PET/CT to optimize treatment management of high-risk stage III and IV melanoma

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CHAPTER 5

The Predictive Value of [^{18}F]FDG PET/CT for Determining Progression-Free Survival in Advanced Stage III–IV BRAF-Mutated Melanoma Patients Treated with Targeted Therapy: What Can Be Learned from Progression?

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ABSTRACT

Purpose

To investigate whether (early) PERCIST response monitoring with [^{18}F]FDG PET/CT is predictive for PFS in unresectable stage III or IV melanoma patients treated with BRAF/MEK inhibitors and to define dissemination patterns at progression with a lesion-based evaluation in direct comparison to baseline to improve our understanding of [^{18}F]FDG PET/CT during BRAF/MEKi.

Methods

This prospective multi-center single arm study included 70 patients with unresectable stage III/IV BRAF-mutated melanoma who underwent contrast-enhanced CT and [^{18}F]FDG PET/CT at baseline and 2 and 7 weeks during treatment with vemurafenib plus cobimetinib and at progression if possible. Tumor response assessment was done with RECIST1.1 and PERCIST. Follow-up PET/CT scans were visually compared to baseline to assess dissemination patterns.

Results

Using RECIST1.1, PFS was not significantly different between the response groups (P=0.26). At 2 weeks, PERCIST median PFS was 15.7 months for patients with complete metabolic response (CMR) versus 8.3 months for non-CMR (P=0.035). The hazard ratio for progression/death in non-CMR vs CMR was 1.99 (95% CI 1.03-3.84, P=0.040) and 1.77 (95% CI 0.91-3.43, P=0.0935) when adjusting for LDH. At 7 weeks, median PFS for PERCIST CMR was 16.7 months versus 8.5 months for non-CMR (P=0.0003). The hazard for progression/death in the non-CMR group was significantly increased (HR=2.94, 95% CI 1.60-5.40, P=0.0005), even when adjusting for LDH (HR=2.65, 95% CI 1.43-4.91, P=0.0020). At week 7, [^{18}F]FDG PET/CT at was false-positive in all four (6%) patients with new FDG-avid lesions but CMR of known metastases. When [^{18}F]FDG PET/CT was performed at progressive disease, 18/22 (82%) patients had progression of known metastases with or without new [^{18}F]FDG-avid lesions.

Conclusion

This study shows that PERCIST response assessment at week 7 is predictive for PFS, regardless of LDH. At 2 weeks, patients with CMR have longer PFS than patients with non-CMR, but different PET parameters should be investigated to further evaluate the added value of early [^{18}F]FDG PET/CT. Disease progression on PET/CT is predominated by progression of known metastases and new [^{18}F]FDG-avid lesions during BRAF/MEKi are not automatically a sign of recurrent disease.

BACKGROUND

Mutations in the BRAF gene (V600E or V600K) are the most common molecular alterations present in 50-60% of patients with unresectable stage III or metastatic melanoma (1, 2). In these patients, targeted therapy with a BRAF- plus MEK inhibitor combination (BRAF/MEKi) is a successful systemic treatment resulting in an overall survival benefit compared to monotherapy with BRAF inhibitors (3-5). Despite good initial response rates, acquired resistance develops in most patients resulting in disease progression. The ability to predict response duration could help to optimize treatment strategy for individual patients by changing to a different therapy such as immune checkpoint inhibitors (ICI's) before resistance occurs. Also, severe adverse events associated with the BRAF/MEKi-combo occur in over one-third of the patients and can lead to treatment discontinuation (4, 6). So, appropriate selection of patients who will benefit from treatment (i.e., have a durable response) with BRAF/MEKi is highly relevant.

The most common prognostic biomarkers for progression-free survival (PFS) and overall survival (OS) in melanoma patients treated with BRAF/MEKi are baseline lactate dehydrogenase (LDH) level, Eastern Cooperative Oncology Group (ECOG) performance status and tumor burden (4, 7, 8). Molecular imaging with [18F]fluorodeoxyglucose ([18F]FDG) Positron Emission Tomography / Computed Tomography (PET/CT) is increasingly used to monitor response and guide therapies. During BRAF/MEKi therapy, melanoma metastases will show a rapid decrease in both lesion size and glucose metabolism, represented by a marked reduction of [18F]FDG accumulation (9). Various studies suggest that reduction in [18F]FDG uptake is a prognostic indicator for PFS or OS in BRAF-mutant metastatic melanoma (9-13).

