Clinical aspects of immunotherapy and targeted therapy of advanced melanoma

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Chapter 6

Vemurafenib for *BRAF* V600 mutated advanced melanoma: results of treatment beyond progression

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ABSTRACT

Background

Selective BRAF inhibition (BRAFi) by vemurafenib or dabrafenib has become approved standard treatment in *BRAF* V600 mutated advanced stage melanoma. While the response rate is high, the response duration is limited with a progression-free survival (PFS) of 5-6 months. Our observation of accelerated disease progression within some patients after stopping vemurafenib treatment has fostered the idea of treatment beyond progression (BRAFi TBP).

Method

In this retrospective study, we analyzed 70 metastatic melanoma patients, treated at our institute, who experienced progression after prior objective response upon treatment with vemurafenib. Thirty-five patients that continued treatment beyond progression are compared with 35 patients who stopped BRAFi treatment at disease progression.

Results

Median overall survival beyond documented progression was found to be 5.2 months versus 1.4 months (95% CI: 3.8-7.4 vs. 0.6-3.4; Log-Rank p = 0.002) in favour of BRAFi TBP. In the multivariate survival analysis, stopping treatment at disease progression was significantly associated with shorter survival (Hazard Ratio: 1.92; 95% CI: 1.04-3.55; p = 0.04).

Conclusion

Our results suggest that continuing vemurafenib treatment beyond progression may be beneficial in advanced melanoma patients, who prior to progression responded to vemurafenib.

INTRODUCTION

Melanoma has a rising incidence in Europe resulting in an estimated 100,300 new diagnoses and yearly 22,200 patients succumb to this disease in 2012 [1, 2]. The progress that has been made in the understanding of melanoma pathogenesis in the past decade has resulted in the development of novel targeted therapies such as vemurafenib and dabrafenib [3-5]. Both drugs inhibit the activity of mutated BRAF proteins, which are observed in 40-60% of cutaneous melanoma [6-9]. Although these selective BRAF inhibitors showed improvement in progression-free survival (PFS) and overall survival (OS), more than 50% of patients will have progressed after five to six months of treatment, highlighting the problem of acquired therapy resistance [10]. A novel combination therapy, consisting of the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib, has been investigated and preliminary results point towards improvement in PFS [11], suggesting that resistance can be postponed by combining two inhibitors of the MAPK pathway.

We clinically observed at our institute that stopping BRAF inhibition (BRAFi) due to disease progression resulted often in an accelerated growth of metastases, and consecutive rapid deterioration and death of the patients. This has raised the question whether continuation of vemurafenib despite disease progression or so-called treatment beyond progression (TBP), could improve overall survival of these patients. Recent data from dabrafenib indicated that this might be indeed the case [12]. The exact mechanism behind accelerated growth of metastases after discontinuing vemurafenib is thus far unknown. One possible explanation may lie in inter-tumoral and intra-tumoral heterogeneity with tumor growth of vemurafenib resistant tumor cells, while other portions of the tumor or other metastases may still be responsive. Stopping vemurafenib based on progressive disease as a result of growth of resistant metastases may lead to sometimes rapid growth of all lesions. A study by Carlino et al. reported a marked increase in the rate of disease progression after withdrawal of MAPK inhibitors (either dabrafenib or the combination of dabrafenib plus trametinib) in patients with *BRAF*-mutant metastatic melanoma treated beyond progression [13]. The same study also showed a slower rate of disease proliferation in resistant melanoma cell lines when continuously exposed to MAPK inhibition. Another, relatively small study with 48 patients with metastatic melanoma showed a potential benefit in treatment beyond progression in patients who showed progression of disease in limited sites only, which was accessible to local therapy [14]. The possible advantages of continuing treatment with vemurafenib have not yet been extensively investigated in melanoma patients, however, results obtained from several studies focusing on other malignancies and other treatments point towards an advantage of TBP [13-21].

Here, we present our retrospective single institution analysis of vemurafenib treatment beyond progression in advanced stage *BRAF* V600 mutated melanoma patients and show a potential beneficial effect of continuation of treatment despite disease progression.

MATERIALS AND METHODS

Patients included in the analysis

This study was undertaken at the Netherlands Cancer Institute – Antoni van Leeuwenhoek. The study included 152 patients with *BRAF*-mutant metastatic melanoma, who are/were treated at our institute with vemurafenib (within the Global Safety Study, 86 patients and on prescription after approval of vemurafenib, 66 patients) between June 2010 and February 2013 [22].

