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Metabolic parameters on baseline and early [^{18}F]FDG PET/CT as a predictive biomarker for resistance to BRAF/MEK inhibition in advanced cutaneous BRAFV600-mutated melanoma

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Abstract

Background [^{18}F]FDG PET/CT plays a crucial role in evaluating cancer patients and assessing treatment response, including in BRAF-mutated melanoma. Metabolic tumor volume (MTV) and total lesion glycolysis (TLG) have emerged as promising alternatives to standardized uptake value (SUV)-based measures for tumor assessment. This study evaluates the predictive value of SUVpeak, MTV, and TLG in predicting progression-free survival (PFS) in advanced BRAF-mutated melanoma treated with BRAF/MEK inhibitors.

Results Seventy-five patients with metastatic melanoma were enrolled in a multi-center trial and treated with vemurafenib/cobimetinib. [^{18}F]FDG-PET/CT scans were performed at baseline, week-2, and week-7. Imaging analysis included SUVpeak, MTV, and TLG of summed metastases, as well as percentage changes over time (Δ). Baseline median PET-parameters were SUVpeak 12.59 (range 3.13–50.59), MTV 159mL (range 0-1897 mL), and TLG 1013 (range 1-13162). Baseline MTV was the strongest predictor ($AUC_{T=6\ months}$=0.714), while early changes in MTV, TLG, and especially week-7 ΔSUVpeak% showed similar or improved performance (P=0.017 vs. baseline SUVpeak). Patients with TLG below the median had significantly prolonged PFS (15.4 vs. 8.5 months, P=0.024). MTV above optimal cutoff (45.3 mL) was associated with an increased risk of progression/death, even after adjusting for LDH, ECOG status, and metastatic sites (HR=2.97, 95% CI 1.17–7.52, P=0.022). At week-2, ΔSUVpeak% was not predictive in a multivariable analysis, but became predictive at week-7 (median ΔSUVpeak%: 64), with a more than three-fold hazard of progression for patients with ΔSUVpeak% below 64% (P=0.0014); PFS was 5.0 months (95% CI: 4.3-NA) for patients below the median versus 14.7 months (95% CI: 9.2–20.2) for those above or with non-quantifiable scans (P=0.0002).

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Median ΔMTV was 95.5% at week-2 and 97.6% at week-7, with significant PFS differences at both time points (week-2: $P = 0.020$, week-7: $P < 0.001$). As expected, TLG mirrored MTV. Patients with MTV increases at week-7 after an initial response at week-2 had a median PFS of 5.3 vs. 12.6 months for those with stable or declining MTV ($P = 0.0023$). Intra-patient metabolic heterogeneity was also associated with outcome, with early reductions in SUVpeak variation between lesions correlating with better PFS.

Conclusion This study supports the use of MTV and TLG as robust predictive markers for PFS in advanced melanoma treated with BRAF/MEK-inhibitors. Monitoring early PET parameters changes can provide valuable insights into therapeutic response and disease progression.

Trial registration Clinicaltrials.gov identifier: NCT02414750. Registered 10 April 2015, retrospectively registered.

Keywords Melanoma, BRAF mutation, Progression-free survival, Positron emission tomography, Metabolic tumor volume, Total lesion glycolysis, Standardized uptake value, Targeted therapy

Background

Melanoma is a major health concern globally due to its aggressive nature and high mortality rate [1]. Fortunately, with the introduction of immune checkpoint inhibitors (ICI) and targeted therapy, the treatment landscape for melanoma patients has revolutionized dramatically over the past decade [2]. Targeted therapy with combined BRAF/MEK inhibitors (BRAF/MEKi) has emerged as effective treatment option for patients harboring a V600E/K mutation in the BRAF gene. This mutation, found in 40–60% of melanomas, activates the Mitogen-activated protein kinase (MAPK) signaling pathway, driving uncontrolled cell growth and survival [3–5]. Clinical trials have shown that BRAF/MEKi offers substantial clinical benefit, with overall response rates (ORR) of approximately 60–70% and median progression-free survival (PFS) of 11–12 months in patients with V600E/K BRAF-mutated melanoma, reinforcing its role in treatment of melanoma [6–10]. Nevertheless, although most patients initially respond to BRAF/MEKi treatment, resistance typically develops within 12–15 months, often due to reactivation of the MAPK pathway or activation of alternative survival pathways [11, 12]. Prediction or early detection of this acquired resistance would provide valuable insights for clinicians to make informed decisions regarding treatment modifications– such as switching to immune checkpoint inhibitors– to optimize patient outcomes.

18F-Fluorodeoxyglucose Positron Emission Tomography / Computed Tomography ([^{18}F]FDG PET/CT) plays a crucial role in the evaluation of cancer patients and has gained considerable attention as a valuable tool for assessing treatment response in various malignancies, including BRAF-mutated melanoma [13–15]. While the predictive value of [^{18}F]FDG PET/CT has been extensively studied in the context of immune checkpoint inhibitor (ICI) therapy [16–26], comparatively fewer studies have investigated this in patients treated with BRAF/MEKi [15, 27, 28]. In PET imaging, the Standardized Uptake Value (SUV) is the primary quantitative measure to assess relative uptake of [^{18}F]FDG in tumors, reflecting their metabolic activity. However, SUV measurements can be influenced by various factors, including patient body weight, blood glucose levels, and the timing between FDG injection and image acquisition [29]. The most common implementations involve SUVmax and SUVpeak, where only the most active voxels of tumor masses are evaluated. These variations and limitations in SUV measurements can undermine their reliability in accurately evaluating treatment response and predicting patient outcomes.

To overcome the limitations of SUV-based measures, metabolic tumor volume (MTV) and total lesion glycolysis (TLG) analysis have emerged as promising alternatives for a more comprehensive tumor assessment of several cancers [30–32]. MTV encompasses the total volume of metabolically active tumor tissue, typically defined by a SUV-threshold. By considering the entire tumor burden rather than focusing on specific tumor areas, MTV provides a holistic perspective on the malignancy's extent and biological behavior. In addition, MTV is less influenced by patient-related and technical factors, making it a more robust parameter for quantitative analysis in clinical practice. Emerging evidence suggests that MTV may serve as a predictive marker in various malignancies, including patients treated with BRAF/MEKi [27, 28]. This predictive capability could enhance risk stratification, treatment planning, and monitoring therapeutic responses.

While MTV only represents the volume of metabolically active tumor tissue, TLG integrates the metabolic tumor volume with its actual metabolic activity (as represented by SUV). It is calculated by multiplying the MTV by the mean SUV within the tumor volume, offering a metric that considers both the volume and the metabolic intensity of the tumors. However, the value of TLG in addition to MTV for predicting treatment response in metastatic melanoma is unknown.

The aim of this study is to assess the predictive value of different metabolic PET parameters on [^{18}F]FDG-PET/

CT at baseline, and during treatment at week-2 and week-7, and determine whether these parameters can serve as indicators for PFS in patients with advanced melanoma undergoing BRAF/MEK-targeted therapy.

Methods

Patients

Seventy-five patients diagnosed with histologically proven advanced or metastatic BRAF-mutated melanoma were enrolled in the REPOSIT-trial (NCT02414750) from March 2015 to February 2019. The study design has been published previously [33]. Briefly, this phase II, multi-center, single arm prospective study included BRAF-mutated unresectable stage IIIC or stage IV American Joint Committee on Cancer Classification (AJCC) 7th edition [34] melanoma patients with measurable lesions according to Response Evaluation Criteria In Solid Tumours version 1.1 (RECIST1.1) [35]. Patients were treated with combined BRAF/MEK inhibitor vemurafenib plus cobimetinib 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. Written informed consent was obtained before inclusion. The study closed enrollment before reaching the anticipated sample size of 90 patients as outlined in the study protocol, due to slow patient accrual. Ultimately, a total of 75 patients were included. Comprehensive data such as patient demographics, clinical, histopathological, imaging, and laboratory data were collected.