To objectively quantify metabolic response with [18F]FDG PET/CT, Wahl et al. introduced the so-called PET Response Criteria in Solid Tumors (PERCIST) (14). Uptake in the "hottest" lesion is quantified at each imaging time point based on the assumption that the most metabolically active lesion defines the clinical status of the patient. Visual interpretation and quantification are used to categorize responses. Visual interpretation of progressive or recurrent disease, however, can be challenging on [18F]FDG PET/CT in this population. Dissemination patterns are highly unpredictable in melanoma as it can metastasize to any tissue, so each new [18F]FDG-avid lesion is suspicious for recurrent disease (15-17). And yet, our clinical experience suggests that new [18F]FDG-avid lesions are most often false-positives when the known tumor lesions remain in remission during BRAF/MEKi. Although quantification of metabolic response with PERCIST, a more objective parameter, has previously been applied to determine efficacy of ICI's in melanoma (18, 19), no studies are available to underline the predictive value of PERCIST in BRAF/MEKi treatment. Therefore, the aim of this study is to investigate the predictive value of (early) metabolic response assessment with PERCIST to predict PFS in patients with unresectable stage III/IV BRAFV600E/K-mutated melanoma treated

with vemurafenib plus cobimetinib (V/C). In addition, a lesion-based evaluation will be performed to define dissemination patterns at progression in direct comparison to baseline, and thus, to improve our understanding of [^{18}F]FDG PET/CT during BRAF/MEKi.

PATIENTS AND METHODS

Patients

Between March 2015 and February 2019, 75 patients with BRAF-mutated melanoma signed informed consent in the REPOSIT-trial (NCT02414750). In this phase II multi-center prospective study, patients with histologically proven BRAF-mutated unresectable stage IIIC or stage IV melanoma were included (20). Patients were treated with vemurafenib 960mg twice daily on day 1-28 and cobimetinib 60mg once daily on day 1-21 until progression or uncontrollable toxicity. Patients were recruited from nine hospitals which are part of the Dutch Melanoma and Skin Cancer group (DMSCG). The study was approved by the local Medical Ethical Committees, and written informed consent was obtained before inclusion. Information on patient demographics, clinical, histopathological, imaging, and laboratory data was collected. Toxicity on BRAF/MEKi was scored according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (21).

Imaging protocol

Baseline [^{18}F]FDG PET/CT and contrast-enhanced CT (ceCT) were performed within 1 month before start of BRAF/MEKi. Follow-up [^{18}F]FDG PET/CT and ceCT scans were performed on day 15 of cycle 1 ('early' week 2), day 21 of cycle 2 ('standard' week 7), and if clinically possible, at progression. Additional ceCT scans were performed every 8 weeks following standard protocol and when progressive disease was suspected.

To enable quantitative evaluation of the different PET/CT scanners used within this multi-center study, [^{18}F]FDG PET/CT scans across sites were acquired according to the European Association of Nuclear Medicine (EANM) guideline for oncology [^{18}F]FDG PET/CT imaging (22, 23) on EARL-accredited scanners. Image reconstruction was performed according to EANM Research Ltd. (EARL) standard 1 (EANM resEARch4Life, https://earl.eanm.org/) (24). Images were acquired from at least base of the skull to thighs at 2-4 min per bed position in a supine position. [^{18}F]FDG PET/CT scans were performed with a Gemini TF PET/CT, TF Big Bore PET/CT, Ingenuity TF PET/CT (all Philips Medical Systems, Netherlands), Siemens Biograph mCT PET/CT (Siemens, Germany). For each patient, [^{18}F]FDG PET/CT scans were performed on the same scanner with a maximum activity difference of 10% compared to baseline.

[^{18}F]FDG PET/CT image analysis

[^{18}F]FDG PET/CT scans were sent for central reviewing by experienced nuclear medicine physicians. Sites of increased uptake were identified for further quantification (BvdH)

and in unclear cases, a second experienced nuclear medicine physician interpreted the data to reach consensus (MS). For quantification, the in-house developed software package ACCURATE was used (25). At each time point, one target lesion was selected, being the lesion with the highest peak standardized uptake value (SUV) corrected for lean body mass (SULpeak). SULpeak was defined as a 1-mL spherical volume of interest (VOI) with the highest uptake within the tumor. The percentage of change in target lesions between baseline and follow-up was computed as follows:

$$100\% \times \frac{Follow\ up\ SULpeak - Baseline\ SULpeak}{Baseline\ SULpeak}$$

To ensure that a decline of [18F]FDG uptake can be measured accurately during therapy, the baseline SULpeak of a target lesions had to be ≥1.5 times the liver SULmean (3-cm spherical VOI in the right upper lobe) plus two times its standard deviation or, in case of liver metastases, >2.0 times the blood pool SULmean (1cm diameter ROI in descending thoracic aorta extended over 2-cm z-axis, taking care not to include in the vessel wall) (14).

Tumor response assessment

Tumor response to BRAF/MEKi was evaluated with ceCT scans of neck, thorax, abdomen and pelvis and with [18F]FDG PET/CT at given time intervals using RECIST v1.1 and PERCIST criteria, respectively (26). For imaging the following classifications were defined: complete metabolic response (CMR), partial metabolic response (PMR), stable metabolic disease (SMD), and progressive metabolic disease (PMD) (27). Subsequently, response was also dichotomous pooled to CMR and non-CMR groups (e.g., PMR, SMD and PMD).

Additionally, follow-up PET/CT scans were visually compared to baseline to assess dissemination patterns. All scans were categorized in three subgroups; a) increased uptake in baseline [18F]FDG-avid lesions only, b) increased uptake in baseline [18F]FDG-avid lesions and new [18F]FDG-avid lesions, c) new [18F]FDG-avid lesions only, while baseline [18F]FDG-avid lesions remain in remission.