Methods

Vemurafenib was given orally at a standard dose of 960 mg twice daily, unless patients experienced toxicities for which dose modification was needed. In one patient with good tolerability, vemurafenib was escalated to a dose of 1200 mg twice daily upon progression of disease. Initially, patients treated in the Global Safety Study were not permitted to continue BRAFi treatment once progression of disease set in. As of the European approval of vemurafenib in 2012, clinicians treating patients in the Global Safety Study have been permitted to continue TBP upon request to and approval by the study monitor. The rationale behind choosing which patients received TBP and which patients would not receive TBP was determined based on multiple factors including: ECOG performance status, nature of disease progression and possibility of other therapies beyond progression. TBP was defined as receiving BRAFi despite progression of disease as measured by RECIST 1.1. During therapy, patients visited the outpatient clinic every four weeks for physical examination and blood sampling. Tumor responses were assessed every eight weeks by CT-scan and in case of brain metastases also by MRI. Nature of disease progression was noted as followed: intracranial versus extracranial, nonvisceral (subcutaneous, bone and lymph node) versus visceral (lung, liver and pancreas), new and/or existing metastases and whether progression of disease was more isolated or generalized. Isolated disease progression was defined as progression with a new or an existing lesion within one site or organ, while the rest of the disease showing at least stable disease. Patient characteristics were obtained from the electronic patient records within our institute.

Statistical analysis

The primary endpoint of our retrospective analyses is OS. We performed two types of OS analyses: traditional OS (OS from start of treatment) and post-progression OS (ppOS)

defined as OS after disease progression according to RECIST 1.1. PFS was measured from the date of vemurafenib commencement until disease progression according to RECIST 1.1. Patients alive at data cut-off are marked as censored in the Kaplan-Meier survival curve. Univariate and multivariate Cox regression methods were used to estimate the hazard ratio (HR) of continuing vemurafenib beyond progression for ppOS. The following known prognostic factors were included in the multivariate analysis: age, serum LDH level, ECOG performance status, M-stage and presence of brain metastases [23-26]. A sensitivity analysis was performed where BRAFi TBP was defined as treatment beyond 0, 7, 14, 21 and 28 days of documented progression. As this was a retrospective case-control study with overall survival as primary endpoint, patients who received subsequent systemic treatment were not censored at the time of starting subsequent treatment.

RESULTS

Patient characteristics within the cohorts

In total 152 patients with *BRAF*-mutant metastatic melanoma were identified. Patients were excluded from this analysis due to absence of measurable disease according to RECIST 1.1, absence of any initial response, or due to ongoing response at data cut-off (see Figure 1). Two patients continued therapy at other institutions and were lost to follow up. From the remaining 70 patients 35 continued vemurafenib treatment (BRAFi TBP) despite disease progression and 35 discontinued vemurafenib at time of progression of disease (no

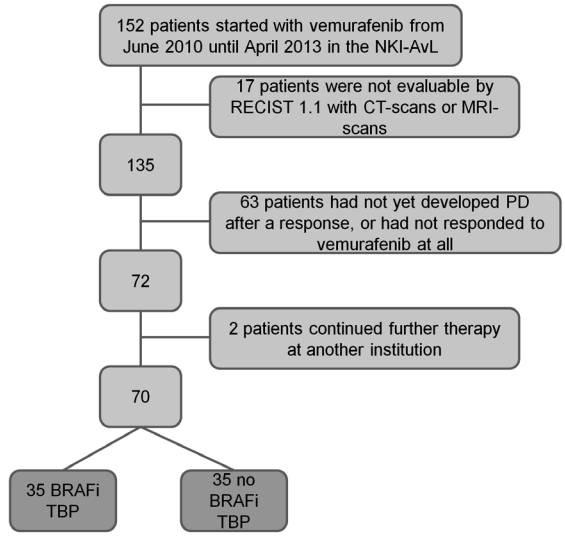


Figure 1. Flow chart showing process of patient selection

BRAFi TBP), thereby serving as a control group. In the BRAFi TBP group 24 patients were in the Global Safety Study and 11 patients received vemurafenib after approval by EMA. For the patients that stopped vemurafenib at disease progression 26 were in the Global Safety Study and 9 received vemurafenib after approval by EMA. Patient characteristics at time of study commencement are shown in Table 1.