Imaging protocol

Patients underwent baseline [^{18}F]FDG PET/CT within one month prior to the initiation of therapy and follow-up [^{18}F]FDG PET/CT on day 15 of Cycle 1 (week-2) and day 21 of Cycle 2 (week-7). Contrast-enhanced CT scans were also performed in accordance with protocol, every eight weeks, and whenever progressive disease was suspected.

PET/CT scans were performed in accordance with the European Association of Nuclear Medicine (EANM) guideline for oncology [18F]FDG PET/CT imaging [29, 36]. Prior to PET/CT scan, patients fasted for at least 4 h and had more than 400 ml fluid intake. Serum glucose levels were below 11.0 mmol/L. [^{18}F]FDG was administered intravenously, with an activity dosage in agreement to the local institutional protocol, ensuring compliance with the EANM Research Ltd. (EARL) standard 1 [37]. Approximately 60 min (range 55–65 min) after administration, PET/CT images were acquired from at least base of the skull to thighs at 2–4 min per bed position in a supine position. A whole-body low-dose CT scan was also conducted for attenuation correction and anatomic localization.

PET/CT scans were performed on a Gemini TF PET/CT, TF Big Bore PET/CT, Ingenuity TF PET/CT (all Philips Medical Systems, Best, the Netherlands), or Siemens Biograph mCT PET/CT (Siemens Healtineers, Erlangen, Germany). For consistency, all PET/CT scans for an individual patient were performed on the same scanner, with a maximum variation of 10% in activity compared to baseline. All participating PET imaging centers were accredited for EARL standard 1 and performed image reconstruction accordingly (EANM resEARch4Life, https://earl.eanm.org/) [37]. This accreditation ensures harmonization of semi-quantitative PET metrics across different scanners, thereby minimizing inter-scanner variability. As a result, no additional normalization or statistical adjustment for scanner type was performed.

Image analysis and response evaluation

The PET/CT scans were sent for central review, where objective evaluation was performed by an experienced nuclear medicine physician (BvdH). Areas of increased uptake were identified for further quantification. In cases of uncertainty, a second experienced nuclear medicine physician (MS) reviewed the data to reach a consensus.

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

Quality control review of all PET/CT scans was conducted to assess their suitability for analysis [38]. Scans were included when SUVmean of the liver fell within the range of 1.3-3.0, measured by placing a spherical VOI with a 3 cm diameter in the right upper lobe of the liver, avoiding malignancies and organ boundaries [39]. In case of extensive liver metastases, the SUVmean blood pool was required to fall within 0.79–2.32, measured by placing several VOIs in the thoracic aorta, ensuring the vessel wall was excluded [39–41]. Scans with SUVmean outside these normal ranges underwent further evaluation of protocol adherence to identify potential errors, and each scan was individually assessed for in- or exclusion.

Baseline and on-treatment analysis of total tumor burden SUVpeak, MTV and TLG

Since at the initiation of the REPOSIT trial there was limited evidence-based data on assessing therapy response and resistance using [^{18}F]FDG PET/CT in patients with unresectable stage IIIc or metastatic melanoma treated with BRAF/MEK inhibitors, the PET imaging analyses were intentionally not pre-specified. Instead, we conducted our analyses in alignment with the most current literature available at the time of the study. For quantification of the PET images, a validated in-house developed software package (ACCURATE) was used [42]. With the total tumor burden tool (TTB) in ACCURATE,

PET-images were automatically delineated using the PET image-based segmentation method SUV40, resulting in a region of interest (ROI) of the summed lesions with a fixed SUV threshold of 4.0 and a volume of > 1mL, see Fig. 1 [43]. The resulting ROI delineation was inspected visually and manually corrected if necessary. SUVpeak, defined as a 1-mL spherical volume of interest with the highest uptake, MTV and TLG were calculated.

On follow-up scans, patients with tumor responses resulting in no measurable metastases (i.e. a complete metabolic response, or with remaining metastatic lesions with a SUV below the threshold of 4.0 and a volume of less than 1mL) were categorized as 'not quantifiable'. For the remaining quantifiable [^{18}F]FDG PET/CT scans, parameters were calculated as the percentage difference compared to baseline using the formula: Δ%=100*(baseline value– week 2 value)/baseline value. Similarly to baseline values, cutoffs were established, and percentage differences were categorized into two groups (above and below cutoff), with the 'not quantifiable' category kept separate. In a separate analysis, patients with a 'not quantifiable' response PET were combined with those showing a Δ% above median (indicating good responders). This combined group (labelled; GroupedSUVpeak, GroupedMTV or GroupedTLG) was compared to the patient group with a Δ% below the median (indicating lesser responders).

Intra-patient heterogeneity analysis

To evaluate intra-patient metabolic heterogeneity, we analyzed the variation in SUVpeak across individual lesions within each patient. All baseline lesions with an SUVmax ≥ 4.0 were included, as this threshold ensures sufficient uptake for the best possible follow-up and assessment of treatment response. For each of these lesions, SUVpeak was measured at baseline, week-2, and week-7. Lesions that were indistinguishable from surrounding tissue at follow-up (i.e., complete metabolic response) were excluded from further analysis.

Statistical analysis

For summarizing patient characteristics, median and range for continuous variables, and frequency and percentage for categorical variables were displayed. PFS was chosen as the primary endpoint over overall survival (OS) due to the relatively small sample size and limited follow-up duration, resulting in a low number of OS events, making PFS a more immediate and robust measure of treatment efficacy in this cohort. PFS was defined as the time from commencement of BRAF/MEKi to disease progression (based on clinical findings and/or

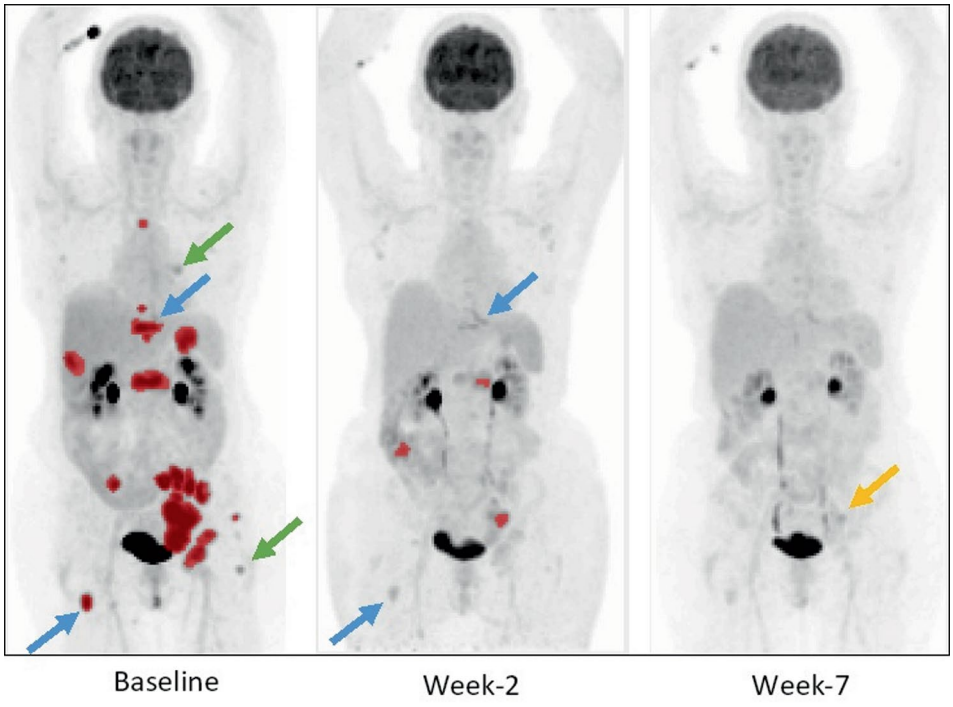

Fig. 1 Anterior Maximum Intensity Projections with semi-automatic delineation of target lesions. Maximum Intensity Projection [^{18}F]FDG PET of a patient at baseline, week-2 and week-7. Delineation of melanoma metastases with a SUV > 4 and volume > 1mL are shown in red. At baseline, some metastases were below the threshold for delineation with Accurate (green arrows; left hilar and left femur). Blue arrows demonstrate metastases that were delineated at baseline, but were below the threshold at week-2, though still visible. At week-7, minimal residual tumor was present (orange arrow), but no metastases could be delineated and the scan was therefore classified as not quantifiable

RECIST1.1) or death from any cause in the absence of progression, whichever occurred first. 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 of start of this new treatment.

