Original Research

MAPKinase inhibition after failure of immune checkpoint blockade in patients with advanced melanoma — An evaluation of the multicenter prospective skin cancer registry ADOREG

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Abstract *Objectives:* Forty to sixty percent of patients with advanced melanoma show primary resistance to PD-1-based immunotherapy, 30–40% of initial responders also progress. Here, we evaluated the outcome of second-line targeted therapy (TT) after progression on PD-1-based immune checkpoint inhibition (ICI) in BRAFV600-mutated melanoma. In addition, we report data on the activity of re-exposure with PD-1-based regimes.

Methods: Patients with advanced (non-resectable stage III or IV, AJCC 2017, 8th edition) melanoma progressing on PD-1-based ICI (nivolumab, pembrolizumab or ipilimumab plus nivolumab) and receiving second-line BRAF plus MEK inhibition were identified from the prospective multicenter skin cancer registry ADOREG.

Results: We identified 108 patients with unresectable stage III or stage IV melanoma progressing on first-line ICI (nivolumab, pembrolizumab or ipilimumab plus nivolumab) and receiving second-line combined BRAF/MEK inhibition. Seventy-three percent of the cohort presented with primary PD-1 resistant disease. Median progression-free survival (PFS) on ICI was 2.6 (95% CI 2.2–2.9) months. Median PFS on subsequent TT was 6.6 (95% CI 5.4–7.8) months. Median OS from start of second-line TT was 16.0 (95% CI 11.2–20.8) months. The 3-year PFS and OS rates on second-line TT were 16% and 30%. The objective response rate (ORR) and disease control rate (DCR) to TT were 42.6% and 55.6%. In patients with brain metastases, the ORR and DCR were 31.4% and 43.1%. Patients without brain metastases showed an ORR and DCR of 52.6% and 66.7%, respectively. Response to first-line ICI was associated with a numerically higher ORR and DCR to second-line TT and improved OS on TT. Twenty-three patients received third-line ICI of whom two patients showed an objective response.

Conclusions: BRAF plus MEK inhibition shows meaningful activity and outcome in patients with advanced melanoma resistant to anti-PD-1-based immunotherapy. Rates of long-term benefit and survival in our study were similar to those reported for treatment-naïve patients receiving first-line MAPKi.

1. Introduction

Immune checkpoint inhibition (ICI) has transformed the outcome of metastatic melanoma in a subset of patients. Though, 40–60% of patients do not respond to ICI (primary resistance) and 30–40% of initial responders progress later (acquired resistance) [1]. MAPK inhibitors remain an important treatment option for patients harbouring a sensitive mutation in the BRAF gene which is found in approximately 50% of patients [2]. First-line BRAF plus MEK inhibition results in rapid and high response rates [3], though the duration of response is limited in the majority of patients with 5-year progression-free survival (PFS) and overall survival (OS) rates of 19% and 34%, respectively [4]. When used in treatment-naïve patients, ipilimumab plus nivolumab is associated with a 5-year PFS and OS of 36% and 52%, and anti-PD-1 monotherapy (nivolumab) with a 5-year PFS and OS of 29% and 44% [1]. This significant long-term benefit for immunotherapy with durable responses and higher OS rates has led to the common administration of ICI as front-line therapy in BRAFV600-mutated melanoma [5]. However, data on the efficacy of second-line targeted therapy (TT) after the failure of ICI and its contribution to survival outcome are limited. The purpose of this study is to examine the activity and long-

term outcome of second-line BRAF plus MEK inhibition after progression on PD-1-based immunotherapy in unresectable stage III and stage IV melanoma.

