frequent non-V600E/K *BRAF* mutations reported in this study were V600R (43%), L597P/Q/R/S (15%), and K601E (11%). All of these are activating mutations with high kinase activity responsible for strong MAPK pathway activation.[24]

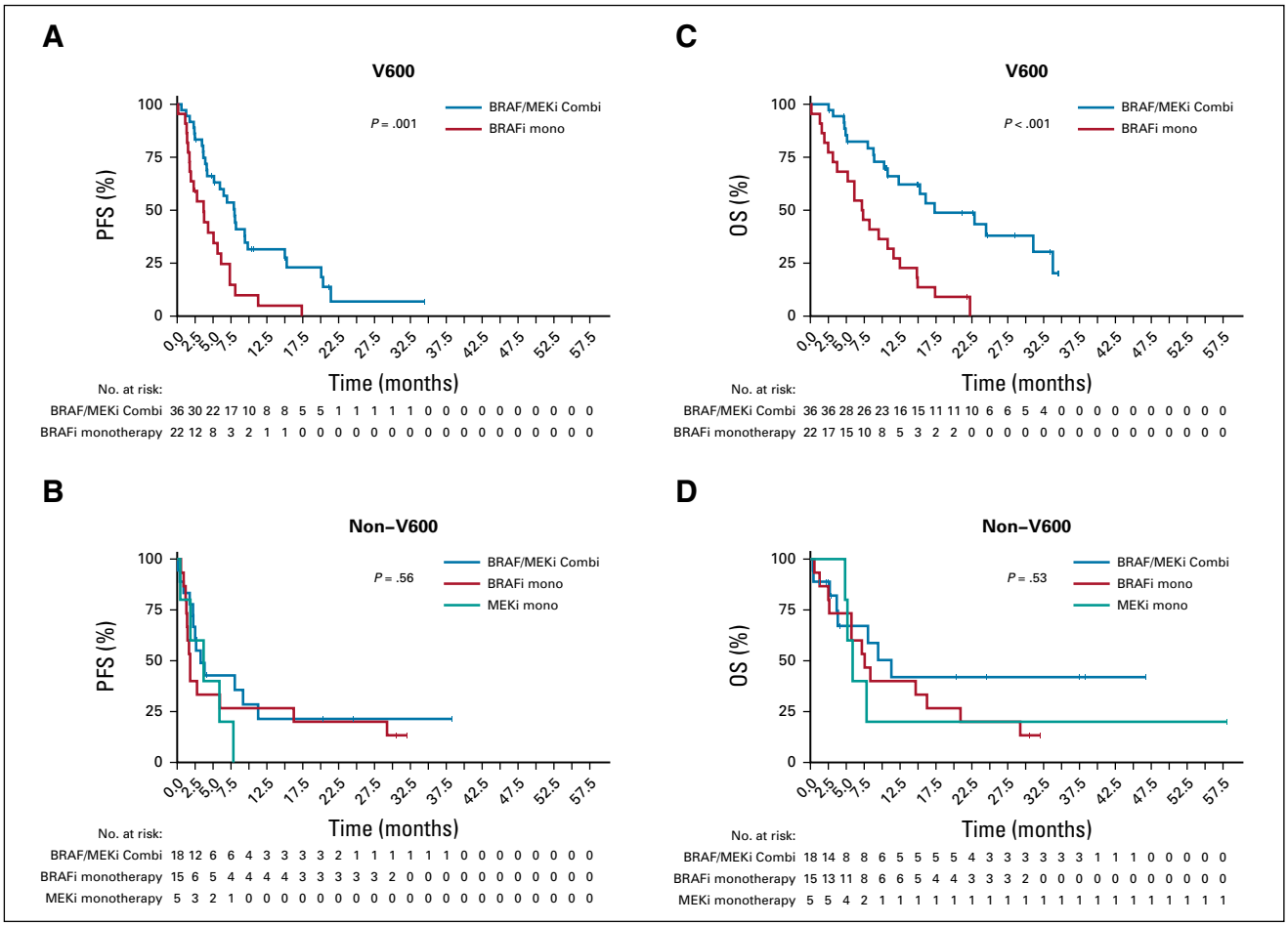

FIG 2. Kaplan-Meier estimates of (A and B) progression-free survival (PFS) and (C and D) overall survival (OS) for the different BRAF genotypes V600 (A and C) and non-V600 (B and D) in reference to their treatment (BRAF inhibitor [BRAFi]/MEK inhibitor [MEKi] combination [blue line], BRAFi monotherapy [red line], and MEKi monotherapy [green line]). In BRAFi/MEKi versus BRAFi alone, there was a significant difference in median PFS for rare V600 mutations (8 months *v* 3.7 months) and OS (17.3 months *v* 7.3 months; $P \leq .05$; A and C). In the non-V600 group, no difference was observed for BRAFi/MEKi combination or monotherapy (B and D). Median PFS was longer for BRAFi/MEKi combination and MEKi monotherapy compared with BRAFi monotherapy.