A Sankey plot was generated to visualize patient-level response dynamics from week-2 to week-7, including only patients with quantifiable PET/CT scans and evaluable RECIST1.1 responses at both time points. Responses were categorized as Stable Disease (SD), Partial Response (PR), Complete Response (CR), or Progressive Disease (PD). The plot was constructed using networkD3 package in R software, with node sizes and flow widths proportional to patient transitions.

Associations between PET parameters (baseline SUVpeak, MTV, TLG; ΔSUVpeak%, ΔMTV%, ΔTLG%) and PFS were assessed by dichotomizing values using both median and optimal cutoff (derived via maximally selected log-rank statistics). Survival curves were generated using Kaplan-Meier analysis, and compared with log-rank tests. The method by Hothorn and Lausen [44] in the R package maxstat was used for approximating the p-value for the comparison between groups based on the optimal cutoff.

Univariable and multivariable Cox regression analyses were performed, hazards ratio (HR) and the corresponding 95% confidence interval (CI) were reported. The likelihood of pairs of nested models was compared using a likelihood ratio test. Multivariable analyses were adjusted for baseline lactate dehydrogenase (LDH) level, Eastern Cooperative Oncology Group (ECOG) performance status and number of metastatic organs at baseline, since these are the most common prognostic biomarkers for PFS and OS in melanoma patients treated with BRAF/MEKi [7, 45, 46].

To evaluate the discriminating potential of SUVpeak, MTV, TLG, and early changes (ΔSUVpeak, %ΔMTV%, ΔTLG%), time-dependent receiver operating characteristic (ROC) curve analyses were performed for survival data using the survivalROC package in R. Area under the curves (AUCs) at 6 months were calculated via the nearest neighbor estimator method of the bivariate distribution of the censoring time and the failure time with moderate smoothing (span = 5%) [47]. To assess whether early metabolic response provided additional predictive value over baseline parameters, AUCs of baseline PET parameters were compared with those of early changes (ΔSUVpeak%, ΔMTV%, ΔTLG%) at week-2 and week-7. This comparison was limited to patients with quantifiable scans at both time points.

A formal correlation matrix between MTV and TLG was not assessed, as TLG is mathematically derived from MTV (TLG = MTV × SUVmean), making the two metrics inherently dependent. Instead, each PET parameter was assessed separately, to determine the strongest predictor of PFS.

Intra-patient metabolic heterogeneity was analyzed using the coefficient of variation (CoV) of SUVpeak across all evaluable lesions per patient at baseline, week-2 and week-7. Relative change in heterogeneity (CoV%) over time was also assessed, and both were tested for association with PFS using Cox models.

Due to the explorative nature of this study and the limited sample size in relation to the number of tests performed, no adjustments for multiplicity were performed except for the adjustment of the *P*-value in the maximally selected log-rank statistics analysis. All *P*-values were 2-sided. 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).

Results

Patient characteristics

Sixty-nine out of 75 patients were included for [^{18}F]FDG PET/CT-analysis, including 36 males and 33 females, with a median age of 63 y (range, 30–88 y). A flow chart of in- and exclusion is presented in Fig. 2, detailed patient demographics are displayed in Table 1. All but 3 (4.3%) patients were diagnosed with stage IV disease, 49 (71.0%) patients had metastases in at least three different tissue types. The median follow-up time among all patients regardless of censoring status was 15.0 months (IQR 9.1 to 24.9 months). In 36 patients (53.7%) treatment ended due to progression and in 17 patients (25.4%) due to adverse events. The median PFS of all included patients was 9.6 months (IQR 8 to 14.9 months).

A total of 212 PET/CT scans were assessed for image quality and quantitative reliability using liver and/or mediastinal blood pool SUVmean, in accordance with EANM guidelines. Liver SUVmean was available in 185 scans across 64 patients, and blood pool SUVmean in 27 scans across 10 patients. In total, 29 scans fell outside the standard reference ranges (21 liver, 8 blood pool). Of these, 12 scans (all liver; including 5 baseline scans) were excluded due to significant deviations without acceptable justification. Seventeen scans with minimal deviations were retained. Additionally, 8 follow-up scans with normal liver SUVmean (5 at week-2, 3 at week-7) were excluded due to prior exclusion of the corresponding baseline scans. As a result, 192 scans from 69 patients were included in the final analysis (see Fig. 1).

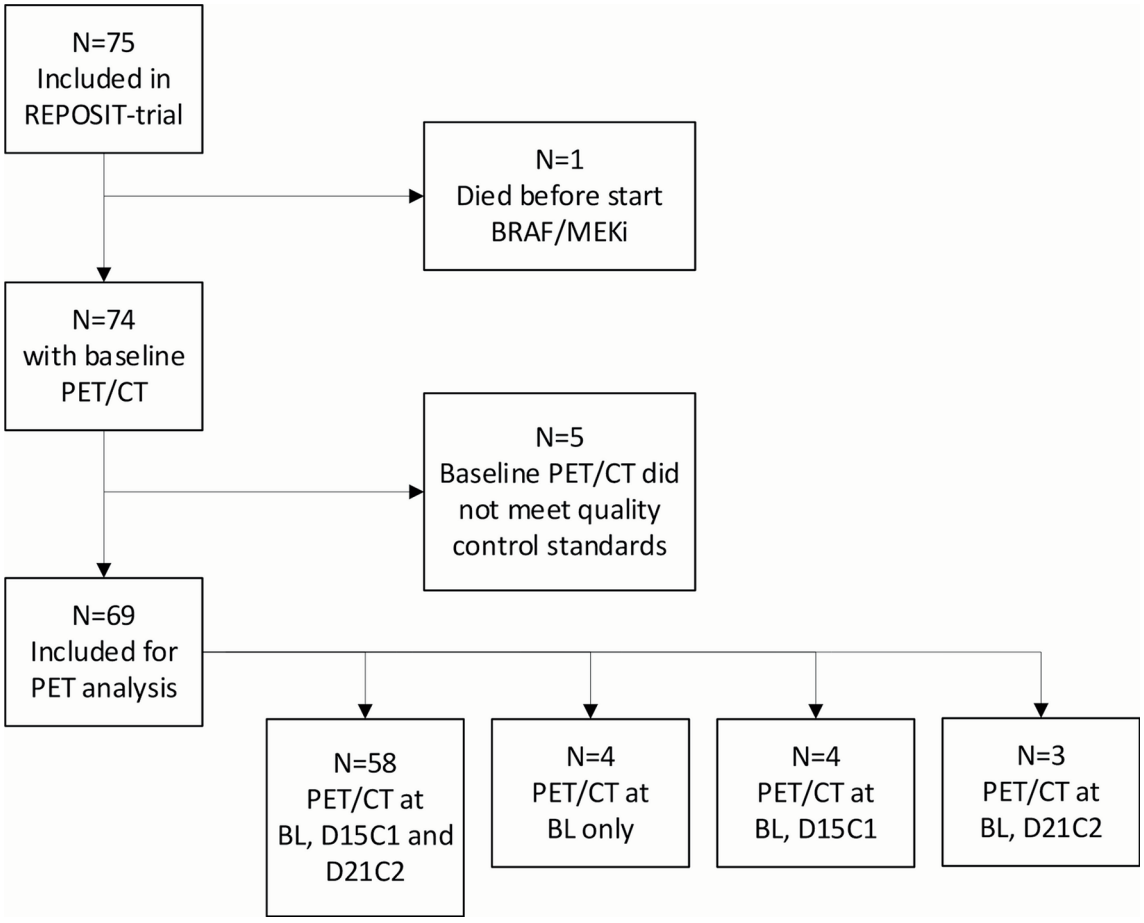

Fig. 2 Flow chart included patients for PET analysis. Flow chart patient inclusion. BL = baseline; D15C1 = Day 15 of Cycle 1; D21C2 = Day 21 of Cycle 2