The median follow-up was 22 months at data cut-off as of February 2014. Twenty-nine patients in the BRAFi TBP group had died, while this was the case for 34 patients in the control group. The patients' characteristics of both cohorts at the time of disease progression are summarized in Table 2. Fifty-nine percent of the patients were men and the mean age of the entire cohort was 55 years. As shown in Table 2, significant imbalances were found concerning the distribution of ECOG performance status ($p < 0.001$), M-stage ($p = 0.011$) and serum lactate dehydrogenase (LDH) level ($p = 0.037$) between the two groups. The presence of brain metastases was similar in both cohorts. In both groups subsequent therapies were started at discretion of the treating physician, which was slightly more frequent (not significant, $p = 0.46$) in the no TBP group (Table 3).

Table 1. Patient characteristics at study commencement

	BRAFi TBP (n = 35) at baseline	**No BRAFi TBP (n = 35) at baseline**	**p value**
Age mean (SD)	**51.5 (13)**	**57.1 (13)**	**0.077**
Gender			**0.628**
Male	**19 (54)**	**22 (63)**	
Female	**16 (46)**	**13 (37)**	
ECOG performance status			**0.106**
0-1	**34 (97)**	**29 (83)**	
2-3	**1 (3)**	**6 (17)**	
Lactate dehydrogenase			**0.216**
0-250 U/L	**20 (57)**	**15 (42)**	
251-500 U/L	**11 (31)**	**10 (29)**	
> 500 U/L	**4 (11)**	**10 (29)**	
Unknown	**0 (0)**	**0 (0)**	
M-stage			**0.152**
M1a	**3 (9)**	**0 (0)**	
M1b	**5 (14)**	**3 (9)**	
M1c	**27 (77)**	**32 (91)**	
Brain metastases			**0.808**
Yes	**22 (63)**	**20 (57)**	
No	**13 (37)**	**15 (43)**	

Abbreviations: BRAFi, BRAF inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; TBP, treatment beyond progression.

Table 2. Patient characteristics at the time of BRAF inhibitor disease progression

	BRAFi TBP (n = 35) at progression	No BRAFi TBP (n = 35) at progression	p value
Age mean (SD)	52.5 (13)	58.2 (13)	0.073
Gender			0.628
Male	19 (54)	22 (63)	
Female	16 (46)	13 (37)	
ECOG performance status			< 0.001
0-1	34 (97)	21 (60)	
2-3	1 (3)	14 (40)	
Lactate dehydrogenase			0.037
0-250 U/L	23 (66)	16 (46)	
251-500 U/L	8 (22)	8 (22)	
> 500 U/L	2 (6)	10 (29)	
Unknown	2 (6)	1 (3)	
M-stage			0.011
M1a	3 (9)	0 (0)	
M1b	4 (11)	0 (0)	
M1c	28 (80)	35 (100)	
Brain metastases			1
Yes	17 (49)	18 (51)	
No	18 (51)	17 (49)	

Abbreviations: BRAFi, BRAF inhibitor; ECOG PS, Eastern Cooperative Oncology Group performance status; TBP, treatment beyond progression.

Nature of disease progression upon BRAFi

Data regarding the nature of disease progression are shown in Table 4. When looking at type of progression of disease 29 of 70 patients (41%) progressed in existing metastases only, while only 9 of 70 patients (13%) had progression of disease due to development of only new metastases. Most patients, 39 of 70 (56%), had generalized progression of disease (i.e. progression in more than one site or organ). There was a significant difference in the two groups: in the BRAFi TBP group 14 of 35 (40%) patients had generalized progression, while this was 25 of 35 (71%) patients in the group that stopped BRAFi treatment at progression (p = 0.015).

When looking more closely, the majority of patients, 42 of 70 (60%), progressed at extracranial sites only, while 10 of 70 patients (14%) progressed only intracranially. Eighteen of 70 patients (26 %) progressed at both intracranial and extracranial sites. Intracranially, 20 of 70 (29%) patients showed progression in existing lesions, while 24 of 70 (34%) patients progressed due to the formation of new metastases. Interestingly, when comparing the site of progression of disease between the two groups there was a significant difference in the nature of progression. In the cohort that stopped BRAFi treatment upon progression

26 of 35 patients (74%) showed progression in existing nonvisceral metastases, while this was only in 16 of 35 patients (46%) in the cohort that had BRAFi TBP (p = 0.03).