V600R mutations are reported in 3% to 7% of BRAF-mutated melanomas, making it the third most common BRAF mutation.[25] ORRs of up to 83% have been reported for treatment with BRAFi monotherapy,[25,26] and with the MEKi trametinib a partial response could be achieved.[27,28] In our cohort of 44 V600R patients with melanoma, the combination of BRAFi/MEKi showed an advantage for response compared with BRAFi monotherapy (55% *v* 27%; $P = 0.11$) and superior PFS (median, 8.0 months *v* 3.8 months; $P = .002$) and OS (median, 22.9 months *v* 7.3 months; $P = .002$). Considering this, ORR, as it is for the whole V600 group, is slightly lower than the ORR of 67% to 76% reported for combined BRAFi/MEKi in BRAF V600E/K-mutated melanomas,[3,12,13] although the 50% inhibitory concentrations in vitro are similar to vemurafenib (9 nM for V600R *v* 10 nM for V600E *v* 7 nM for V600K).[29] However, clinical outcome, especially PFS, seemed to be inferior (median, 8.0 months for V600R *v* 12.1 to 14.9 months for V600E/K in the respective registration trials; Appendix Fig A4, online only).[3-5] Of note, our cohort had a similar proportion of patients with elevated LDH (our cohort, 43%; CoBRIM, 46%; Combi-D, 34%) and 75% of patients were treated first line. In a pooled subgroup analysis of the Combi-D and -V trials, patients with a V600K mutation revealed significantly lower PFS and OS in multivariable analysis compared with V600E.[22] This might be a result of differences in gene expression between V600E and V600K melanomas, with V600E depending mostly on the MEK/ERK pathway, whereas in V600K alternative pathways, such as phosphoinositide (PI) 3-kinase (PIK3-AKT), gain a more important role.[23] In addition, V600E and V600D-mutated BRAF have been shown to be two- to four-fold more active than V600R or V600K.[2,6,30-33]

Non-V600 mutations have been reported to demonstrate responsiveness to BRAFi/MEKi as well. L597Q/R/S are neighboring oncogenic mutations on the *BRAF* gene with

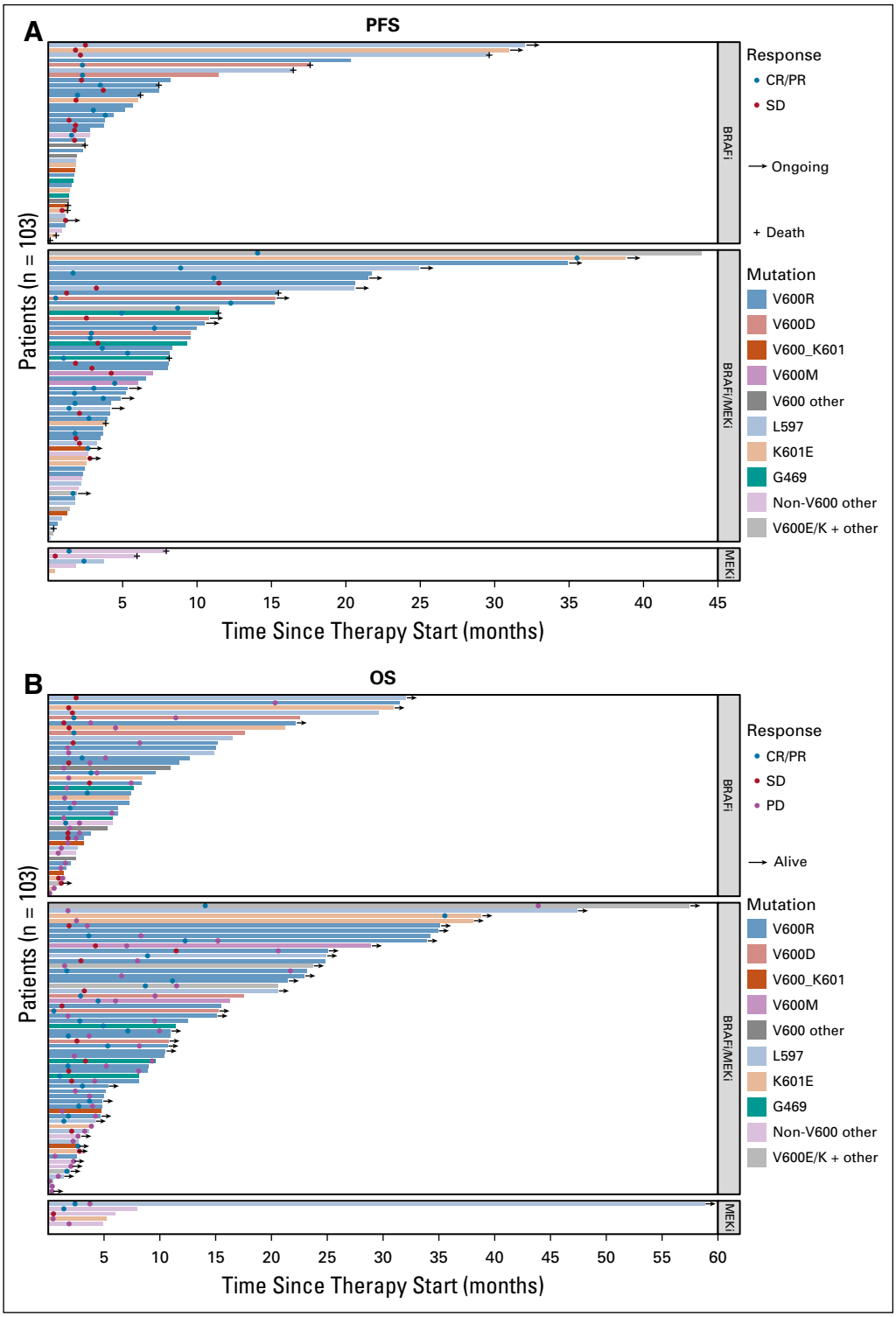

FIG 3. Swimmer plots demonstrating (A) progression-free survival (PFS) and (B) overall survival (OS) with corresponding therapy (BRAF inhibitor [BRAFi] monotherapy, BRAFi/MEK inhibitor [MEKi] combination, or MEKi monotherapy) for each patient with rare *BRAF* mutation included in this study. Patients with the longest PFS (> 22 months) had a V600R-, K601E-, or L597P/S-mutated melanoma (A). In general, none of the single cases achieved durable PFS. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

a 138-fold increased kinase activity (compared with a 500-fold activity increase in V600E).[34] L597P has not been functionally or clinically validated, but is considered likely oncogenic as a result of its vicinity to L597Q/R/S.[35-37] Ectopic expression of L597Q/R/S has shown elevated phospho-MEK and -ERK, and tumor regression was shown for L597S and L597Q in vitro and in vivo when treated with MEKi.[24,35] This finding was clinically supported in two case reports in patients with L597R- and L597S-mutated melanoma.[24] One report showed a response of L597R to BRAFi with vemurafenib.[38] On the contrary, Kim et al[39] could not confirm efficacy for either vemurafenib or trametinib in two patients with L597R-mutated melanoma. Finally, Dankner et al[40] generated patient-derived xenografts bearing an