Evaluation of baseline [^{18}F]FDG PET/CT

The predictive power of baseline [^{18}F]FDG PET/CT for progression

The distribution of baseline and on-treatment PET parameters is shown in Supplementary Fig. 1, where histograms are used to illustrate the spread of SUVpeak, MTV, and TLG values across the cohort.

At baseline, all patients presented with metastases that met the criteria of a SUV threshold > 4.0 and a volume > 1 mL, were included for automated delineation of ROIs to determine SUVpeak, MTV and TLG. Median SUVpeak was 12.59 (range 3.13–50.59); median MTV was 159mL (range 0-1897 mL) and median TLG was 1013 (range 1-13162). Time-dependent ROC curve analyses demonstrated that MTV had the best predictive performance for identifying patients at risk of progression at 6 months, as evidenced by its highest $AUC_{T=6\ months}$ =0.714 among the evaluated metrics. For TLG, $AUC_{T=6\ months}$ =0.685, while for SUVpeak $AUC_{T=6months}$ =0.598.

Baseline [^{18}F]FDG PET parameters using median and optimal cutoff values

Using median SUVpeak (12.6) as a cutoff, median PFS for patients with a SUVpeak below the median, was 14.7 months, versus 9.2 months for patients with a SUVpeak above the median (P = 0.064), see Fig. 3A. For patients with MTV > 159.2mL (median cutoff), the median PFS was 8.0 months, compared to 14.9 months for those with MTV ≤ 159mL, P = 0.094 (Fig. 3B). For TLG, the median PFS for patients with a TLG above the median of 1013.2 was significantly higher than for those with TLG below the median (8.5 vs. 15.4 months, P = 0.024), Fig. 3C, but only in univariable analyses. Results from univariable and multivariable Cox regression analysis for these baseline PET parameters are displayed in Table 2.

When using maximally selected log-rank statistics, no significant PFS differences were found between groups for SUVpeak. With the estimated best cutoff of 10.9, the median PFS for patients with SUVpeak ≤ 10.9 was 16.8 months vs. 8.8 months SUVpeak > 10.9, P = 0.32, as shown in Fig. 3D. For MTV, a significant difference in PFS was found with a cutoff of 45.3 mL: the median PFS for patients with MTV > 45.3mL was 8.5 months vs.

Table 1 Patient demographics

Characteristic	*N* = 69 patients
	Frequency (%)
Sex	
Male	36 (52.2%)
Female	33 (47.8%)
Age in years (median (range))	63 (30–88)
ECOG performance status	
0	39 (56.5%)
1	30 (43.5%)
AJCC 7th edition	
Locally advanced (Stage IIIc)	3 (4.3%)
Metastatic (Stage IV)	66 (95.7%)
Number of metastasis	
Median (Q1-Q3)	12 (6–34)
Min-max	1-128
Number of metastatic sites	
<3	20 (29.0%)
≥3	49 (71.0%)
LDH	
≤ ULN	34 (50.7%)
> ULN	33 (49.3%)
[^{18}F]FDG PET/CT scans	
Baseline	69 (100%)
Day 15 Cycle 1	62 (89.9%)
Day 21 Cycle 2	61 (88.4%)

ECOG = Eastern Cooperative Oncology Group; AJCC = American Joint Committee on Cancer; LDH = Lactate dehydrogenase; ULN = Upper limit of normal

21.6 months for those with MTV ≤ 45.3mL, $P = 0.021$, as shown in Fig. 3E. At this cutoff, the sensitivity and positive predictive value from the time-dependent ROC curve at 6 months were 1.00 and 0.81, respectively, whereas using the median cutoff of 159.2mL, sensitivity decreased to 0.64. Patients with MTV > 45.3 mL had a hazard of progression more than three times higher (HR = 3.53, 95% CI 1.50–8.36, $P = 0.021$), as detailed in Table 2. The prolonged PFS remained significant in the multivariable analysis adjusted for baseline LDH level, ECOG performance status and number of metastatic sites at baseline (HR = 2.97, 95% CI 1.17–7.52, $P = 0.022$). Best cutoff for TLG was estimated to be 268, but since the patient distribution above and below this threshold was the same to the MTV cutoff (14 patients below and 55 patients above), the results for TLG were consistent with those for MTV, Fig. 3F.

Evaluation of on-treatment [^{18}F]FDG PET/CT

During BRAF/MEKi treatment, [^{18}F]FDG PET/CT performed at week-2 revealed in 23 (37.1%) patients a SUV < 4.0 in all remaining metastases, preventing automated delineation of ROI. These scans were classified as 'not quantifiable' and were considered good responders. At week-7, the number of not quantifiable scans increased to 32 (52.5%). Figure 1 provides an example of a patient with a not quantifiable scan at week-7. A Sankey plot illustrating transitions in RECIST1.1-defined treatment responses between week-2 and week-7 is presented in Supplementary Fig. 2. Of the patients with evaluable data at both time points (n = 63), 36 remained in the same category (24 in PR, 12 in SD), while other patients showed conversion from PR to SD (n = 1), PR to CR (n = 1) and SD to PR (n = 25).

Predictive power of early metabolic changes

When evaluating the predictive power of early metabolic changes, the relative change in SUVpeak (ΔSUVpeak%), MTV (ΔMTV%) and TLG (ΔTLG%) at week-2 and week-7 were assessed in the subgroup of patients with quantifiable scans. At week-2, $AUC_{T=6months}$ was [0.638] for ΔSUVpeak%, [0.727] for ΔMTV% and [0.731] for ΔTLG%. At week-7, $AUC_{T=6months}$ was [0.722] for ΔSUVpeak%, [0.736] for ΔMTV% and [0.735] for ΔTLG%. No significant differences in AUC were found for week-2 and week-7 compared to baseline, except for ΔSUVpeak% ($P = 0.017$).

Changes in early and late [^{18}F]FDG PET parameters on treatment

For the optimal cutoff percentage difference determined with maximally selected log-rank statistics the outcome was similar compared to the median percentage difference for both MTV and TLG. Therefore, for on-treatment results, we focused on the median percentage difference (median Δ%) PET parameters. Kaplan-Meier curves for PFS with MTV and TLG grouped according to the optimal cutoff are summarized in Supplementary Fig. 3.

Percentage change from baseline SUVpeak

Kaplan-Meier curves for PFS, stratified by the percentage change in SUVpeak from baseline and a separate not quantifiable group, are presented in Fig. 4. The median percentage difference of SUVpeak at week-2 (median $\Delta SUVpeak\%_{week-2}$) was 61% (range: -5–100%), and at week-7 (median $\Delta SUVpeak\%_{week-7}$) it was 64% (range −53–100%). The not quantifiable group had the longest median PFS at both time-points (Fig. 4A and C). When combining the not quantifiable group with patients who had a ΔSUVpeak% above median, the PFS for this GroupedSUVpeak was significantly longer compared to ΔSUVpeak% below the median at week-7, but not at week-2 ($P = 0.0002$ versus $P = 0.056$, respectively), see Fig. 4B and D. These results were corroborated in multivariable analyses (see Table 3).