Statistical analysis

Patients' characteristics were summarized as either mean ± standard deviation or median and range for continuous variables, and frequency and percentage for categorical variables. PFS was defined as the time from commencement of vemurafenib/cobimetinib to disease progression (based on clinical findings and/or RECIST1.1), or death from any cause in the absence of progression. OS was defined as time from commencement of vermurafenib/cobimetinib to death from any cause. Patients without any of these events before the end of follow-up were censored at the date last known to be alive and progression/recurrence-free. Patients starting non-protocol treatment were censored at the date the new treatment was initiated. For survival analyses, week 2 and week 7 were considered landmark points, so only patients without an event up to that time were included.

The Kaplan-Meier method was used to generate survival curves, and log-rank tests were used to compare them. Univariable and multivariable Cox regression analyses were performed, with hazard ratio (HR) and corresponding 95% confidence interval (CI) being reported. Multivariable analyses were adjusted for LDH, ECOG performance status and the number of metastatic organs at baseline. LDH upper limits of normal (ULN) were normalized for the reference range at each participating center.

Statistical analyses were performed using R statistical software (version 4.2.0 The R Foundation for Statistical Computing, Vienna, Austria) and SAS statistical software package (version 9.4. SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the 75 patients with signed informed consent in the REPOSIT trial, two patients died before week 2, and in two patients, treatment was discontinued before week 2 due to adverse events. One patient was excluded because baseline PET/CT was not performed according to EARL. Demographics of the 70 remaining patients are summarized in **Table 1**.

Table 1. Patient characteristics.

	N=70 patients
Characteristic	**Frequency (%)**
Sex	
male	39 (55.7%)
female	31 (44.3%)
Age in years (median (range))	61 (53-69)
Ethnicity	
Caucasian	70 (100%)
ECOG performance status	
0	38 (54.3%)
1	32 (45.7%)
Type of primary tumor	
locally advanced (Stage IIIc)	3 (4.3%)
metastatic (Stage IV)	67 (95.7%)
LDH	
missing	3
LDH ≤ ULN	35 (52.2%)
LDH > ULN	32 (47.8%)
Number of metastatic sites	
<3	19 (27.1%)
≥3	51 (72.9%)

ECOG= Eastern Cooperative Oncology Group; LDH=lactate dehydrogenase; ULN=upper limit of normal.

Median follow-up time among all patients regardless of censoring status was 16 months (range 3.7-57.1 months). Forty-eight (69%) patients had progressive disease while on BRAF/MEKi based on clinical findings and/or RECIST1.1. There were 55 deaths observed during the study. Median PFS and OS were 9.86 months (95% CI 8.05-15.2) and 17.6 months (95% CI 13.9-22.6), respectively. Observed toxicity are listed in **Supplementary Table S1**.

RECIST1.1 response classification

Of the 70 patients with RECIST1.1 evaluation at week 7, 54 (77%) had partial response (PR) and 15 (21%) had stable disease (SD). One patient (2%) had complete response (CR) and remained recurrence-free at the end of follow-up (54.4 months). There was no significant difference in PFS between the RECIST response groups (log-rank P=0.26).

PERCIST response classification

[18F]FDG PET/CT scans were performed in 67 patients at week 2 and 7, in one patient [18F]FDG PET/CT was performed only at week 2 and in two patients only at week 7. Median time between start of treatment and [18F]FDG PET/CT or ceCT was 14 days (range 12-19 days) and 48 days (range 45-62 days), respectively.

Early response classification at week 2 (n=68) revealed 19 patients with CMR, 46 patients with PMR and three patients with SMD. No patient had PMD. Median PFS was 15.7 months (95% CI 14.7-NA) for patients with CMR, compared to 8.3 months (95% CI 6.7-13.9) for PMR and 7.6 months (95% CI 3.3-NA) for SMD (**Figure 1A**). When grouping PMR and SMD together as non-CMR, PFS was significantly longer in the CMR group (median PFS 15.7 months for CMR versus 8.3 months for non-CMR, log-rank P=0.035), see **Figure 1B**.

By using standard response classification at week 7 (n=69), a total of 31 patients were classified as CMR, 33 patients as PMR, four patients as SMD and one patient with PMD (**Table 2**). Median PFS was 16.7 months for patients with CMR (95% CI 10.9-NA), 8.5 months for PMR (95% CI 6.2-13.9) and 7.6 months for SMD (95% CI 3.4-NA), see **Figure 1C**. At week 7, PFS was also significantly different between these groups (log-rank P=0.0016). In the only patient (#328) with PMD, the dosage of vemurafenib was halved within 11 days after treatment initiation due to adverse events. Here, [18F]FDG PET/CT at week 2 showed SMD and at week 7 PMD based on increase of [18F]FDG uptake of existing intra-abdominal metastases. One week later the patient went off study and started a different BRAF/MEKi, 13 months later the patient progressed and died. Comparing CMR with non-CMR revealed a more favorable PFS for patients with early CMR (median 16.7 months, 95% CI 10.9-NA for CMR versus 8.5 months, 95% CI 7.1-12.6 for non-CMR, log-rank P=0.0003) (**Figure 1D**).