L597S mutation that responded to BRAFi, MEKi, and BRAFi/MEKi combination with superiority for the combination. The same group reported a response to treatment with BRAFi/MEKi combination in two patients with L597S-mutated melanoma.[40] In our study, we included 15 patients with L597P/Q/R/S mutation with no response to BRAFi monotherapy, one (L597Q) to MEKi monotherapy, and only two of nine (both L597S) to combination treatment (Appendix Table A3). No significant advantage for PFS or OS was observed for the combination over BRAFi or MEKi monotherapy in Kaplan-Meier analysis (Appendix Fig A3, online only), although this comparison is limited by low numbers.

In several case reports, patients with *BRAF* K601E-mutated melanoma did not benefit from either BRAFi or BRAFi/MEKi combination therapy[39,41]; however, a response to the MEKi trametinib was reported by Kim et al[39] in one patient and by Bowyer et al[36] in three of four patients.[28,36] We included 11 patients with a K601E mutation. Here, response rates were low, with none of the patients treated with BRAFi (zero of six) or MEKi (zero of one) monotherapy experiencing a response and only one of four treated with combination therapy (Appendix Table A3). With a median PFS of only 3.3 months for combination treatment, clinical efficacy for non-V600-mutated melanoma has to be stated as low and hence targeted therapy should be considered with care.

Of interest, five patients with a G469 mutation were included in the study. Two of two did not experience a response to BRAFi monotherapy, but two of three treated with BRAFi/MEKi experienced a response. Of note, these are the first reports on responses to BRAFi/MEKi in patients harboring this mutation. In vitro, G469V/A-mutated cell lines from melanoma and non–small-cell lung cancer, respectively, were growth inhibited by MEKi with or without BRAFi, but not by BRAFi monotherapy.[33] As G469 mutations are the second most frequent *BRAF* mutation in non–small-cell lung cancer,[14] this might be of great interest for the treatment of such patients.

In intermediate activating mutants, or class II mutations, such as the V600 neighboring mutants K601E, L597, and G469A, suboptimal catalytic kinase efficiency has been demonstrated. Differences in activity to high-activity mutations of class I may rely on the bulkiness of the amino acids that occur as substitutes in the activation segments of the protein.[16] In these mutations, activation of wild-type C-RAF and the subsequent elevation of ERK activity seem to play an important role in pathogenesis. Reports that elevated C-RAF protein levels are associated with drug resistance to BRAFi in melanoma support this hypothesis.[42]

Limitations of this study are the retrospective collection of data and the heterogeneity of patients as from 20 different cancer centers worldwide over a time period of more than 8 years. In addition, some mutations are rare, leading to small sample sizes for some of the reported mutations. The locally performed *BRAF* testing resulted in a variety of genomic sequencing methods with a variance in sensitivity and specificity for *BRAF* mutation detection; however, all methods used show a sensitivity of more than 90% for the detection of *BRAF* mutants and can be considered reliable in the diagnosis of *BRAF* single mutants. For the double *BRAF* mutations (V600E/K plus another mutation), there is a risk of detection failure on the basis of misleading next-generation sequencing mutation calling software.

To our knowledge, this is to date the largest collection of data on the clinical experience with the treatment of BRAFi and/or MEKi therapy in patients carrying a *BRAF* mutation other than V600E/K. In summary, as the BRAFi/MEKi drug combination results in less toxicity than either BRAFi or MEKi alone, its use in patients with advanced melanoma with rare V600 *BRAF* mutations should be considered. High response rates were observed with rare *BRAF* V600-mutated melanomas but, as previously reported for V600K mutations,[6,23] median PFS was shorter compared with that reported for V600E.[11,43,44] Non-V600-mutated melanomas do not respond to BRAFi monotherapy and reveal a short median PFS to combination treatment. However, considering the still small sample size for mutations in other locations than V600, results from this study should be interpreted with caution.

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Targeted Therapy in Advanced Melanoma With Rare *BRAF* Mutations

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APPENDIX

BRAF Sequencing

As patients from cancer centers around the world were included, sequencing methods to detect *BRAF* mutations differed, comprising deep sequencing (next generation sequencing [NGS]; 21%, 22/103), high resolution melting (HRM; 9%, 9/103), pyrosequencing (3%; 3/103), real time polymerase chain reaction (PCR; 21%, 22/103), and Sanger sequencing (33%; 34/103; method not specified in 13 subjects). We did not reconfirm the *BRAF* mutation results from the centers centrally. The capability to activate the MAPK pathway of different *BRAF* mutants and translocations was determined by and their outcome compared to the current literature.[14,15,19]