2. Materials and methods

2.1. Patient cohort and data acquisition

The database of the prospective multicenter skin cancer registry ADOREG was queried for patients with unresectable stage III or stage IV melanoma progressing on first-line PD-1-based ICI and receiving second-line BRAF plus MEK inhibition. Exclusion criteria comprised prior adjuvant treatment with anti-PD-1 or BRAF plus MEK inhibitors, ocular or mucosal melanomas and BRAF mutations other than V600E or V600K. Clinicopathological and demographic data as well as therapy specific outcomes were extracted from the ADOREG registry. ADOREG is a multicentric registry of the German Dermatologic Oncology Group (DeCOG) prospectively collecting real-world data of patients with skin cancer treated at skin cancer centres. On 1st January, 2022, 57 centres were actively recruiting patients into ADOREG, and 8856 patients with melanoma had been enrolled [6]. Details are provided at https://www.hautkrebsregister.de/en.

All local institutions were queried for a follow-up of progression status, survival status and subsequent treatment lines between May and August 2021. Patients documented according to the AJCC 2009 staging system (7th edition) were re-classified into the AJCC classification (8th edition, 2017).

Patients were enrolled into ADOREG after written informed consent after approval by central (University Hospital Essen, 14-5921-BO) and local ethics committees. The study was conducted according to the Declaration of Helsinki.

2.2. Definition of end-points

End-points were the best overall response (BOR) according to RECIST 1.1 [7], objective response rate (ORR), disease control rate (DCR), intracranial and extracranial PFS and OS. OS was calculated from the initiation of TT until the date of death (OS TT) and from the initiation of ICI until the date of death (OS ICI). Further end-points were the BOR, ORR, DCR, PFS and OS on third-line ICI for patients being re-exposed to ICI. Patients not reaching a progression or survival event were censored at the last documented follow-up. ORR was calculated by dividing the sum of patients achieving a CR or PR (BOR according to RECIST 1.1) from all patients assessed. DCR was calculated by dividing the sum of patients achieving a CR or PR or SD (RECIST 1.1) from all patients. Treatment-related adverse events (AEs) were categorised according to the common toxicity criteria.

2.3. Statistical analysis

The statistical analysis was performed by applying Fisher's exact, Wilcoxon test and Mann-Whitney-U test as appropriate using SPSS (IBM, version 28.0). The survival analysis was conducted using the Kaplan−Meier method, the log-rank test was used for curve comparison. Median follow-up was calculated using the reverse Kaplan−Meier method. For multivariate analyses, Cox proportional hazards regression models were used. A two-sided p-value <0.05 was considered statistically significant. Plotting was performed by applying R (V4.1.1), python (V3.9.4) and matplotlib (V3.4.1).

3. Results

3.1. Study population

A total of 108 patients from 19 participating institutions with unresectable stage III and stage IV melanoma failing first-line ICI (nivolumab, pembrolizumab or ipilimumab plus nivolumab) and receiving second-line combined BRAF plus MEK inhibition were identified in the ADOREG registry (Table 1). All patients started first-line ICI between August 2015 and January 2020. Database was locked 31st August, 2021.

Fifty-nine (54.6%) patients were male, 86.1% (n = 93) of patients had a cutaneous primary melanoma, 9% of patients presented with a melanoma of unknown primary and in 4.6% of patients, the primary melanoma was not classified (n = 5). Ninety-seven patients harboured a BRAF V600E (89.9%) and 11 patients harboured a BRAF V600K mutation (10.2%). Nineteen patients (17.6%) had stage III disease when starting ICI, only 4 patients remained in stage III prior to second-line TT. The disease progression from first-to second-line therapy is reflected in a significant shift of the M-category. One-third of the cohort (34.3%) presented with brain metastases before ICI, 47.2% showed M1d disease prior to second-line treatment. Prior to ICI, only 13.9% had an ECOG performance status ≥ 1 compared to 28.7% prior to TT. An elevated LDH was observed in 44.4% pre-ICI versus 61.1% prior to TT (Supplementary Table 1).