When comparing early (week 2) with standard (week 7) PERCIST response classification, 51 (76.1%) patients had an unchanged PERCIST classification, see **Table 2**. In twelve

(17.9%) patients ongoing response resulted in an improved PERCIST classification at week 7 and in 4 (6.0%) patients' response had worsened.

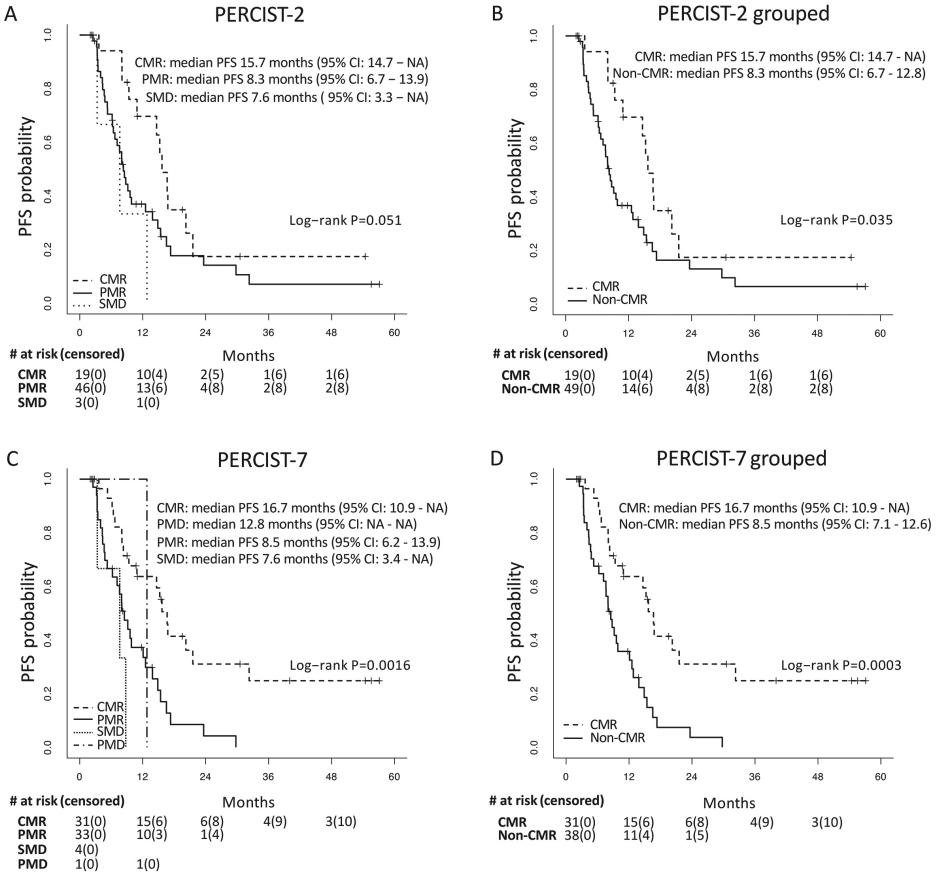

Figure 1. *Kaplan-Meier progression-free survival curves by PERCIST week 2 (A, B) and week 7 (C, D) scans. CMR=complete metabolic response; PMR=partial metabolic response; SMD=stable metabolic disease; PMD=progressive metabolic disease; PFS=progression-free survival; CI=confidence interval; NA=not avalilable.*

Table 2. Change of PERCIST from week 2 to week 7.

	PERCIST week 7				
Variable	CMR (N=31)	PMR (N=33)	SMD (N=4)	PMD (N=1)	Missing (N=1)
PERCIST week 2					
CMR (N=19)	19 (100.0%)	0 (0%)	0 (0%)	0 (0%)	0
PMR (N= 46)	11 (24.4%)	31 (68.9%)	3 (6.7%)	0 (0%)	1
SMD (N=3)	0 (0%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0
Missing (N=2)	1	1	0	0	0

CMR=complete metabolic response; PMR=partial metabolic response; SMD=stable metabolic disease; PMD=progressive metabolic disease.

Table 3. Cox regression analysis results for PFS from week 2 and week 7.

Variable	No. patients	Univariable analysis			Multivariable analysis		
		HR	95% CI	P	HR	95% CI	P
PERCIST 2							
CMR	19	1.0 (ref.)			1.0 (ref.)		
Non-CMR	46	1.99	1.03-3.84	0.040*	1.77	0.91-3.43	0.0935
LDH							
≤ULN	34	1.0 (ref.)			1.0 (ref.)		
>ULN	31	2.69	1.48-4.89	0.0012*	2.50	1.37-4.56	0.0029*
ECOG PS							
0	34	1.0 (ref.)					
1	31	0.98	0.56-1.73	0.9558	-	-	-
No. organs							
<3	40	1.0 (ref.)					
≥3	25	1.67	0.93-3.01	0.086	-	-	-
PERCIST 7							
CMR	31	1.0 (ref.)			1.0 (ref.)		
Non-CMR	35	2.94	1.60-5.40	0.0005*	2.65	1.43-4.91	0.0020*
LDH							
>ULN	35	1.0 (ref.)			1.0 (ref.)		
≤ULN	31	2.08	1.17-3.68	0.012*	1.74	0.97-3.11	0.0613
ECOG PS							
0	36	1.0 (ref.)					
1	30	1.02	0.58-1.80	0.58	-	-	-
No. organs							
<3	51	1.0 (ref.)					
≥3	15	1.33	0.69-2.56	0.40	-	-	-