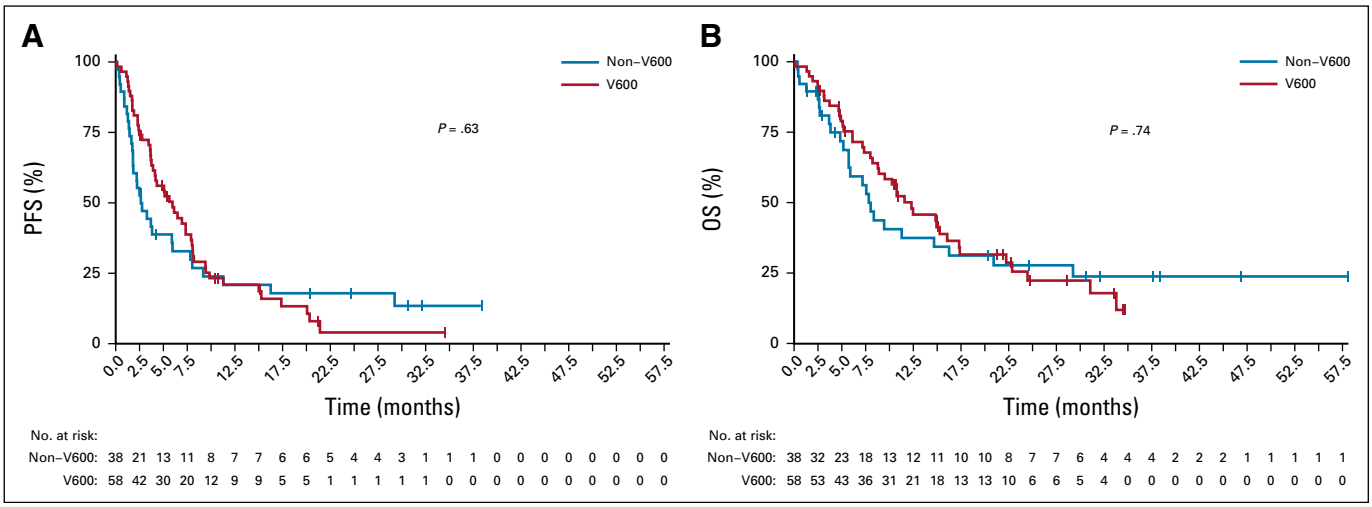

FIG A1. Kaplan Meier estimates for the whole patient group (V600 and non-V600) for (A) progression-free survival (PFS) and (B) overall survival (OS). Median PFS was generally lower in patients with non-V600 melanoma. Both groups showed no significant difference in overall PFS and OS likely due to small numbers of patients. Median PFS was 6.0 months for patients with V600 and 2.6 months for non-V600 mutated melanoma ($P = .63$); median OS was 12.3 months for V600 and 7.8 months for non-V600 melanomas ($P = .74$)

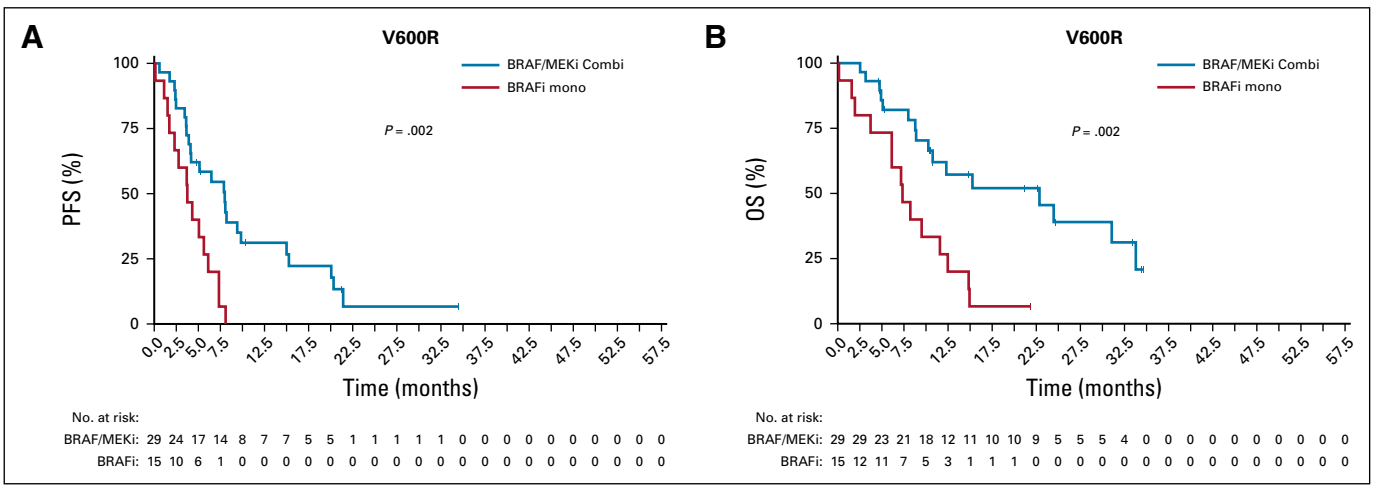

FIG A2. Kaplan Meier estimates for V600R mutated patients with BRAFi/MEKi combination and BRAFi monotherapy for (A) progressiom-free survival (PFS) and (B) overall survival (OS). Results from this cohort resembled the overall V600 group with a significant advantage of the combination therapy compared with monotherapy for PFS (median 8.0 months *v* 3.8 months; $P = .002$) and OS (median 22.9 months *v* 7.3 months, $P = .002$).