Sixty-seven patients (62.0%) were treated with PD-1 inhibitors (24.1% nivolumab, 38.0% pembrolizumab) and 41 patients (38.0%) received ipilimumab plus nivolumab. Dabrafenib plus trametinib was administered in 84.3%, encorafenib plus binimetinib in 7.4% and vemurafenib plus cobimetinib in 8.3% (Table 1).

3.2. Treatment outcomes

More than two-thirds of the patients in our cohort (n = 79) showed primary resistance towards PD-1 inhibition. Of the 19 patients progressing after an initial

Table 1
Treatment characteristics.

	PD-1-based ICI	BRAF + MEK
	Median (95%-CI)	*Median (95%-CI)*
Duration (months) [range]	2.6 [0−44.1]	7.3 [0.4−55.7]
PFS (months)	2.6 (2.2−2.9)	6.6 (5.4−7.8)
Intracranial PFS (months)		9.2 (4.3−14.2)
OS (months) from the start of therapy	23.9 (19.7−28.1)	16.0 (11.2−20.8)
Time to TT (days) [range]	27 [0−648]	
	n (%)	*n (%)*
Drug		
Nivolumab	26 (24.1)	
Pembrolizumab	41 (38.0)	
Ipilimumab + Nivolumab	41 (38.0)	
Drug		
Dabrafenib + Trametinib		91 (84.3)
Encorafenib + Binimetinib		8 (7.4)
Vemurafenib + Cobimetinib		9 (8.3)
BOR (total cohort)		
CR	2 (1.9)	9 (8.3)
PR	17 (15.7)	37 (34.3)
SD	8 (7.4)	14 (13.0)
PD	79 (73.1)	47 (43.5)
Unknown	2 (1.9)	1 (0.9)
BOR (Pts with brain metastases)	n = 37 (100%)	n = 51 (100%)
CR	1 (2.7)	3 (5.9)
PR	7 (18.9)	13 (25.5)
SD	3 (8.1)	6 (11.8)
PD	26 (70.3)	29 (56.9)
unknown	0	0
Intracranial BOR		n = 51 (100%)
CR		4 (7.8)
PR		11 (21.6)
SD		10 (19.6)
PD		21 (41.2)
Unknown		2 (3.9)
Not applicable		3 (5.9)
BOR (Pts without brain metastases)	n = 71 (100%)	n = 57 (100%)
CR	1 (1.4)	6 (10.5)
PR	10 (14.1)	24 (42.1)
SD	5 (7.0)	8 (14.0)
PD	53 (74.6)	18 (31.6)
Unknown	2 (2.8)	1 (1.8)
Toxicity		
G1	16 (14.8)	13 (12.0)
G2	12 (11.1)	32 (29.6)
G3	17 (15.7)	28 (25.9)
G4	2 (1.9)	0

ICI, immune checkpoint inhibition; TT, targeted therapy; CI, confidence interval; PFS, progression-free survival; OS, overall survival; BOR, best overall response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; Pts, patients; G, grade.

response, two patients had achieved a complete response (CR), 17 patients had a partial response (PR) on first-line ICI. The median PFS on ICI was 2.6 (95% CI 2.2−2.9) months. In patients with acquired ICI resistance, the median PFS on ICI was 7.7 (95% CI 4.4−11.0) months (Table 2).

Second-line TT was administered after a median of 27 days after stopping ICI (range 0−648). The ORR for TT was 42.6% with CR observed in 8.3% and PR in 34.3% of patients. The DCR was 55.6%. The ORR and DCR in patients without brain metastases were 52.6% and 66.7%. An objective response and disease control in patients with brain metastases (n = 51) occurred in 31.4% and 43.1%, respectively. The median PFS and OS on TT was 6.6 (95% CI 5.4−7.8) and 16.0 (95% CI 11.2−20.8) months (Table 1, Fig. 1A+B). The PFS rates were 16% at 3 years and 13% at 5 years and OS rates were 30% at 3 years and 27% at 5 years (Fig. 1A+B). The median OS from the start of ICI was 23.9 (95% CI 19.7−28.1) months (Table 1, Fig. 1C).