CMR=complete metabolic response; LDH=lactate dehydrogenase; ULN=upper limit of normal; ECOG PS= Eastern Cooperative Oncology Group performance status; HR=hazard ratio; CI=confidence interval; *= p<0.05. Five out of the initial 70 patients excluded for 2-week analysis due to missing 2-week PERCIST measurement, LDH or number of organs. Four out of the initial 70 patients excluded for 7-week analysis due to missing 7-week PERCIST measurement, LDH or number of organs.

Multivariable analysis for PFS

In the univariable analysis the number of metastatic sites at baseline and ECOG were not predictive for PFS and therefore not considered for the multivariable analysis. At week 2, the hazard ratio of progressive disease/death was significantly higher for patients PERCIST classified as non-CMR than CMR (HR=1.99, 95% CI 1.03-3.84, P=0.040) and for elevated LDH than normal LDH (HR=2.69, 95% CI 1.48-4.89, P=0.0012) in an univariable analysis, see **Table 3**. When adjusting for baseline LDH, the hazard ratio for PERCIST was no longer significant (HR=1.77, 95% CI 0.91-3.43, P=0.0935). At week 7, the hazard of progressive disease/death for PERCIST non-CMR versus CMR was 2.94 (95% CI 1.60-5.40, P=0.0005). When adjusting for baseline LDH, PFS remained significantly worse for non-CMR versus CMR (HR=2.65, 95% CI 1.43-4.91, P=0.0020).

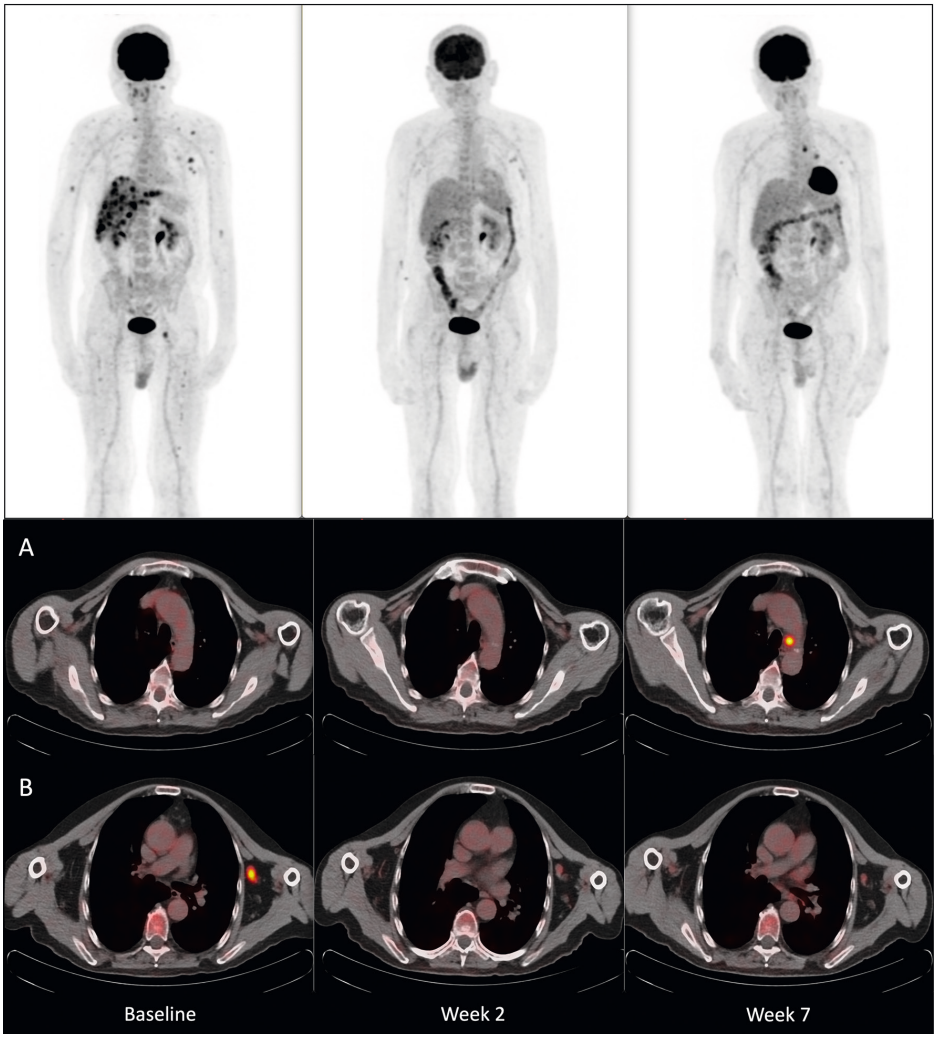

Figure 2. *Maximum Intensity Projection and transaxial [^{18}F]FDG PET/CT at baseline (left), week 2 (middle) and week 7 (right). PET/CT at baseline shows multiple [^{18}F]FDG-avid metastases in lymph nodes (left axilla), liver and bone (right humerus, pelvis, left and right femur). All metastases show decreased [^{18}F]FDG uptake corresponding with response to treatment 2 weeks after initiation. At week 7, new [^{18}F]FDG-avid lymph nodes are shown subaortic (A) and left hilus, while other sites remain in complete metabolic remission (B).*