Table 3. Management after progression of disease from BRAF inhibitor

Factor	No. of Patients (%)			
	Total	BRAFi TBP	No BRAFi TBP	p value
Total	70 (100)	35 (50)	35 (50)	
Treatment after vemurafenib				0.46
Ipilimumab	20 (29)	10 (29)	10 (29)	
DTIC	3 (4)	1 (3)	2 (6)	
Temozolomide	1 (1)	0 (0)	1 (3)	
Anti PD-1	2 (3)	1 (3)	1 (3)	
Tumor Infiltrating Lymphocytes	1 (1)	0 (0)	1 (3)	
None	43 (61)	23 (65)	20 (57)	
Disease progression amenable to local				0.03
treatment	38 (54)	14 (40)	24 (69)	
No	32 (46)	21 (60)	11 (31)	
Yes				
Disease progression treated locally				0.02
No	39 (56)	14 (40)	25 (71)	
Yes	31 (44)	21 (60)	10 (29)	
Patients with intracranial disease progression				
n = 28				-
Intracranial surgery	28 (100)	13 (100)	15 (100)	
No	0 (0)	0 (0)	0 (0)	
Yes				
Intracranial SRS				0.21
No	26 (93)	11 (85)	15 (100)	
Yes	2 (7)	2 (15)	0 (0)	
Intracranial WBRT				0.11
No	18 (64)	6 (46)	12 (80)	
Yes	10 (36)	7 (54)	3 (20)	
Patients with extracranial disease progression				
n = 60				0.05
Extracranial surgery	52 (87)	23 (77)	29 (97)	
No	8 (13)	7 (23)	1 (3)	
Yes				
Extracranial XRT				0.75
No	48 (80)	25 (83)	23 (77)	
Yes	12 (20)	5 (17)	7 (23)	

Abbreviations: BRAFi, BRAF inhibitor; SRS; stereotactic radiosurgery; TBP, treatment beyond progression; WBRT, whole-brain radiotherapy; XRT, radio-therapy.

Local treatment after BRAFi progression of disease

Twenty-eight patients had intracranial disease progression. Of those 28 patients 12 (43%) received local treatment to progressing sites. Two patients (7%) received stereotactic radiotherapy and 10 patients (36%) received whole-brain radiotherapy. No patient had

intracranial surgery for intracranial disease progression. Of 60 patients who had extracranial disease progression, 20 (33%) received local treatment for progressing sites. Eight patients (13%) underwent local surgery and 12 patients (20%) received local radiotherapy. When comparing the BRAFi TBP group to the group who stopped BRAFi treatment upon progression there was a borderline significant difference in patients who underwent extracranial surgery (7 versus 1 patient $p = 0.05$) in favor of the TBP group.

Table 4. Nature of BRAFi progression of disease

Factor	No. of Patients (%)			
	Total	BRAFi TBP	No BRAFi TBP	p value
Intracranial/extracranial disease progression	42 (60)	22 (63)	20 (57)	0.941
Extracranial only	10 (14)	5 (14)	5 (14)	
Intracranial only	18 (26)	8 (23)	10 (29)	
Extracranial and intracranial				
Type of progression				0.016
Existing lesion	29 (41)	19 (54)	10 (29)	
New lesion	9 (13)	6 (17)	3 (9)	
New and existing lesions	32 (46)	10 (29)	22 (63)	
Isolated*	31 (44)	21 (60)	10 (29)	0.015
Generalized	39 (56)	14 (40)	25 (71)	
Site of progression				
Visceral existing				1
No	35 (50)	17 (49)	18 (51)	
Yes	35 (50)	18 (51)	17 (49)	
Visceral new+				0.133
No	56 (80)	31 (89)	25 (71)	
Yes	14 (20)	4 (11)	10 (29)	
Nonvisceral existing+				0.03
No	28 (40)	19 (54)	9 (26)	
Yes	42 (60)	16 (46)	26 (74)	
Nonvisceral new+				1
No	52 (74)	26 (74)	26 (74)	
Yes	18 (26)	9 (26)	9 (26)	
Brain existing				1
No	50 (71)	25 (71)	25 (71)	
Yes	20 (29)	10 (29)	10 (29)	
Brain new				0.45
No	46 (66)	25 (71)	21 (60)	
Yes	24 (34)	10 (29)	14 (40)	

Abbreviations: BRAFi, BRAF inhibitor; TBP, treatment beyond progression.

* Isolated progression of disease was defined as progression in a new or existing lesion within one site or organ, where the rest of disease showing at least stable disease.

+ Visceral disease included lung, liver and pancreas; nonvisceral included subcutaneous, bone and lymph node disease.