Using the not quantifiable group as reference for good response, patients with ΔSUVpeak% below the median had a worse PFS at both time-points ($\Delta SUVpeak\%_{week-2}$:

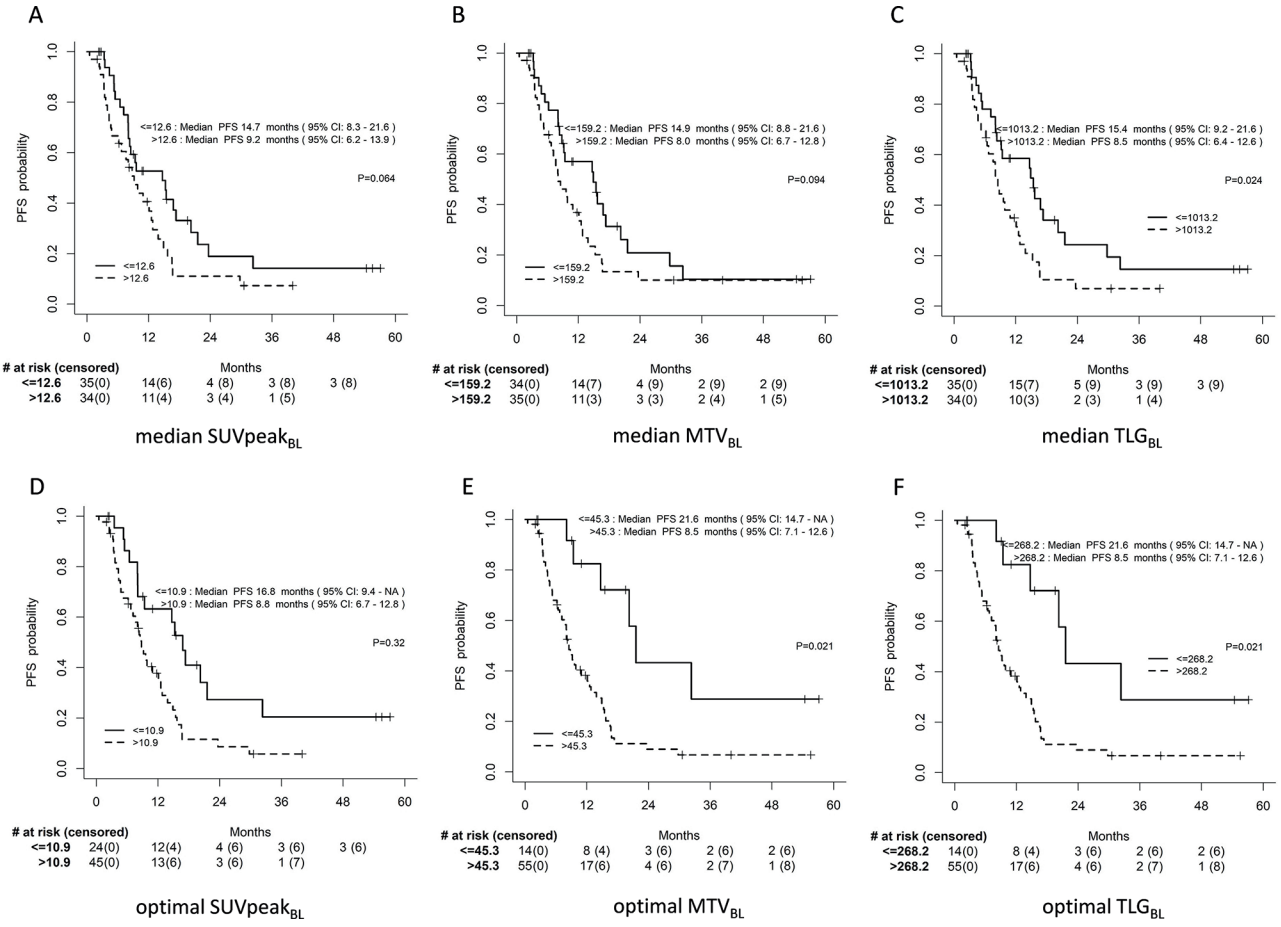

Fig. 3 Kaplan-Meier progression-free survival by SUVpeak, MTV and TLG at baseline. Kaplan-Meier progression-free survival curves by baseline median of SUVpeak (**A**), MTV (**B**) and TLG (**C**) and optimal cutoff of SUVpeak (**D**), MTV (**E**) and TLG (**F**). SUV = standard uptake value; MTV = metabolic tumor volume; TLG = total lesion glycolysis; BL = baseline

HR = 2.51, 95% CI 1.21–5.18, $P = 0.048$; ΔSUVpeak%$_{week-7}$: HR = 4.00, 95% CI 1.88–8.48, $P = 0.0003$), though only the latter remained significant in multivariable analyses. This was also true for week-7 when not quantifiable was grouped with patients having a ΔSUVpeak% above the median (GroupedSUVpeak), at detailed in Table 3. It should be noted that this model had a poorer fit for the data than when no grouping was done, according to likelihood ratio test results.

Percentage change from baseline metabolic tumor volume

At both week-2 ($\Delta\%_{week-2}$) and week-7 ($\Delta\%_{week-7}$), the groups specified by percentage change below and above median MTV were similar as those for TLG, resulting in the same outcomes for both PET-parameters. As a results, we focused on changes in MTV only. Figure 5 displays Kaplan-Meier curves for PFS, stratified by median percentage changes in MTV during treatment. See Supplementary Fig. 3 for Kaplan-Meier PFS by optimal cutoff for percentage change in MTV. Among the quantifiable scans, the median percentage change in MTV at week-2 (median ΔMTV%$_{week-2}$) was 96% (range 22–100%), and at week-7 (median ΔMTV%$_{week-7}$) this was 98% (range −41–100%). Significant differences in PFS were seen between the 3 groups at both week-2 and week-7 ($P = 0.020_{week-2}$; $P < 0.0001_{week-7}$), see Fig. 5A and C. When combining not quantifiable with patients having a ΔMTV% above median, the PFS for this combined group (GroupedMTV) was significantly longer compared to those with ΔMTV% below median (median 13.9 vs. 6.9 months at week-2, and 14.7 vs. 4.5 months at week-7), see Fig. 5B and D. See Supplementary Fig. 4 for the results of TLG.

With the not quantifiable group used as reference for good response, at both time points the hazard of a PFS event was significantly higher for patients with ΔMTV% below the median, but not for patients with ΔMTV% above the median; HR = 2.83 (95% CI 1.33–6.03, $P = 0.0070$) vs. HR = 1.65 (95% CI 0.79–3.43, $P = 0.1804$) at week-2, and HR = 4.7 (95% CI 2.19–10.07, $P = 0.0001$) vs. HR = 1.36 (95% CI 0.66–2.80, $P = 0.3988$) at week-7, see Table 3. Only results for week-7 remained significant

Table 2 Univariable and multivariable Cox regression results for progression-free survival SUVpeak, MTV and TLG at baseline