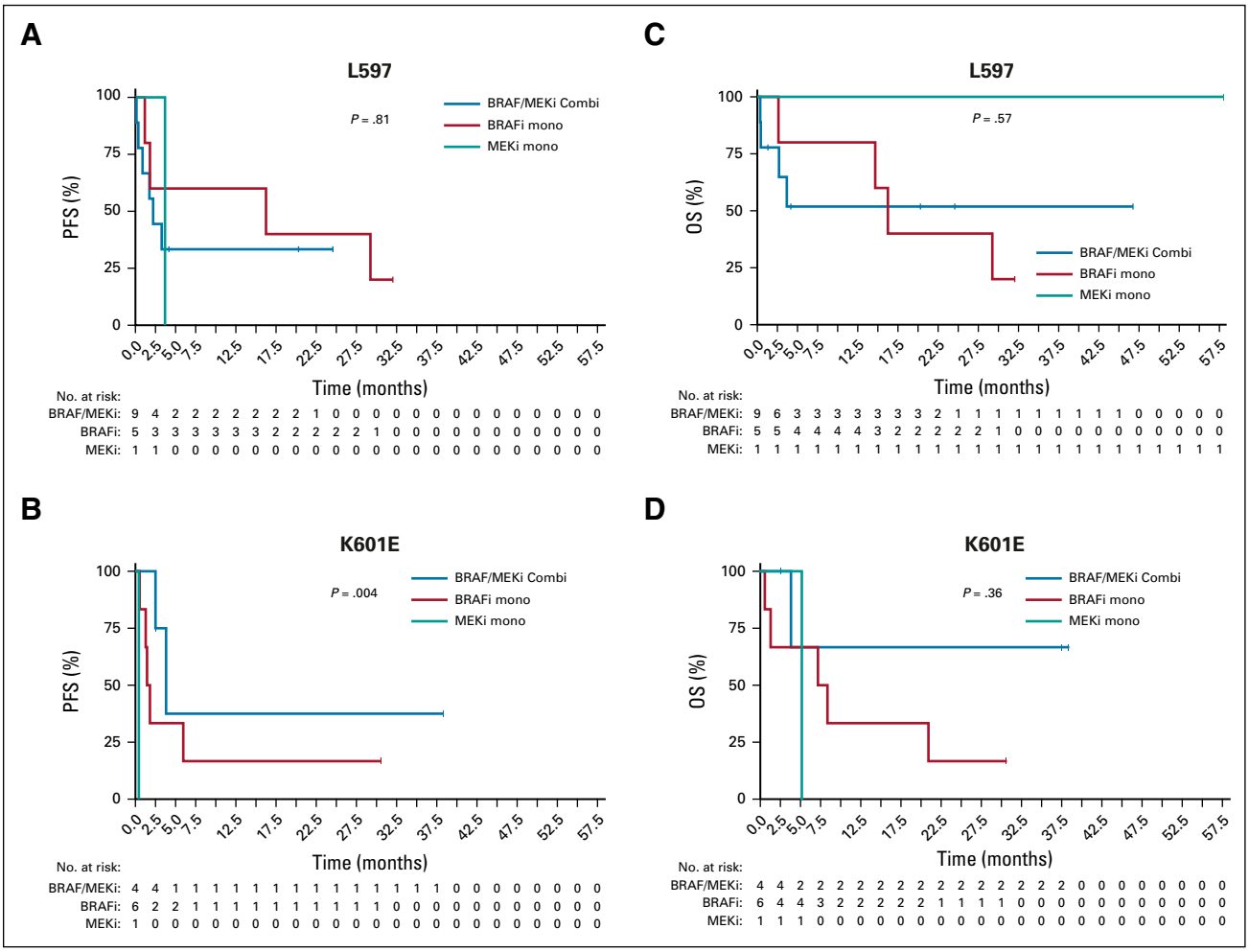

FIG A3. Kaplan Meier estimates for (A, C) L597 and (B, C) K601E mutated patients with BRAFi/MEKi combination and BRAFi or MEKi monotherapy for (A, B) progression-free survival (PFS) and (C, D) overall survival (OS). For non-V600 melanoma the two most frequent mutations were L597P/Q/R/S (15/38, 40%) and K601E (11/38, 29%). No significant differences were detected between the outcome for these unique non-V600 mutations compared with the full non-V600 cohort (PFS: L597 (P = .81); K601E (P = .004); OS: L597 (P = .57); K601E (P = .36).