As expected, patients responding to TT (CR or PR) had improved outcome with a median PFS of 11.8 (95% CI 6.0−17.6) and OS of 29.4 (95% CI 19.8−39.0) months (Supplementary Table 2). Patients with brain metastases prior to TT showed a median PFS on TT of 6.3 (95% CI 5.0−7.6) compared to 7.4 (95% CI 4.9−9.9) months in patients without brain metastases. The median OS TT in patients with brain metastases was 13.7 (95% CI 11.2−16.2) compared to 21.4 (95% CI 11.0−31.7) months in patients without intracranial disease. At a median follow-up of 34.1 (95% CI 28.7−39.5)

Table 2
Clinicopathological characteristics of patients with primary or acquired resistance to ICI.

	Primary resistance to PD-1-based ICI (PD)	Acquired resistance to PD-1-based ICI (CR + PR)	P
Individual patients	*n = 79 (%)*	*n = 19 (%)*	
BOR to BRAF + MEK TT			0.49[#]
CR	5 (6.3)	3 (15.8)	
PR	29 (36.7)	7 (36.8)	
SD	8 (10.1)	3 (15.8)	
PD	36 (45.6)	6 (31.6)	
unknown	1 (1.3)	0	
M-stage at start of BRAF + MEK TT			0.38[#]
M0	3 (3.8)	1 (5.3)	
M1a	4 (5.1)	1 (5.3)	
M1b	4 (5.1)	2 (10.5)	
M1c	32 (40.5)	4 (21.1)	
M1d	36 (45.6)	11 (57.9)	
	median (95%-CI)	*median (95%-CI)*	
Duration ICI (months) [range]	2.1 [0−13.3]	8.9 [0.7−44.1]	0.00[$]
PFS ICI (months)	2.3 (1.9−2.6)	7.7 (4.4−11.0)	0.00[*]
OS ICI (months)	20.6 (15.8−25.4)	42.1 (−)	0.02[*]
Duration TT (months) [range]	7.2 [0.4−55.7]	8.2 [0.5−49.0]	0.98[$]
PFS TT (months)	6.3 (5.0−7.6)	8.3 (0.0−19.4)	0.25[*]
OS TT (months)	15.4 (11.1−19.7)	29.4 (9.0−49.7)	0.18[*]