		Univariable			Multivariable		
	No. patients	**HR**	**95% CI**	***P*#**	**HR**	**95% CI**	***P*##**
SUVpeak using median							
≤ 12.6	35	1.0 (ref.)			1.0 (ref.)		
> 12.6	34	1.67	0.96–2.91	0.064	1.58	0.88–2.85	0.13
SUVpeak using optimal cutoff							
≤ 10.9	24	1.0 (ref.)			1.0 (ref.)		
> 10.9	45	2.20	1.19–4.05	0.32	1.89	0.97–3.66	0.0602
MTV using median							
≤ 159.2	34	1.0 (ref.)			1.0 (ref.)		
> 159.2	35	1.60	0.92–2.77	0.094	1.21	0.61–2.40	0.58
MTV using optimal cutoff							
≤ 45.3	14	1.0 (ref.)			1.0 (ref.)		
> 45.3	55	3.53	1.50–8.36	0.021*	2.97	1.17–7.52	0.022*
TLG using median							
≤ 1013.2	35	1.0 (ref.)			1.0 (ref.)		
> 1013.2	34	1.88	1.08–3.28	0.024*	1.42	0.72–2.80	0.31
TLG using optimal cutoff							
≤ 268.2	14	1.0 (ref.)			1.0 (ref.)		
> 268.2	55	3.53	1.50–8.36	0.021*	2.97	1.17–7.52	0.022*

All patients presented with metastases that met the criteria of an SUV threshold > 4.0 and a volume > 1 mL, which were included for the automated delineation of regions of interest (ROIs) to determine SUVpeak, MTV and TLG

Log-rank test p-value. When maximally selected log-rank statistics are used to determine the optimal cutoff, the p-value is approximated using the method by Hothorn and Lausen

From multivariable Cox regression model adjusted for LDH, ECOG performance status and the number of metastatic organs at baseline

Of note: Due to the different tests used for obtaining p-values (log-rank with or without approximated p-value using the method by Hothorn and Lausen for the univariable analysis, Wald test for multivariable analysis) comparison between univariable and multivariable analyses should focus on HR estimates

* p-value < 0.05 (for median cutoff only). SUV = standard uptake value; MTV = metabolic tumor volume; TLG = total lesion glycolysis

in multivariable analyses. The hazard of a PFS event remained significant when not quantifiable was grouped with median ΔMTV% above the median (Grouped MTV) in both univariable and multivariable analyses for week-2 and week-7, see Table 3. A likelihood ratio test indicated that this model was a worse fit for the data than the ungrouped model though.

On-treatment increase of metabolic tumor volume

At week-2 and week-7, all patients revealed a decrease in MTV compared to baseline. However, when PET/CT of week-7 was compared to week-2, an increase in MTV was measured in 9/58 (15.5%) patients, illustrated in Fig. 6. Median PFS of these 9 patients was 5.3 months compared to 12.6 months of the other patients with stable or ongoing decrease of MTV ($P = 0.0023$), see Fig. 7. A multivariable Cox regression analysis displayed a high HR but power was limited (HR = 2.34, 95% CI 0.96–5.74, $P = 0.062$).

Intra-patient heterogeneity

In total, 69 patients with 620 evaluable baseline lesions met the inclusion criteria for the intra-patient heterogeneity analysis. At baseline, the median CoV of SUVpeak per patient was 0.4 (range 0 to 1.09). After 2 weeks of therapy, the median ΔCoV% was 45.3% (range – 142.2 to 100.0%), based on 46 patients and 200 lesions included for analysis. Finally, at 7 weeks, 40 patients and 132 lesions were available for analysis and the median ΔCoV% was 48.7% (range – 132.3 to 100.0%).

Cox regression analysis showed worsened PFS when there was less than 45.3% decrease in intra-patient heterogeneity from baseline to week-2 (HR = 2.88, 95% CI 1.27–6.56, $P = 0.012$). Predictive power was also good ($AUC_{T=6\ months}$=0.744). For baseline CoV and ΔCoV% between baseline and week-7 no statistically significant differences were obtained, but results were indicative of worse PFS for greater intra-patient heterogeneity at baseline, and for increases in intra-patient heterogeneity from baseline to week-7 (see Table 4, and Fig. 8 for details).

Discussion

[^{18}F]FDG PET/CT has emerged as a powerful imaging tool for evaluating treatment response and predicting outcomes in various cancers, including BRAF-mutated melanoma [13–15]. In this study, we present the results of baseline [^{18}F]FDG PET parameters, such as SUVpeak, MTV, and TLG, in predicting PFS in patients with advanced BRAF-mutated melanoma undergoing BRAF/MEKi therapy. Additionally, the potential of these parameters as early indicators of treatment resistance was assessed.

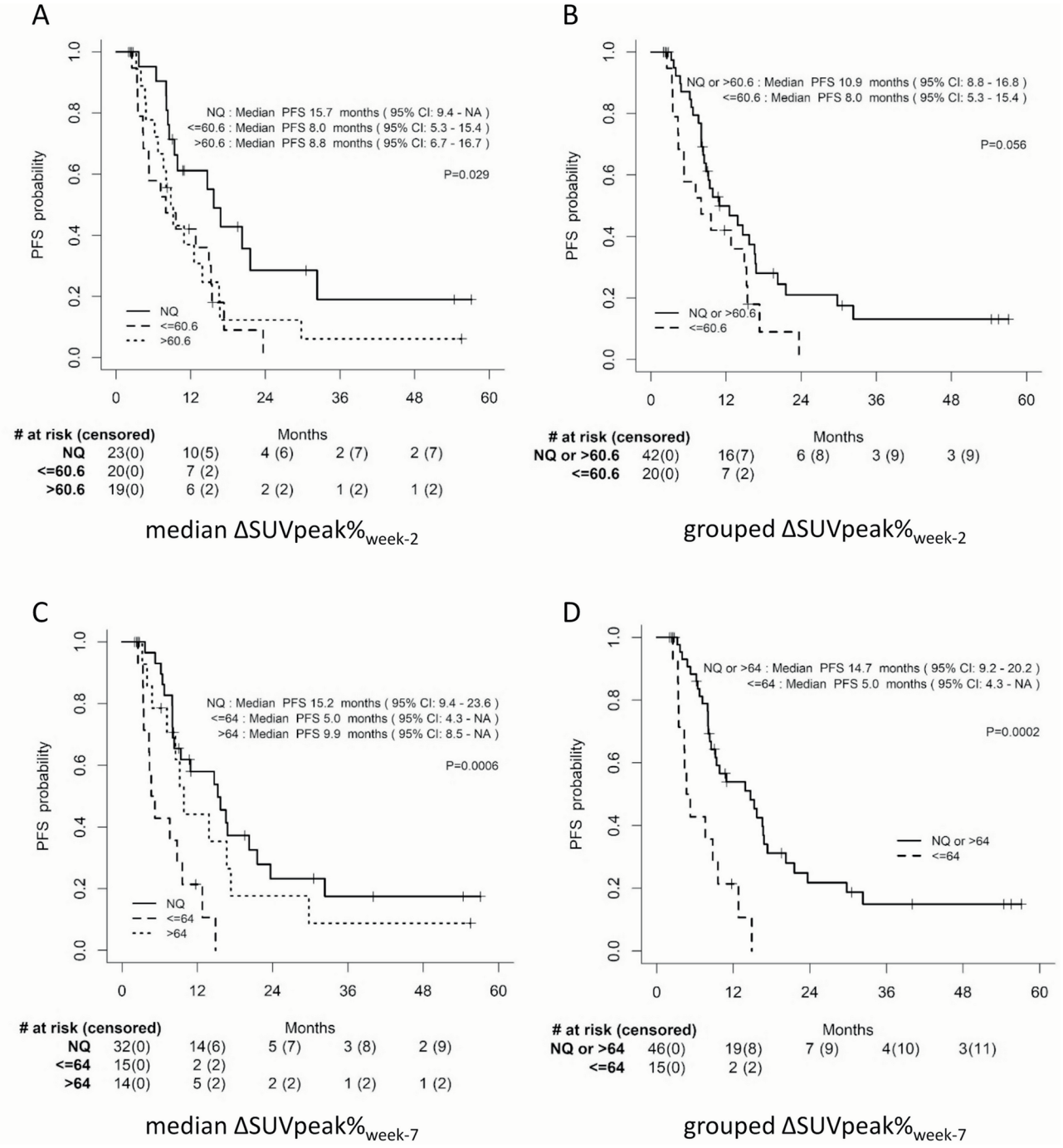

Fig. 4 Kaplan-Meier progression-free survival by on treatment median percentage difference SUVpeak from baseline. Kaplan-Meier progression-free survival curves by 2-weeks median % change SUVpeak (**A**), 2- weeks grouped SUVpeak (**B**), 7-weeks median % change SUVpeak (**C**), and 7-weeks grouped SUVpeak (**D**). SUV = standard uptake value; NQ = Not quantifiable