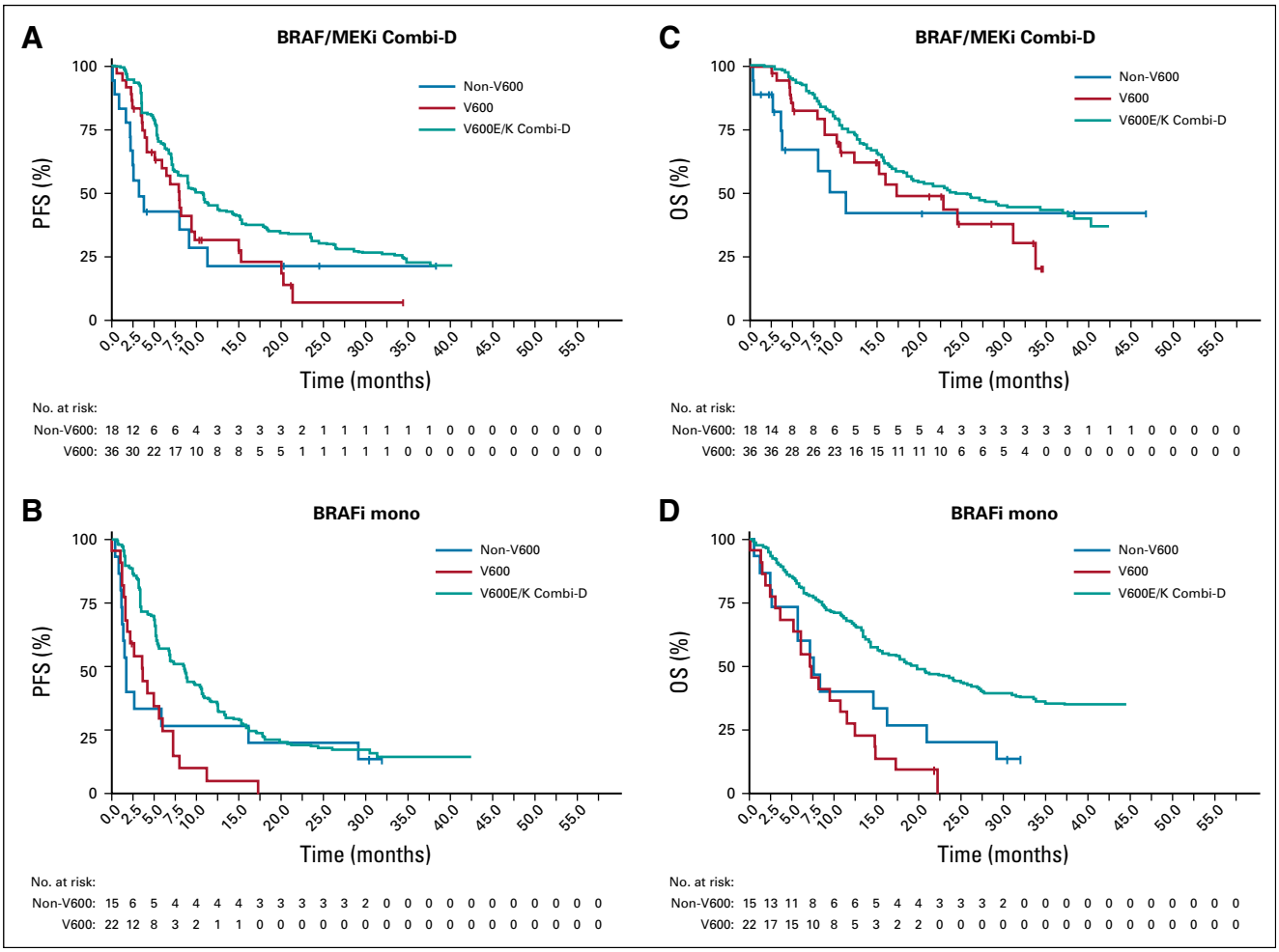

FIG A4. Overlay of Kaplan-Meier curves from this study (V600, red line; non-V600, blue line) and from Combi-D study (Novartis; V600E/K, green line) for orientating comparison of survival outcomes of rare *BRAF* mutations compared to V600E/K: (A,C) Kaplan Meier estimates for BRAFi/MEKi combination; (B,D) BRAFi monotherapy. Clinical outcome of patients with rare *BRAF* mutations appeared inferior to patients with BRAF V600E/K mutations. For overall survival (OS) it has to be noted that Combi-D allowed for cross-over to BRAFi/MEKi combination after progression on BRAFi monotherapy. PFS, progression-free survival.

TABLE A1. Results of Multivariable Cox Regression Analysis V600

	V600			
	Unweighted		IPTW Approach	
Covariable	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
PFS				
Therapy (reference BRAFi)	0.42 (0.22 to 0.80)	0.008	0.45 (0.26 to 0.80)	.006
Age	1.02 (1.00 to 1.05)	0.11	1.02 (0.99 to 1.05)	.13
Sex	0.49 (0.23 to 1.03)	0.060	0.59 (0.27 to 1.31)	.20
LDH	1.09 (0.59 to 2.01)	0.79	1.11 (0.62 to 1.98)	.73
BRAFfirstline	0.80 (0.38 to 1.68)	0.55	0.56 (0.24 to 1.31)	.18
OS				
Therapy	0.21 (0.10 to 0.45)	< 0.001	0.21 (0.10 to 0.43)	< .001
Age	1.01 (0.98 to 1.05)	0.37	1.01 (0.98 to 1.04)	.56
Sex	0.65 (0.29 to 1.46)	0.29	0.79 (0.33 to 1.86)	.58
LDH	2.10 (1.03 to 4.28)	0.040	2.35 (1.16 to 4.76)	.018
BRAFfirstline	0.40 (0.19 to 0.86)	0.019	0.30 (0.12 to 0.73)	.008

NOTE. Reference for sex is male, for lactate dehydrogenase (LDH) is normal and for BRAFfirstline is "No". Reported are the unweighted approach and the inverse probability of treatment weighting (IPTW) approach to account for the observational nature of the data. The IPTW approach uses propensity scores to obtain unbiased estimates of average treatment effects in observational studies. Propensity scores reflect the probability of treatment assignment conditional on baseline covariates, hence, conditional on the propensity score, the distribution of observed baseline covariates is balanced between the treatment arms. For the IPTW approach, the inverse of the propensity score is used as weight in the Cox regression model.

Abbreviations: OS, overall survival; PFS, progression-free survival.

TABLE A2. Results of Multivariable Cox Regression Analysis Non-V600

	Non-V600					
	Unweighted		IPTW approach			
Covariable	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
PFS						
Therapy (BRAF/MEKi vs. BRAFi)	0.28 (0.09 to 0.82)	.020	0.27 (0.10 to 0.68)	.006		
Therapy (BRAF/MEKi vs. MEKi)	0.26 (0.07 to 0.98)	.047			0.30 (0.12 to 0.77)	.013
Age	1.02 (0.99 to 1.05)	.17	1.02 (1.00 to 1.04)	.059	1.00 (0.96 to 1.05)	.84
Sex	1.07 (0.44 to 2.59)	.88	1.12 (0.52 to 2.45)	.77	1.95 (0.73 to 5.19)	.18
LDH	4.28 (1.65 to 11.07)	.003	5.77 (2.10 to 15.82)	< .001	2.35 (0.72 to 7.67)	.16
BRAFfirstline	0.91 (0.37 to 2.24)	.83	0.82 (0.40 to 1.65)	.57	1.15 (0.42 to 3.18)	.79
OS						
Therapy (BRAF/MEKi vs. BRAFi)	0.21 (0.06 to 0.72)	.014	0.28 (0.11 to 0.72)	.009		
Therapy (BRAF/MEKi vs. MEKi)	0.25 (0.05 to 1.12)	.070			0.72 (0.22 to 2.37)	.59
Age	1.04 (1.01 to 1.08)	.018	1.04 (1.01 to 1.06)	.018	1.06 (0.97 to 1.17)	.21
Sex	0.83 (0.28 to 2.42)	.73	1.03 (0.43 to 2.49)	.94	1.75 (0.44 to 6.89)	.43
LDH	3.62 (1.35 to 9.69)	.011	3.97 (1.69 to 9.28)	.001	1.13 (0.18 to 6.90)	.90
BRAFfirstline	0.65 (0.26 to 1.65)	.36	0.68 (0.26 to 1.77)	.43	0.28 (0.03 to 2.82)	.28