Fisher's exact test (#), Mann-Whitney-U test ($) or log-rank test (*) used for comparison.
ICI, immune checkpoint inhibition; TT, targeted therapy; CI, confidence interval; PFS, progression-free survival; OS, overall survival; BOR, best overall response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; G, grade.
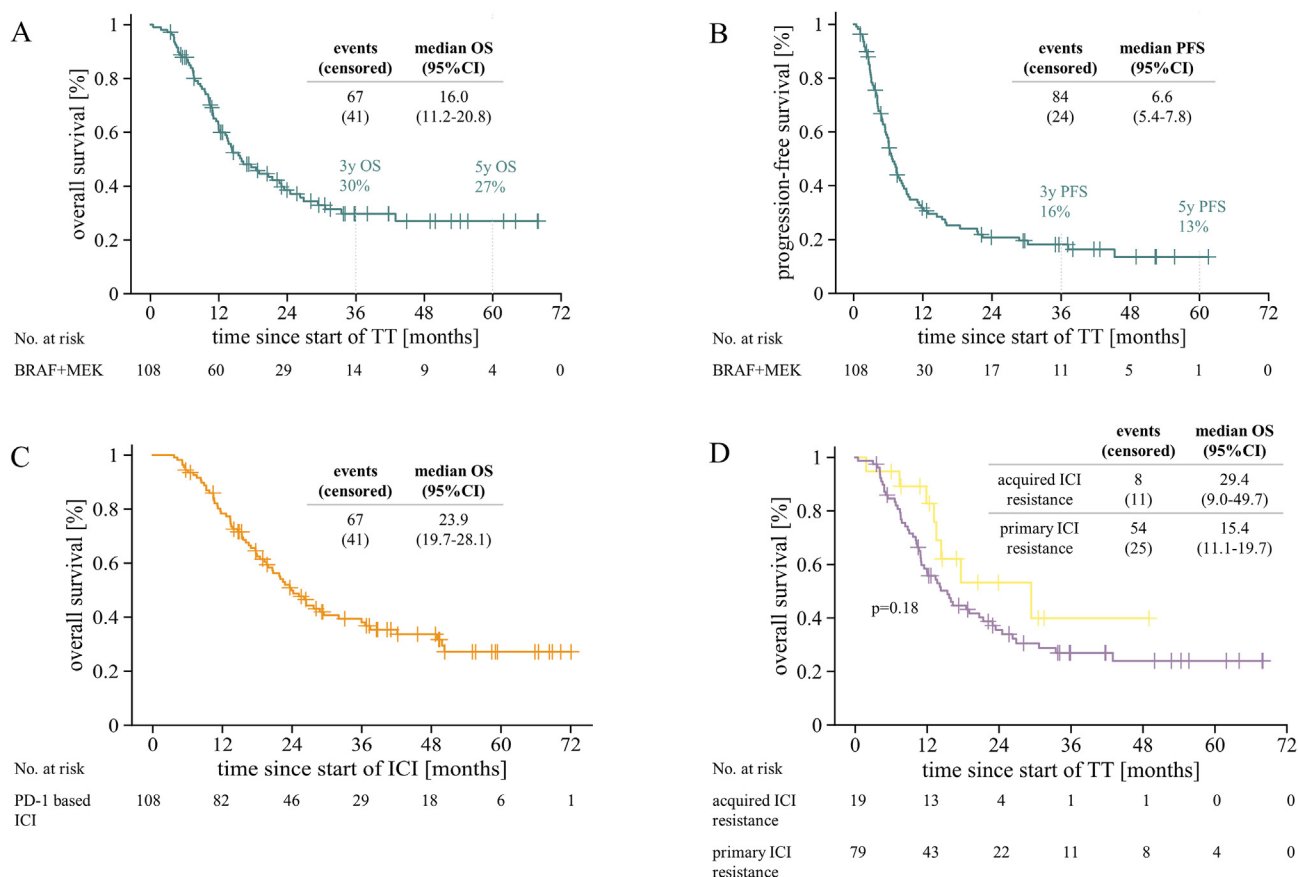
Fig. 1. **(A) (B)** Kaplan−Meier estimates of overall and progression-free survival in 108 patients with advanced melanoma from the start of second-line targeted therapy after progression on ICI. **(C)** Kaplan−Meier estimates of overall survival in 108 patients with advanced melanoma progressing on ICI and receiving second-line targeted therapy from the initiation of first-line ICI. **(D)** Kaplan−Meier estimates of overall survival in 98 patients stratified based on the resistance mechanism to first-line ICI: Acquired resistance (complete response and partial response as BOR to ICI (CR + PR)) versus primary resistance (progressive disease as BOR to ICI (PD)). Patients with stable disease (SD) or unknown response data on ICI were excluded. BOR, best overall response; ICI, immune checkpoint inhibition.

months after start of second-line TT, 26 patients (24.1%) were still on treatment. Any AEs on TT were reported in 68.5% of patients. Grade 3 events occurred in 25.9% of patients. Most commonly, pyrexia in 21.3%, myalgia in 13.9% and rash in 13.9% (Supplementary Table 3).