Baseline SUVpeak, MTV and TLG

At baseline, the ability of median SUVpeak (12.59) to predict PFS was modest, with an AUC of 0.598 at six months. While lower baseline SUVpeak values suggested a trend toward prolonged PFS (median 14.7 vs. 9.2 months), the difference was not statistically significant ($P = 0.064$). Similarly, a cutoff of 10.9 showed a potential survival benefit, but without statistical significance ($P = 0.32$). The limited predictive value of SUVpeak may be due to its focus on metabolic activity within a small tumor region, thus overlooking tumor heterogeneity and total disease burden. Additionally, SUVpeak is influenced by technical factors such as image acquisition and

Table 3 Univariable and multivariable Cox regression results for progression-free survival SUVpeak, MTV and TLG on treatment

	No. patients	Univariable			Multivariable		
		HR	95% CI	$P^{\#}$	HR	95% CI	$P^{\#\#}$
On treatment Baseline – 2 weeks							
SUVpeak % difference using median							
Not quantifiable	23	1.0 (ref.)			1.0 (ref.)		
> 60.6	19	2.08	1.01–4.28	0.013*	1.61	0.73–3.55	0.24
≤ 60.6	20	2.51	1.21–5.18	0.048*	1.74	0.77–3.90	0.18
SUVpeak Grouped							
Not quantifiable + SUVpeak > 60.6	42	1.0 (ref.)			1.0 (ref.)		
≤ 60.6	20	1.79	0.98–3.30	0.056	1.33	0.69–2.59	0.40
MTV % difference using median							
Not quantifiable	20	1.0 (ref.)			1.0 (ref.)		
> 95.6	21	1.65	0.79–3.43	0.18	0.82	0.36–1.84	0.63
≤ 95.6	21	2.83	1.33–6.03	0.0070*	2.09	0.92–4.74	0.077
MTV Grouped							
Not quantifiable + MTV > 95.6	41	1.0 (ref.)			1.0 (ref.)		
≤ 95.6	21	2.15	1.17–3.96	0.012*	2.36	1.21–4.60	0.012*
TLG % difference using median							
Not quantifiable	20	1.0 (ref.)			1.0 (ref.)		
> 97.3	21	1.68	0.81–3.49	0.17	0.98	0.44–2.16	0.96
≤ 97.3	21	2.75	1.29–5.86	0.0089*	2.02	0.87–4.71	0.104
TLG Grouped							
Not quantifiable + TLG > 97.3	41	1.0 (ref.)			1.0 (ref.)		
≤ 97.3	21	2.07	1.12–3.82	0.017*	2.05	1.04–4.05	0.039*
On treatment Baseline – 7 weeks							
SUVpeak % difference using median							
Not quantifiable	32	1.0 (ref.)			1.0 (ref.)		
> 64	14	1.43	0.69–2.98	0.33	1.77	0.83–3.77	0.14
≤ 64	15	4.0	1.88–8.48	0.0003*	4.58	1.92–10.91	0.0006*
SUVpeak Grouped							
Not quantifiable + SUVpeak > 64	46	1.0 (ref.)			1.0 (ref.)		
≤ 64	15	3.56	1.76–7.19	0.0002*	3.75	1.66–8.46	0.0014*
MTV % difference using median							
Not quantifiable	31	1.0 (ref.)			1.0 (ref.)		
> 97.6	15	1.36	0.66–2.80	0.3988	1.28	0.61–2.69	0.52
≤ 97.6	15	4.70	2.19–10.07	0.0001*	3.68	1.46–9.29	0.0057*
MTV Grouped							
Not quantifiable + MTV > 97.6	46	1.0 (ref.)			1.0 (ref.)		
≤ 97.6	15	4.19	2.07–8.48	< 0.0001*	3.28	1.40–7.68	0.0062*
TLG % difference using median							
Not quantifiable	31	1.0 (ref.)			1.0 (ref.)		
> 98.6	15	1.36	0.66–2.80	0.40	1.39	0.66–2.95	0.38
≤ 98.6	15	4.70	2.19–10.07	0.0001*	4.41	1.68–11.62	0.0027*
TLG Grouped							
Not quantifiable + TLG > 98.6	46	1.0 (ref.)			1.0 (ref.)		
≤ 98.6	15	4.19	2.07–8.48	< 0.0001*	3.74	1.54–9.07	0.0036*

Patients without metastases meeting the criteria of an SUV threshold > 4.0 and a volume > 1 mL were classified as not quantifiable. SUV = standard uptake value; MTV = metabolic tumor volume; TLG = total lesion glycolysis

[#] Log-rank test p-value

[##] From multivariable Cox regression model adjusted for baseline SUVpeak/MTV/TLG, LDH, ECOG performance status and the number of metastatic organs at baseline