NOTE. Reference for sex is male, for lactate dehydrogenase (LDH) is normal, and for BRAFfirstline is "No". Reported are the unweighted approach and the inverse probability of treatment weighting (IPTW) approach to account for the observational nature of the data. The IPTW approach uses propensity scores to obtain unbiased estimates of average treatment effects in observational studies. Propensity scores reflect the probability of treatment assignment conditional on baseline covariates, hence, conditional on the propensity score, the distribution of observed baseline covariates is balanced between the treatment arms. For the IPTW approach, the inverse of the propensity score is used as weight in the Cox regression model. In the IPTW approach, two analyses were performed, one for each comparison of mono- and combination therapy, resulting in two sets of parameter estimates.

Abbreviations: OS, overall survival; PFS, progression-free survival.

TABLE A3. Response Rates and Median PFS/OS Based on the *BRAF* Genotype (full list)

BRAF Genotype	Treatment	No.	ORR, No. (%)	DCR, No. (%)	Median PFS, Months (95% CI)	Median OS, Months (95% CI)
V600	Total	58	26 (45)	42 (72)	6.0 (4.1 to 8.1)	12.3 (8.9 to 17.4)
	BRAFi	22	6 (27)	12 (55)	3.7 (1.9 to 7.3)	7.3 (5.2 to 12.5)
	MEKi	0	—	—	—	—
	BRAFi/MEKi	36	20 (56)	30 (83)	8.0 (5.1 to 15.0)	17.3 (12.3 to NA)
V600R	Total	44	20 (46)	33 (75)	5.1 (3.9 to 8.1)	11.6 (8.8 to 24.5)
	BRAFi	15	4 (27)	9 (60)	3.8 (2.3 to 7.3)	7.3 (6.1 to 14.8)
	MEKi	0	—	—	—	—
	BRAFi/MEKi	29	16 (55)	24 (83)	8.0 (4.2 to 15.3)	22.9 (10.8 to NA)
V600D	Total	5	4 (80)	5 (100)	17.4 (11.3 to NA)	17.4 (17.3 to NA)
	BRAFi	2	2 (100)	2 (100)	14.3 (11.3 to NA)	19.8 (17.4 to NA)
	MEKi	0	—	—	—	—
	BRAFi/MEKi	3	2 (67)	3 (100)	NA (9.4 to NA)	17.3 (NA to NA)
V600_K601 (_E/delinsE)	Total	4	1 (25)	1 (25)	1.5 (1.3 to NA)	3.1 (1.3 to NA)
	BRAFi	2	0	0	1.5 (1.3 to NA)	2.2 (1.3 to NA)
	MEKi	0	—	—	—	—
	BRAFi/MEKi	2	1 (50)	1 (50)	1.3 (1.3 to NA)	4.7 (NA to NA)
V600M	Total	2	1 (50)	2 (100)	6.5 (6.0 to NA)	16.1 (16.1 to NA)
	BRAFi	0	—	—	—	—
	MEKi	0	—	—	—	—
	BRAFi/MEKi	2	1 (50)	2 (100)	6. 5 (6.0 to NA)	16.1 (16.1 to NA)
V600G	BRAFi	1		PD	PFS = 1.9	OS = 5.2
V600L	BRAFi	1		PD	PFS = 2.5	OS = 2.5
V600_S602delinsDT	BRAFi	1		PD	PFS = 1.4	OS = 10.7
Non-V600	Total	38	7 (18)	18 (47)	2.6 (1.8 to 7.8)	7.8 (5.7 to 20.9)
	BRAFi	15	0	6 (40)	1.8 (1.4 to 29.2)	7.6 (5.7 to 29.2)
	MEKi	5	2 (40)	3 (60)	3.7 (1.8 to NA)	5.9 (5.2 to NA)
	BRAFi+MEKi	18	5 (28)	9 (50)	3.3 (2.2 to NA)	11.3 (3.8 to NA)
L597P/Q/R/S	Total	15	3 (20)	8 (53)	3.3 (1.7 to NA)	16.3 (3.7 to NA)
	BRAFi	5	0	3 (60)	16.3 (1.8 to NA)	16.2 (14.7 to NA)
	MEKi	1	1	1	3.7	OS > 57.8
	BRAFi/MEKi	9	2 (22)	4 (44)	2.2 (0.9 to NA)	NA (2.7 to NA)
K601E	Total	11	1 (9)	5 (45)	2.5 (1.5 to NA)	8.4 (3.8 to NA)
	BRAFi	6	0	3 (50)	1.6 (1.3 to NA)	7.8 (1.3 to NA)
	MEKi	1	0	0	PFS = 0.4	OS = 5.2
	BRAFi/MEKi	4	1 (25)	2 (50)	3.8 (2.5 to NA)	NA (3.8 to NA)
G469R/S/A/T170delinsAK	Total	5	2 (40)	3 (60)	8.0 (1.6 to NA)	8.0 (7.6 to NA)
	BRAFi	2	0	0	1.5 (1.4 to NA)	6.6 (5.7 to NA)
	MEKi	0	—	—	—	—
	BRAFi/MEKi	3	2 (67)	3 (100)	9.2 (8.0 to NA)	9.4 (8.0 to NA)
G593D	BRAFi	1		PD	PFS = 0.9	OS = 2.5
D594G	MEKi + Sorafenib	1		PD	PFS = 1.8	OS = 4.8
A598V	BRAFi/MEKi	1		PD	PFS = 2.2	OS > 2.2
1596_1597insTAC	BRAFi/MEKi	1		PD	PFS = 2.6	OS > 2.8