Systemic treatment after BRAFi progression of disease

As previously described, at time of progression of disease according to RECIST 1.1, 35 of 70 patients (50%) continued treatment with vemurafenib. Twenty of 70 patients (29%) did not receive any subsequent treatment and 15 (21%) received other therapies such as ipilimumab, dacarbazine, temozolomide, anti-PD1 or tumor infiltrating lymphocytes. Of the 35 patients who continued BRAFi treatment despite progression of disease 12 (34%) eventually received other systemic treatment when progression was not manageable anymore with vemurafenib. Subsequent treatment included ipilimumab, dacarbazine and anti PD-1. There was no significant difference between the two groups regarding subsequent systemic treatment. The median duration of continued BRAFi TBP was 103 days (range 13-401). The median number of cycles (4 weeks vemurafenib) given in the BRAFi TBP group was 4 (range 1-14).

Clinical outcomes

Median PFS for all 70 patients was 5.2 months (Figure 2A) and thus comparable to the data observed in the phase 3 study and the global safety study [22, 24]. Median PFS within the BRAFi TBP group was significantly longer than that of patients in the no BRAFi TBP group (5.6 months vs. 4.0 months, CI: 4.4-7.5, 3.7-5.5; Log-Rank p = 0.02) (Figure 2B). This may have been the result of the difference in ECOG PS and serum LDH levels between the two groups at the start of the vemurafenib treatment. Results from the global safety study point towards a shorter PFS for these subgroups [22]. This translated also into a significantly longer median OS (Figure 2C), namely 12.8 months in the TBP group versus 6.3 months in the control group (Log-Rank p = 0.0001). The median ppOS (Figure 2D) of these groups was 5.2 versus 1.4 months (95% CI: 3.8-7.4, 0.6-3.4; Log-Rank p = 0.002), respectively. Comparing both groups in a univariate survival analysis for several of the identified prognostic markers (see Table 5), stopping vemurafenib upon progression was significantly associated with a shorter ppOS (HR 2.16; 95% CI: 1.30, 3.57; p = 0.002), as was ECOG performance status of 2 or 3, the presence of brain metastases and the serum LDH levels of 251-500 U/L and > 500 U/L. Male gender and M-stage (M1b and M1c) were also associated with a shorter ppOS, but this was not statistically significant (p > 0.05). No additional toxicities were seen in the TBP group. To decrease the possibility that the ppOS benefit evolves solely from imbalances within the cohorts, a multivariate Cox regression analysis was performed adjusting for the identified imbalances in the cohorts, such as: age, performance status, serum LDH level, M-stage and presence of brain metastases, since these are known prognostic factors for melanoma survival. Applying this analysis stopping treatment at time of progression was independently and still significantly associated with shorter ppOS (Table 6, HR 1.92; 95% CI: 1.04, 3.55; p = 0.04). Serum LDH levels higher than 500 U/L and the presence of brain metastases were also significantly associated with shorter ppOS, but not M-stage M1c and a serum LDH level between 251 and 500 U/L. It is noteworthy that the HR for TBP was hardly altered when comparing univariate with multivariate analysis (HR 2.16 versus HR 1.92).