3.3. Biomarkers associated with the activity of TT stratified by prognostic characteristics

Patients showing an objective response to TT had a lower M-category before TT (p = 0.06) (Supplementary Table 2). The LDH prior to TT was not associated with response rates to TT (p = 0.83) but significantly correlated with progression-free (p = 0.01) and OS (p = 0.00). In our cohort, patients with normal LDH prior to TT (n = 40) had a median PFS and OS on TT of 11.1 (95% CI 4.3−17.8) and 30.7 (95% CI 18.5−42.9) months compared to 5.8 (95% CI 4.8−6.9) and 12.0 (95% CI 9.0−15.0) months in patients with elevated LDH (n = 66) (Supplementary Fig. 1A+B). The LDH prior to ICI was not associated with OS. In a multi-variate Cox regression model, the LDH (elevated versus normal) was significantly associated with OS TT (p = 0.00, HR 2.59 (95% CI 1.46−4.60)) and PFS TT (p = 0.00, HR 2.55 (95% CI 1.53−4.26). The response to TT (CR + PR versus PD) correlated significantly with OS TT (p = 0.02, HR 0.53 (95% CI 0.31−0.90)) and PFS TT (p = 0.00, HR 0.31 (95% CI 0.19−0.52)). M-category (M1a-c versus M1d), however, showed no significant association with OS TT (p = 0.11) or PFS TT (p = 0.31).

3.4. Response rate and OS stratified by primary versus acquired resistance to ICI

Acquired ICI resistance correlated by trend with a longer PFS and OS on TT than primary ICI resistance: 8.3 (95% CI 0−19.4) versus 6.3 (95% CI 5.0−7.6) (p = 0.25) and 29.4 (95% CI 9.0−49.7) versus 15.4 (95% CI 11.1−19.7) months (p = 0.18) (Fig. 1D).

Further, acquired ICI resistant tumours showed numerically a higher ORR and DCR to TT than primary ICI resistant tumours (52.6% and 68.4% vs. 43.0% and 53.2%), (p = 0.49) (Table 2).
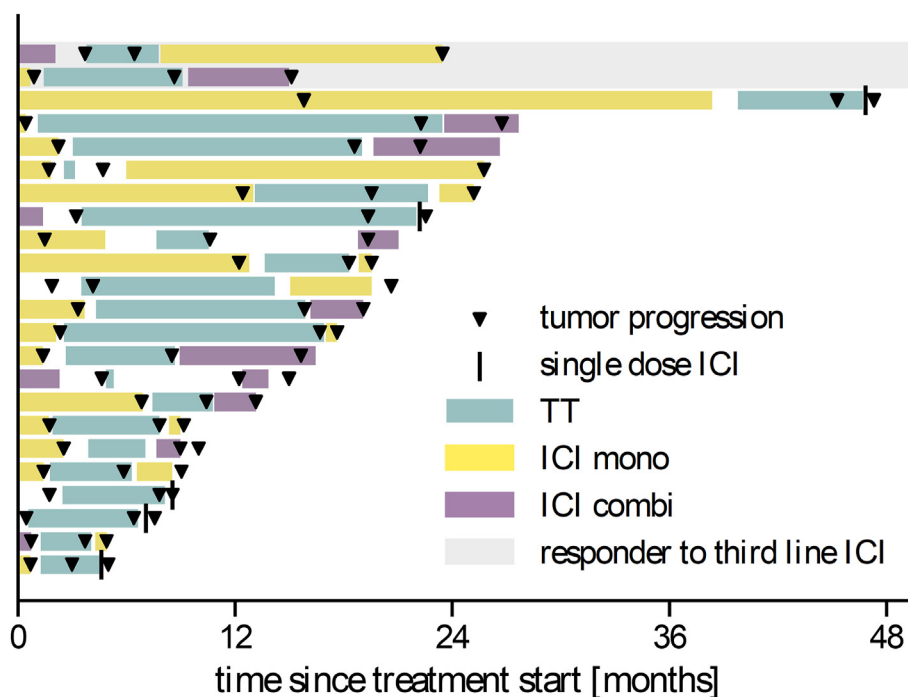
Fig. 2. Swimmer plot showing individual progression-free survival of 23 patients who received third-line ICI who progressed on first-line ICI and second-line TT. ICI, immune checkpoint inhibition; TT, targeted therapy.