PET-based dissemination patterns at progression

At week 2, no progression was detected in the whole study population. At week 7, four patients (6%) revealed new [^{18}F]FDG-avid lesions, but in a multidisciplinary tumor board it was decided to neglect these findings, based on the complete metabolic response of all other known lesions and the clinical assessments of the patients, which were favorable of a continuous response. Patients' follow-up with imaging (ceCT) confirmed that the 'new [^{18}F]FDG-avid lesions' detected at week 7 were false-positive lesions.

These four patients remained on BRAF/MEKi therapy and had a PFS of 5 months, 17 months, 3 years, and >4.5 years. **Figure 2** provides an example of one such case (**Supplementary Table S2** provides detailed reporting on all four patients).

At the time of clinically and/or RECIST confirmed progression, [18F]FDG PET/CT scans were made in 22 patients, of which 18 patients showed progression according to PERCIST criteria with increased uptake in baseline [18F]FDG-avid lesions only. In 6/18 patients [18F]FDG PET/CT also showed new [18F]FDG-avid lesions. In the remaining 4/22 patients MRI was performed on clinical indication and revealed brain metastases, while on [18F]FDG PET/CT all known lesions remained in CMR. No patients had progression based solely on new lesions outside the brain.

DISCUSSION

In this study, we showed that CMR according to PERCIST response evaluation on [18F]FDG PET/CT 7 weeks after initiation of combined vemurafenib plus cobimetinib is a valuable predictive biomarker for PFS in patients with unresectable stage IIIc or metastatic stage IV BRAF-mutated melanoma. At both week 2 and week 7, a longer median PFS was seen in patients with CMR compared to patients with non-CMR (median 8.5 vs. 16.7 months), though when adjusting for LDH in a multivariable analysis results remained significant only at week 7, albeit with a relatively strong hazard ratio in the analyses for week 2. RECIST1.1 response assessment could not predict PFS.

Dissemination patterns

The lesion-based evaluation of the 22 patients with proven progression revealed that progression of known metastases is the most prominent pattern of progression, and that no patients presented with solely new lesions (outside the brain). All false-positive new lesions in our study were identified as [18F]FDG-avid lymph nodes. As with other ICI's, these false-positive nodes can be regarded as reactive lymph nodes in the lymphatic drainage basin of metastatic sites caused by stimulation of the immune response (28, 29). Indeed, Wilmott et al. demonstrated an increase of tumor infiltrating lymphocytes already 7 days after commencement of BRAF inhibitors. On [18F]FDG PET/CT, these reactive lymph nodes may show increased [18F]FDG uptake and are seen generally in the first weeks after treatment initiation with BRAF/MEKi (30). So, our results show that new [18F]FDG-avid lesions (especially lymph nodes) during BRAF/MEKi are not automatically a sign of disease progression, and that images should always be evaluated in their clinical context.

Patient stratification with [18F]FDG PET/CT

Previous studies have investigated the use of [18F]FDG PET/CT for pre-therapeutic prognostic stratification or for monitoring metabolic response in patients with melanoma treated with targeted therapy (9-13). McArthur et al. were the first to report

on a dose escalation study in BRAFi-naive patients with advanced BRAFV600-mutated melanoma treated with vemurafenib alone, that greater reductions in [^{18}F]FDG uptake at day 15 tended to have a longer PFS (9). The subsequent Phase IB trial (BRIM7), in which vemurafenib was combined with cobimetinib, again showed that patients with an CMR on PET/CT early in their first cycle had a longer PFS (10).

Other groups have also evaluated the role of [^{18}F]FDG PET/CT to better identify patients who will have a durable response to targeted therapy. Annovazzi et al. (n=57) showed a prolonged PFS in patients achieving a CMR on [^{18}F]FDG PET/CT at 2-6 months from the start of treatment compared to those with PMR (median PFS 42.9 vs 8.8 months, P=0.009) (13). The patients analyzed received a BRAFi as a single agent or combined with a MEKi. Carlino et al. (n=23) used [^{18}F]FDG PET/CT at baseline and 2 weeks after start of dabrafenib to investigate whether response heterogeneity predicts clinical outcome (11). A heterogeneous response was defined as partial or complete metabolic response of target lesions in the presence of new or metabolically progressive lesion(s). The presence of response heterogeneity (n=6) was correlated with a shorter median time to progression (TTP) of 3.0 months (95% CI 0.6-5.4) compared to patients with a CMR or PMR of >90% lesions (median TTP 7.4 months, 95% CI 6.5-8.3, P=0.032). Finally, Schmitt et al. (*n*=24) investigated in a retrospective evaluation the prognostic impact of [^{18}F]FDG PET/CT performed at baseline and after 3 weeks of dabrafenib plus trametinib or vemurafenib plus combimetinib (12). The authors found that for the least responsive tumor (i.e., lesion with lowest difference in SUVmax), the change in SUVmax was associated with PFS (HR=1.34, 95% CI 1.06-1.71, P=0.01).