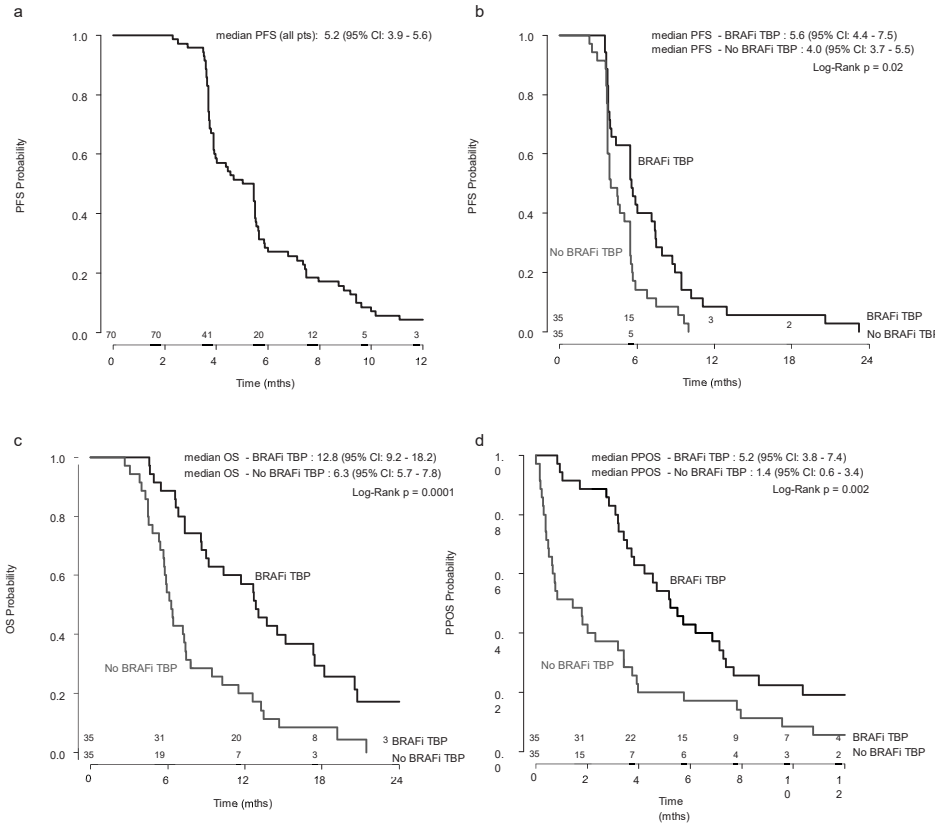


Figure 2. Kaplan-Meier curves of progression-free and overall survival in months

(a) Progression-free survival curve of total sample, (b) progression-free survival curve categorized by patients BRAFi TBP and no BRAFi TBP, (c) overall survival curve categorized by patients BRAFi TBP and no BRAFi TBP, (d) overall survival curve from the time of progression of disease. Numbers above the time-line represent the patients who are at risk at that time.

Abbreviations: BRAFi, BRAF inhibitor; OS, overall survival; PFS, progression-free survival; PPOS, post-progression overall survival; TBP, treatment beyond progression.

Sensitivity analysis was performed to see whether the number of days of BRAFi TBP used to define the cohort receiving TBP would influence overall survival from the date of progression of disease. Overall survival remained statistically different for the two groups when defining BRAFi TBP as treatment > 28 days ($p < 0.001$), > 21 days ($p < 0.001$), > 14 days ($p < 0.001$), > 7 days ($p < 0.001$) and > 0 days ($p < 0.001$). We also analyzed cost implementation of TBP. In The Netherlands one vemurafenib tablet of 240mg costs €40. This would add up to €320 a day for full dose vemurafenib. Based on a median ppOS of 5.2 months in the TBP group, TBP would add an additional "cost" of approximately €48,000-.

Table 5. Univariate analysis of post-progression overall survival (n = 70)

	Univariate analysis		
	HR	**95% CI**	**p value**
Treatment with vemurafenib			
TBP	**1**		
No TBP	**2.16**	**1.30 - 3.57**	**0.003**
Age			
Per year	**1.00**	**0.98 - 1.02**	**0.76**
ECOG performance status			
0-1	**1**		
2-3	**3.58**	**1.96 - 6.51**	**< 0.0001**
Lactate dehydrogenase			
0-250 U/L	**1**		
251-500 U/L	**1.26**	**0.68 - 2.34**	**0.460**
> 500 U/L	**5.36**	**2.50 - 11.47**	**< 0.0001**
M-stage			
M1a	**1**		
M1b	**1.97**	**0.37 - 10.85**	**0.434**
M1c	**1.71**	**0.42 - 7.01**	**0.457**
Brain metastases			
No	**1**		
Yes	**1.67**	**1.00 - 2.77**	**0.05**

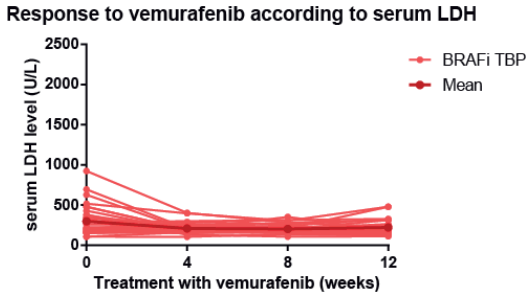
Abbreviations: CI, Confidence Interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, Hazard Ratio; TBP, treatment beyond progression;

Table 6. Multivariate analysis of post-progression overall survival (n = 70)