3.5. Subsequent therapies after second-line TT

A total of 48 patients (44.4%) received subsequent therapies after second-line TT of whom 28 patients received one line, 8 patients two lines and 12 patients ≥ three additional lines of treatment. As shown in Fig. 2, third-line immune checkpoint blockade was given to 23 patients. A response on third-line ICI was observed in two patients and disease stabilisation in another patient. One patient showing a PR on third-line ipilimumab plus nivolumab had primary resistance towards first-line nivolumab and a PR on second-line TT. Another patient having a CR on third-line nivolumab achieved SD on first-line ipilimumab plus nivolumab and PD on second-line TT. One patient achieving disease stabilisation on third-line nivolumab showed PD after one dose of ipilimumab plus nivolumab and PD on second-line TT (Supplementary Fig. 2). Five patients had rapid disease progression after one single dose of third-line ICI (Fig. 2). The median PFS and OS after the initiation of third-line ICI were 2.4 (95% CI 0−4.9) and 11.1 (95% CI 2.1−20.1) months (Supplementary Table 4).

4. Discussion

In this study, we show that TT has meaningful clinical activity in anti-PD-1 resistant, BRAFV600 E/K-mutated, advanced melanoma. So far, data on the activity of combined TT after ICI failure have been reported in small and/or heterogeneous patient cohorts

and from subgroup analyses. In a retrospective analysis, Xia *et al.* reported a response rate of 66% (n = 35) on second-line BRAF ± MEK inhibition in prior anti-PD-1 progressors. The median PFS on TT was 4.1 months (95% CI 2.4−6.8) and median OS 13.6 months (95% CI 10.2-not reached) [8]. Patrinely *et al.* reported an ORR of 58.6% on TT after anti-PD-1 progression (n = 41), including an ORR of 70.4% for BRAF/MEK naïve patients [9] while Amaral *et al.* reported a response rate of 62.6% on second-line TT (n = 17) in melanoma primary resistant to PD-1-based ICI [10]. Saab *et al.* reported an OS of 15.6 months on BRAF plus MEK inhibition after anti-PD-1-based ICI [11]. Similar to our cohort, patients with elevated LDH levels were found to have a shorter median time on TT than those with normal LDH values (4.3 months versus 11 months; p = 0.04) [11]. All these investigations in smaller groups of patients did not further specify baseline characteristics of the cohort limiting the comparability of efficacy. The first presentation of the DREAMseq study (NCT02224781) recently reported an ORR of 48% (n = 11/23) on dabrafenib plus trametinib in patients failing first-line ipilimumab plus nivolumab [12]. Further, a post hoc analysis of the Keynote-006 trial (NCT01866319) of patients receiving BRAF ± MEK inhibition after pembrolizumab just recently reported a response rate of 43.2% in BRAFi ± MEKi-naïve patients (CR n = 3, PR n = 3) [13]. Our data resemble the observations from these two prospective clinical trials since ORR in our cohort was achieved by 42.6% and disease control by 55.6% of patients. The PFS on TT

was 6.6 (95% CI 5.4−7.8) and the OS TT was 16.0 (95% CI 11.2−20.8) months.

In our cohort, the 3-year PFS and OS rates were 16% and 30% and the 5-year PFS and OS rates were 13% and 27% at a median follow-up of 34.1 (95% CI 28.7−39.5) months. When comparing our results with the COMBI-d/v trials (NCT01584648, NCT01597908) reporting a 3-year PFS and OS of 22% and 44% and a 5-year PFS and OS of 19% and 34% on first-line treatment with dabrafenib plus trametinib [4,14], the long-term benefit of targeted agents in a subgroup of patients employed second-line seems unaffected by upfront immunotherapy.