When comparing our study with literature, we prospectively included the largest population in which all patients were treated with the same schedule of treatment enclosing a BRAF inhibitor (vemurafenib) combined with a MEK inhibitor (cobimetinib) and in which [^{18}F]FDG PET/CT was performed at consistent time points. Regarding other studies, these studies vary in study design with different treatment strategies and timing of imaging at follow-up. Consequently, it remains difficult to compare these studies in this respect.

When combining the results of our study with literature, it seems that [^{18}F]FDG PET/CT response assessment at 7-8 weeks after BRAF/MEKi is indeed valuable. Patients with a CMR at 7-8 weeks after treatment initiation are far more likely to have a durable response, thus suggesting effective inhibition of the ERK-pathway. Though the results of our study could not predict resistance in individual patients, we revealed that awareness of early development of resistance is warranted in patients with a non-CMR early or 7 weeks after treatment commencement. Also, no patients at week 2 and only one patient at week 7 revealed PMD. This finding is highly relevant, since in some patients BRAF/MEKi is given as bridging treatment (often for 8 weeks) to reduce tumor load before switching to ICI. Thus, short-term induction treatment with BRAF/MEKi is safe for achieving a quick and effective response in this setting.

Methodology of PET-based response assessment

In all aforementioned studies, metabolic response was assessed using the European Organisation for Research and Treatment of Cancer (EORTC) criteria for PET response, which have been widely used in clinical practice (31). In our study we used the more recently introduced PERCIST response assessment. The difference in these methodologies lies in the identification of the single target lesion on baseline and follow-up; EORTC measures consistently the same lesion, whereas PERCIST measures the hottest lesions in each scan. Though, studies in other tumor types have shown that response assessment by EORTC criteria and PERCIST may lead to similar response classifications, this has to be elucidated for targeted therapy in advanced melanoma (32).

For PET/CT response assessment in patients treated with ICI's, several different response criteria have been proposed, such as PET Response Evaluation Criteria for Immunotherapy (PERCIMT) (33), immunotherapy-modified PERCIST (imPERCIST) (19), and immune PERCIST (iPERCIST) (34). Though these criteria seem better than PERCIST to assess response in these patients, an optimal evaluation method for patients treated with targeted therapy has yet to be established.

Early response assessment

Besides patient stratification early after the initiation of therapy, [18F]FDG PET/CT at week 2 in addition to the more common evaluation at week 7 could have the additional value to early identify those patients with an increase in glucose metabolism as a sign of (early) resistance. Indeed, metabolic decline in [18F]FDG uptake shortly after the initiation of targeted therapy can already be impressive as was reported by McArthur et al., who revealed a decrease in SUVmax of 58-94% at week 2 (10). Thus, with only a decline of 30% needed to be classified as PERCIST PMR, this group of patients can be heterogeneous with a wide variety of decline in SUV. Early [18F]FDG PET/CT at week 2 can unveil patients with metastases that show already an increase in SUVmax at week 7 compared to week 2, but remain classified as PMR compared to baseline.

Therefore, the additional value of early [18F]FDG PET/CT is still not fully clarified. Further research should focus on identifying patients with reactivation of glucose metabolism in metastases at week 7, after initial response at week 2.

Study limitations

This study has several limitations. First, the number of patients included in our study is relatively small and, as a result, evaluation with PERCIST of separate response groups is difficult. To overcome this problem, we combined response groups by comparing CMR to non-CMR. Though ideally larger cohorts are needed to better evaluate the prognostic value of PERCIST response assessment, this study was designed as a multicenter trial and to our knowledge is the largest study in which [18F]FDG PET/CT is prospectively studied in patients treated with BRAF/MEKi until progression. In the changed treatment

landscape in which immunotherapy has become the preferred first-line treatment in these patients, it is unlikely to investigate the value of PET/CT in a larger cohort with BRAF/MEKi as first-line treatment. Second, we chose timing of response imaging early at week 2 and after two treatment cycles at week 7. Though like our results, also in literature (early) response assessment with [^{18}F]FDG PET/CT seems predictive for PFS, the timing of response imaging is currently not consequently done at set intervals and varies from 13 days to 6 months. We suggest that a time point of 7 weeks is adequate for early response prediction, and that future studies could incorporate this time point for prediction of PFS with [^{18}F]FDG PET/CT.

Finally, we did not perform a follow-up PET/CT to confirm or exclude PMD in our patients with new lesions at week 7. Instead, we instantly defined these new lesions as true or false-positive and used regular follow-up with ceCT for evaluation. Though it might have been more suitable to perform follow-up with PET/CT, we believe that it's also important to provide appropriate care in a patient-orientated, effective manner and avoid unnecessary costs.