	Multivariate Cox regression analysis		
	HR	**95% CI**	**p value**
Treatment with vemurafenib			
TBP	**1**		
No TBP	**1.92**	**1.04 - 3.55**	**0.04**
Age			
Per year	**1.02**	**1 - 1.04**	**0.11**
ECOG performance status			
0-1	**1**		
2-3	**1.65**	**0.79 - 3.47**	**0.18**
Lactate dehydrogenase			
0-250 U/L	**1**		
251-500 U/L	**0.95**	**0.48 - 1.87**	**0.88**
> 500 U/L	**3.85**	**1.63 - 9.07**	**0.002**
M-stage			
M1a	**1**		
M1b	**2.39**	**0.4 - 14.2**	**0.34**
M1c	**0.7**	**0.15 - 3.18**	**0.64**
Brain metastases			
No	**1**		
Yes	**2.39**	**1.25 - 4.59**	**0.01**

Abbreviations: CI, Confidence Interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, Hazard Ratio; TBP, treatment beyond progression;

a



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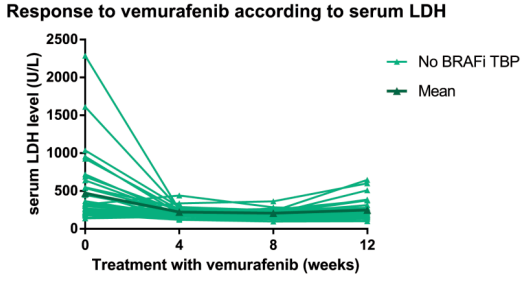

Figure 3. Serum LDH levels during treatment with vemurafenib
(a) Serum LDH levels of BRAFi TBP
(b) Serum LDH levels of no BRAFi TBP
Abbreviations: BRAFi, BRAF inhibitor; LDH, lactate dehydrogenase; TBP, treatment beyond progression.

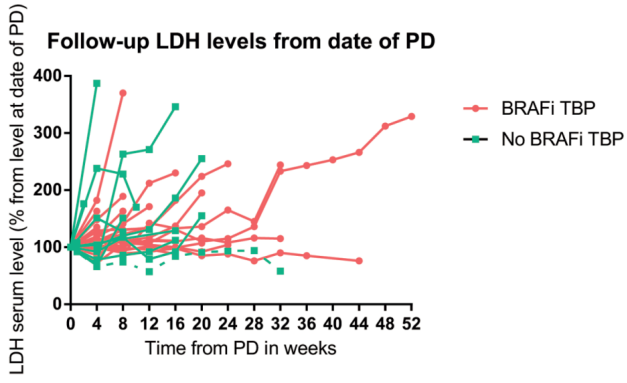

Figure 4.
Abbreviations: BRAFi, BRAF inhibitor; LDH, lactate dehydrogenase; PD, progression of disease; TBP, treatment beyond progression.

DISCUSSION

Although vemurafenib and dabrafenib have revolutionized the treatment of *BRAF*-mutated melanoma, early drug resistance and subsequent disease progression hamper long-term benefit for these patients [24, 27]. Traditionally, treatment is discontinued once progression is documented. This is especially true for classical therapies like chemotherapy with cytotoxic drugs, and this was similarly implemented in the vemurafenib versus dacarbazine phase 3 trial and initially in the Global Safety Study [24, 28, 29]. However, in the era of immunotherapy and targeted therapy, this strategy may need revision [15, 17, 18, 20, 21]. It was our clinical observation in patients treated with vemurafenib that discontinuation oftentimes lead to accelerated disease progression. Therefore, we switched our strategy and kept patients on vemurafenib (after permission of the EAP study monitor) despite progression.

In this retrospective and exploratory analysis presented here, we investigated whether BRAFi TBP could be beneficial for *BRAF* V600 mutated melanoma patients treated with vemurafenib, who initially responded to treatment. We found that BRAFi TBP was, in a multivariate analysis, significantly and independently associated with a relative reduction of nearly 50% in the risk of death, leading to a prolonged median OS after progression of 5.2 months as compared to 1.4 months in the group that stopped treatment. These data are in line with data observed for treatment with dabrafenib, the second recently approved selective BRAF inhibitor [12].

Our findings correspond also with those from other studies, which have investigated treatment beyond disease progression with targeted therapies in other malignancies [15, 17, 18, 21, 30]. For example, TBP with bevacizumab in patients with metastatic colorectal carcinoma and trastuzumab in patients with breast cancer improved OS [15, 18]. Similar results have also been found for NSCLC patients treated with EGFR inhibitors [21]. A recently published article by Chan et al. analyzing the effects of extended BRAF inhibition after progression of disease in patients with metastatic melanoma, discovered a prolonged overall survival even after adjusting for potential prognostic factors [31]. Yet other preclinical data, using xenograft models, suggest a possible adverse effect of continued BRAFi TBP. A study by Hartsough et al. discovered that growth and signaling of *in vivo* and *in vitro* derived RAF inhibitor-resistant cell lines that expressed *BRAF* V600E splice variants grew more efficiently in the presence of a BRAFi compared to without the inhibitor [32]. Another study by Thakur et al. showed that vemurafenib-resistant melanoma become drug dependent for their continued proliferation. Stopping vemurafenib treatment here led to regression of drug-resistant tumors [33]. These data, however, do need validation in humans. Furthermore, other possible BRAFi resistance mechanisms may not have