The question how to sequence TT and ICI in treatment-naïve BRAF mutant melanoma is addressed in several ongoing clinical trials (NCT02631447, NCT02224781, NCT03235245, NCT02902029). The randomised phase II SECOMBIT trial evaluates the efficacy of ipilimumab plus nivolumab followed by encorafenib plus binimetinib and the reverse sequence. In a preliminary report after a median follow-up of 32 months, no statistically significant difference was observed between the groups [15]. The DREAMseq trial is now the first prospective head-to-head study comparing first-line MAPKi and ICI [12]. The 2-year OS for the group starting with ipilimumab plus nivolumab was significantly higher with 72% than 52% in the dabrafenib plus trametinib group [12]. Although the low ORR towards first-line MAPKi and the low rate of cross-over to the intended second-line therapy in DREAMseq raise some questions, upfront ICI treatment seems the superior sequence in patients with advanced BRAF mutant melanoma.

As expected, patients with favourable prognostic markers showed improved outcome. Our cohort included several patients with poor prognosis. Intracranial disease was present in 47.2% of patients when starting TT. The ORR in this group was only 31.4%. Normal levels of LDH prior to TT were associated with a significantly longer PFS TT and OS TT. In a pooled extended survival data from COMBI-d and COMBI-v trial, 25% of patients with a normal baseline LDH level remained progression-free after 5 years compared with 8% of patients with an elevated LDH level at baseline [4]. Interestingly, LDH levels prior to ICI had no impact on the OS ICI and OS TT. Thus, the usage of first-line ICI in patients with elevated LDH seems to be without disadvantage for the overall outcome.

Twenty-three patients in our cohort received third-line ICI of whom two patients experienced a response. Of note, one patient responded to third-line ipilimumab plus nivolumab after first-line nivolumab which is an accepted treatment escalation strategy post anti-PD-1 progression [16]. Our data indicate that ICI re-exposure after progression on TT has rather limited activity.

Clinical, translational and murine studies previously reported that resistance to MAPKi mediates cross-resistance to immunotherapy in melanoma [17−19]. A favourable tumour microenvironment enriched for T cell inflammation markers such as interferon-γ, however, facilities the activity of both MAPKi and ICI [20,21]. In our study, we observed that patients with acquired ICI resistance showed improved PFS and OS on TT. Although this finding must be validated, our data support the hypothesis that there might be a shared phenotype of responders.

Our study has some limitations. The real-world registry setting and the patient number does not allow to exclude a selection bias. This must be particularly taken into account when performing subgroup analyses stratifying patients into small groups and groups of different sizes. As 60% of patients show primary resistance towards PD-1 inhibition and about 30−40% develop resistance after an initial response, it is not surprising to have a smaller subset of acquired PD-1 resistance than primary resistance. The reported 5-year outcomes have to be recognised with caution as the median follow-up is 34.1 months and a large number of cases are censored. Additionally, it is not inconceivable that treatment-related AEs are underreported in this registry. Moreover, adjuvant therapies were not incorporated in this analysis. The impact of adjuvant treatments on subsequent therapies in patients relapsing on adjuvant therapies presenting with metastatic disease remains to be determined. The optimal therapeutic strategies in this group are under investigation and might differ from our results looking at therapy-naïve patients with metastatic melanoma.

5. Conclusion

Second-line TT has antitumour activity in patients with BRAFV600-mutant melanoma failing ICI. Similar to first-line use, a minority of patients achieves long-term disease control on second-line TT. While a response to first-line ICI might be associated with higher activity of second-line MAPKi, ICI re-exposure given as third-line therapy seems to have very limited activity. Rates of long-term benefit and survival in our study were similar to those reported for treatment-naïve patients receiving first-line MAPKi.

Credit author statement

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Conflict of interest statement

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Appendix A. Supplementary data

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