CONCLUSION

In conclusion, our prospective multi-center study revealed that disease progression on PET/CT is predominated by progression of known metastases and new [^{18}F]FDG-avid lesions during BRAF/MEKi are not automatically a sign of recurrent disease. Though patients with CMR on [^{18}F]FDG PET/CT at week 2 have a longer PFS than patients with non-CMR, different PET parameters should be investigated to further evaluate the added value of early [^{18}F]FDG PET/CT after the initiation of therapy. Regardless of LDH, PERCIST response assessment at week 7 is predictive for PFS and could be considered as marker for early response prediction in future studies.

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SUPPLEMENTARY

Supplemental 1 - Adverse Events

Toxicity on BRAF/MEKi was observed in all 70 patients; the highest grade of adverse events was grade 1 for 27% of patients, grade 2 for 27%, grade 3 for 40% and grade 4 for 3% of patients. Two patients died for reasons related to treatment (grade 5 toxicity). The most frequent toxicities were rash (60%), fatigue (51%), diarrhea (36%), photosensitivity reaction (34%) and malaise (29%), see **Table 2**. In 18 patients, treatment was stopped prematurely due to adverse events. Of those, 7 patients continued a different BRAF/MEKi.

Supplemental 2 – Cases of false positive 'new [18]FDG-avid lesions'

The first patient had a primary melanoma on the abdomen in history and was diagnosed with mediastinal and left axillary lymph node and multiple liver and bone metastases during follow-up when entering the study (see Figure 2, #321). At week 7, new [18F]FDG-avid lymph nodes were found in the left axilla and mediastinum, while all known metastases remained in CMR. Progression could not be confirmed on follow-up ceCT imaging every four months thereafter and the patient was still in remission at the last date of follow-up 4.5 years later.

The second patient (#339) had predominantly bone metastases of an unknown primary melanoma when entering the study. New [18F]FDG-avid lymph nodes were detected in the neck at week 7, which could not be confirmed on follow-up imaging and the patient remained in remission of known metastases for 17 months.

A third patient (#342) had resection of a melanoma of the chest and an axillary lymph node metastasis in history and entered the study with metastases in lymph nodes, lung, liver, muscle and peritoneum. [18F]FDG PET/CT at week 7 revealed new mediastinal [18F]FDG-avid lymph nodes and CMR of the former [18F]FDG-avid metastases. Five months later the patient had progressive disease due to brain metastases, while the new [18F]FDG-avid lymph nodes at week 7 could not be confirmed at follow-up and other known visceral metastases remained in remission.

The fourth patient (#118) with a primary melanoma on the back in history and metastases in subcutaneous tissue, lymph nodes, liver, spleen and bone, developed a new [18F]FDG-avid subcutaneous nodule of the upper back, which was presumed to be a lymph node or subcutaneous metastasis. No other [18F]FDG-avid lesions were found, and the subcutaneous nodule could not be confirmed. Three years later the patient received radiotherapy on a melanoma metastasis in the spine as a first sign of progression.

Table S1. Worst grade treatment-related adverse events.

	N=70 patients	
	Frequency (%)	
Most common adverse events (occurring in ≥10% of all grades)	All Grades	Grade ≥3
Rash*	42 (60%)	10 (14%)
Fatigue	36 (51%)	3 (4%)
Diarrhea	25 (36%)	6 (9%)
Photosensitivity reaction	24 (34%)	-
Malaise	20 (29%)	2 (3%)
Edema	16 (23%)	1 (1%)
Eye symptoms#	16 (23%)	1 (1%)
Nausea	16 (23%)	-
Decreased appetite/ weight loss	15 (21%)	-
Fever	15 (21%)	2 (3%)
Arthralgia	14 (20%)	-
Liver function test abnormality^	10 (14%)	3 (4%)
Skin disorder other than rash or tumor$	10 (14%)	2 (3%)
Vomiting	10 (14%)	1 (1%)
Alopecia	7 (10%)	-
Other Adverse Events with known association with BRAF or MEK inhibition		
Keratoacanthoma	4 (6%)	-
Decreased ejection fraction	3 (4%)	1 (1%)
Squamous cell carcinoma / atypical squamous proliferation	2 (3%)	1 (1%)
Prolonged QT interval	1 (1%)	-

*Combined terms: includes (in decreasing order of reported incidence) the preferred terms rash ((non)-acneiform-, maculo-, papular, maculopapular-), pruritus, erythema, erythema multiforme, erythema nodosum, dermatitis, erythroderma, folliculitis.
#Combined terms: includes (in decreasing order of reported incidence) the preferred terms vision symptoms, uveitis, retinopathy, eye pain, occlusion vena retinae, blepharitis, dry eyes, red eyes, watering eyes.
^Combined terms: includes (in decreasing order of reported incidence) the preferred terms bilirubin concentration increase, aspartate aminotransferase concentration increase, alkalin aminotransferase concentration increase, Gamma glutamyltransferase increase concentration.
$Combined terms: includes (in decreasing order of reported incidence) the preferred terms skin and disorders, skin infection, skin pain, skin ulceration, skin urticaria, eczema hand, dry skin.