these effects on continued BRAFi TBP [34]. Our analysis in melanoma patients here does not support these findings from animal models proposing treatment discontinuation to be more beneficial. Furthermore, we did not observe any patient showing spontaneous regression after stop of BRAFi treatment.

While BRAFi treatment is showing impressive results regarding objective response rate (ORR), unfortunately there does not appear to be a plateau in overall survival as is seen with immunotherapy [35-37]. We therefore believe that TBP with a BRAFi should be reserved as a last line treatment, or should be considered as first line treatment in patients with high tumor burden, who most likely do not benefit from immunotherapy at all [38]. Treatment beyond progression will add additional costs to the health budget, but if we are able to select patients more carefully that will benefit from TBP, an additional of € 48,000.- for a median OS benefit, may still be worthwhile. Perhaps that on the basis of emerging technologies, such as cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA), we will be able to select patients that fit the group benefitting from TBP better, however further research is needed.

We are aware of the retrospective character of our analysis that might have been biased, not only by the small sample size, but also by the physicians' decision regarding continuation or discontinuation of vemurafenib depending on patients' choice, site of disease progression or treatment possibilities beyond progression. Although no variation between the groups was found in the number of patients that received subsequent treatment with, for example, ipilimumab, the physicians' decision has clearly led to imbalances between the two groups in other patients' characteristics, such as a significant difference in ECOG performance status. Also a significant difference in PFS was seen between the two groups. To minimize selection bias we conducted a sensitivity analysis that still showed a significant difference in overall survival when patients, who initially received BRAFi TBP, but who deteriorated within one month of treatment, were excluded. To analyze the possible difference in tumor biology between the two groups we compared changes of LDH upon treatment with vemurafenib. LDH has been identified as a prognostic factor and is thought to correlate with tumor metabolism [26, 39-41]. In both groups we found a normalization of the mean LDH upon vemurafenib treatment (mean serum LDH at week 8 was 203 U/L in the group treated beyond progression versus 207 U/L in the control group, $p = 0.453$) indicating no differences in the changes of tumor metabolism upon treatment (Figure 3). Interestingly baseline LDH was higher in the no BRAFi TBP group (mean LDH 470 U/L versus 311 U/L, $p = 0.066$), representing possibly a higher tumor load at treatment initiation. Considering these prognostic factor imbalances, however, this did not reduce the strong HR observed for BRAFi TBP in the multivariate analysis indicating that the imbalances in our groups had only a minor effect on the HR for BRAFi TBP.

Only a well controlled and randomized setting could provide better balanced groups and prove the benefit of treatment beyond progression in a completely unbiased setting. This study, however, would be ethically very challenging and therefore not feasible.

Since we clinically observed that once patients stopped vemurafenib, they tended to have an accelerated course of disease, we compared the serum LDH levels after progression of patients stopping vemurafenib with that of patients continuing the treatment. No significant difference was found in the rate of increase of LDH levels after stopping vemurafenib between these groups of patients (Figure 4). Since LDH levels have been considered a measurement of tumor load, these data suggest that upon stopping vemurafenib at progression, changes in LDH levels are an insufficient predictor of progressive disease.

While pretreatment serum LDH levels are prognostic factors for patients with metastatic melanoma, serum LDH levels can indicate the tumor response to vemurafenib in patients with metastatic melanoma [26, 39, 41]. We found no significant differences in LDH decrease upon treatment to vemurafenib, pointing towards similar tumor biology and thus similar initial response to selective BRAFi in the two groups. However, we cannot rule out that the lack of a difference in LDH decline was the result of the small sample size.

Our data suggest that BRAFi TBP can benefit melanoma patients, who initially responded to treatment. In the light of lack of alternative treatment options, which is not uncommon for these patients, our data suggest that BRAFi TBP with vemurafenib could be considered. A retrospective subgroup analysis of the global safety study cohort, could confirm these results, or at least give us more insight information [22]. In addition quality of life analyses should be performed. Identification of biomarkers to identify patients that benefit from TBP could round-up such analyses.

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