(continued on following page)

TABLE A3. Response Rates and Median PFS/OS Based on the *BRAF* Genotype (full list) (continued)

BRAF Genotype	Treatment	No.	ORR, No. (%)	DCR, No. (%)	Median PFS, Months (95% CI)	Median OS, Months (95% CI)
T599_V600insT	BRAFi	1		PD	PFS = 2.8	OS = 5.7
BRAF translocation. MET T9921	MEKi	1		PR	UNK	OS = 7.8
BRAF translocation. Fusion: KIAA1549-BRAF	MEKi	1		SD	UNK	OS = 5.9
V600E/K + other						
V600E + V600V	BRAFi/MEKi	1		CR	PFS = 43.2	OS > 56.5
V600E + V600R	BRAFi/MEKi	1		PD	PFS = 0.3	OS > 0.4
V600E + V600M	BRAFi/MEKi	1		PR	PFS = 11.3	OS = 20.3
V600K + V600R	BRAFi/MEKi	1		PR	PFS > 1.9	OS > 1.9
V600E + K601E	BRAFi/MEKi	1		PD	PFS = 1.4	OS > 29.3
V600E + W604C	BRAFi	1		SD	PFS > 1.1	OS > 1.1
V600E + F610L	BRAFi/MEKi	1		PD	PFS = 2.0	OS > 2.1

NOTE. NA is given when the upper limit of the 95% CI for the median cannot be determined. The CI is defined by drawing a horizontal line at 0.5 on the plot of the confidence bands of the survival curve.

Abbreviations: CR, complete response; DCR, disease control rate; NA, not determined; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression free survival; PR, partial response; SD, stable disease; UNK, unknown.

TABLE A4. Patient Characteristics for Selected *BRAF* Genotypes

***BRAF* Genotype**	**K601E (n = 11)**	**L597P/Q/R/S (n = 15)**	**V600R (n = 44)**	***P***
Median age, years (range)	54.8 (26.1-89.4)	59.3 (44.2-90.0)	63.8 (40.6-81.6)	0.03
Sex				.65
Female	3 (27)	4 (27)	8 (18)	
Male	8 (73)	11 (73)	36 (82)	
LDH				.56
Elevated	4 (36)	8 (53)	19 (43)	
Normal	6 (54)	7 (47)	20 (45)	
Missing	1 (9)	0	5 (11)	
BRAFfirstline				.46
No	1 (9)	2 (13)	10 (23)	
Yes	10 (91)	13 (87)	34 (77)	
Type of melanoma				.30
Cutaneous	10 (91)	15 (100)	37 (84)	
Mucosal	0	0	0	
Unknown primary	1 (9)	0	7 (16)	
Melanoma subtype (cutaneous)				.019
NM	2 (20)	7 (47)	16 (43)	
SSM	7 (70)	2 (13)	9 (24)	
ALM	0	0	0	
LMM	1 (10)	0	0	
Other	0	0	2 (5)	
Missing	0	6 (40)	10 (27)	
Tumor thickness (cutaneous), mm (range)	1.28 (0.23-7)	1.95 (0.8-9)	2.5 (0.4-19)	.09
Ulceration (cutaneous)				.94
Yes	4 (40)	6 (40)	15 (41)	
No	4 (40)	5 (33)	10 (27)	
Missing	2 (20)	4 (27)	12 (32)	

NOTE. Values are presented as No. (%) unless otherwise indicated.

Abbreviations: ALM, acral lentiginous melanoma; LDH, lactate dehydrogenase; LMM, lentigo maligna melanoma; NM, nodular melanoma; SSM, superficially spreading melanoma.

TABLE A5. Patient Characteristics in V600 and Non-V600 Patients by Therapy

Therapy	BRAF/MEKi (n = 54)	BRAFi mono (n = 37)	MEKi mono (n = 5)	*P*
Median age, years (range)	62.3 (19.4-90.0)	60.4 (26.1-81.6)	62.9 (43.7-65.9)	.74
Sex				.79
Female	16 (30)	8 (22)	1 (20)	
Male	38 (70)	29 (78)	4 (80)	
LDH				.54
Elevated	25 (46)	15 (41)	1 (20)	
Normal	23 (43)	20 (54)	4 (80)	
Missing	6 (11)	2 (5)	0	
Therapy firstline				.077
No	10 (19)	10 (27)	3 (60)	
Yes	44 (81)	27 (73)	2 (40)	
Type of melanoma				.045
Cutaneous	48 (89)	29 (78)	4 (80)	
Mucosal	0	0	1 (20)	
Unknown primary	6 (11)	8 (22)	0	
Melanoma subtype (cutaneous)				.075
NM	22 (46)	7 (24)	1 (25)	
SSM	11 (23)	11 (38)	2 (50)	
ALM	0	0	1 (25)	
LMM	0	1 (3)	0	
Other	3 (6)	1 (8§)	0	
Missing	12 (25)	9 (40)	0	
Tumor thickness (cutaneous), mm (range)	2.40 (0.25-10)	2.50 (0.23-19)	1.68 (1.2-9)	.85
Ulceration (cutaneous)				.31
Yes	22 (46)	8 (28)	3 (75)	
No	15 (31)	11 (38)	1 (25)	
Missing	11 (23)	10 (34)	0	

NOTE. Values are presented as No. (%) unless otherwise indicated.

Abbreviations: ALM, acral lentiginous melanoma; LDH, lactate dehydrogenase; LMM, lentigo maligna melanoma; NM, nodular melanoma; SSM, superficially spreading melanoma.