* p-value < 0.05

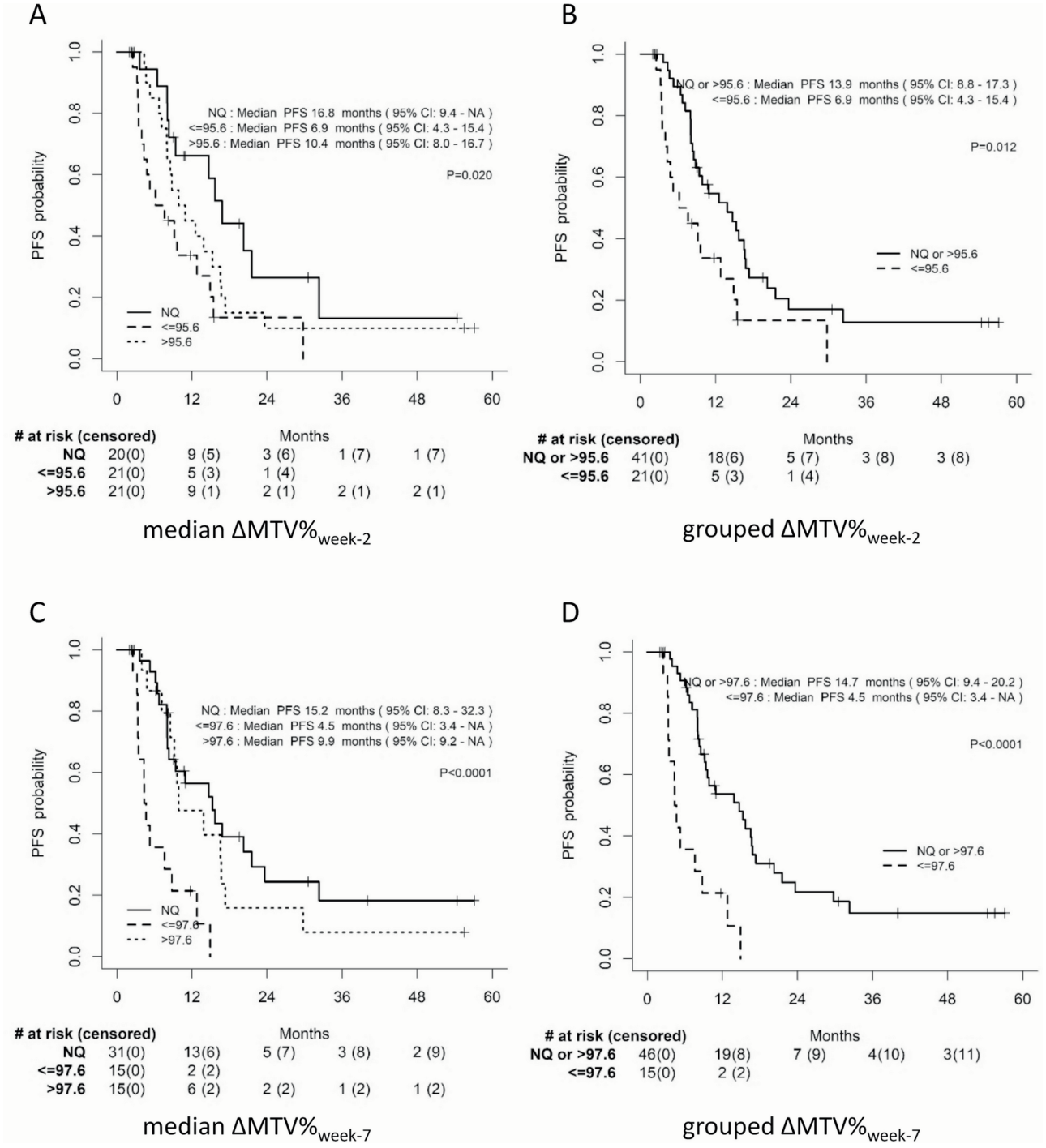

Fig. 5 Kaplan-Meier progression-free survival by on-treatment median percentage difference MTV from baseline. Kaplan-Meier progression-free survival curves by 2-weeks median % change MTV (**A**), 2-weeks grouped MTV (**B**), 7-weeks median % change MTV (**C**), and 7-weeks grouped MTV (**D**). MTV = metabolic tumor volume; NQ = Not quantifiable

reconstruction methods, as well as biological factors like glucose metabolism [29].

Though moderate, our study findings indicate that MTV had a higher predictive value for PFS (AUC = 0.714 at six months) than SUVpeak. Patients with a baseline MTV below 45.3mL had significantly longer PFS (21.6 vs. 8.5 months, $P = 0.021$), while those with MTV above this threshold experienced a threefold increased risk of progression (HR = 3.53). The multivariable analysis also indicated prolonged PFS in patients with a baseline MTV below 45.3 mL. However, it is important to note that the corresponding p-value cannot be directly approximated

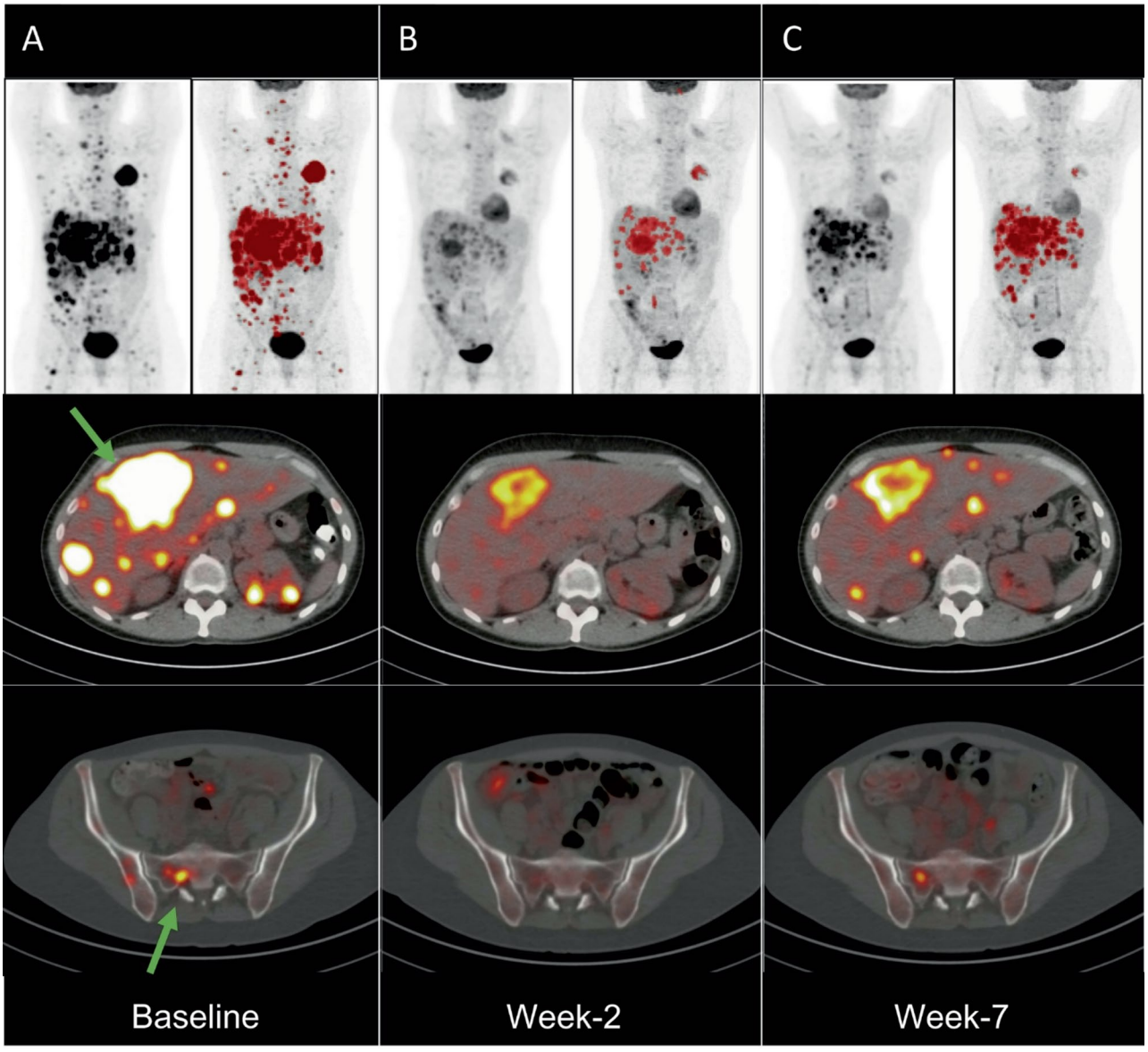

Fig. 6 [^{18}F]FDG PET/CT of a patient with early increased metabolic tumor volume after initial response. [^{18}F]FDG PET/CT of a patient at baseline (**A**), week-2 (**B**) and week-7 (**C**). Maximum Intensity Projection without and with (in red) total tumor burden delineation (upper images). Transaxial fusion images of the liver (middle images) and pelvis (lower images). The images demonstrate a decrease in metabolic tumor volume at week-2, but an increase in MTV at week-7, however with an MTV still less than baseline (green arrows and tumor delineation in red)

for selecting the optimal cutoff due to the multivariable nature of the analysis, which limits its interpretability. Nonetheless, these findings demonstrate MTV's potential to better capture overall disease burden and predict long-term outcomes.

As expected, TLG closely mirrored MTV in its association with PFS, reflecting their mathematical relationship. Given this dependency, we evaluated each parameter separately and proceeded with MTV for multivariable analysis based on its superior predictive performance. In contrast to SUVpeak, MTV provides a more comprehensive view of the total volume of metabolically active tumor tissue, capturing volumetric changes across all metastatic sites rather than focusing on selected lesions.

In literature, two studies investigated baseline MTV on [^{18}F]FDG PET as a predictor of survival following BRAF/MEK inhibition in patients with advanced BRAFV600-mutated melanoma. McArthur et al. prospectively evaluated 35 BRAFi/MEKi-naïve melanoma patients treated with vemurafenib and cobimetinib [27]. As in our study, they observed significant early and improving metabolic responses during therapy. [^{18}F]FDG PET scans, performed during the first two treatment cycles (day 10–15 and day 35–49), showed marked reductions in tumor

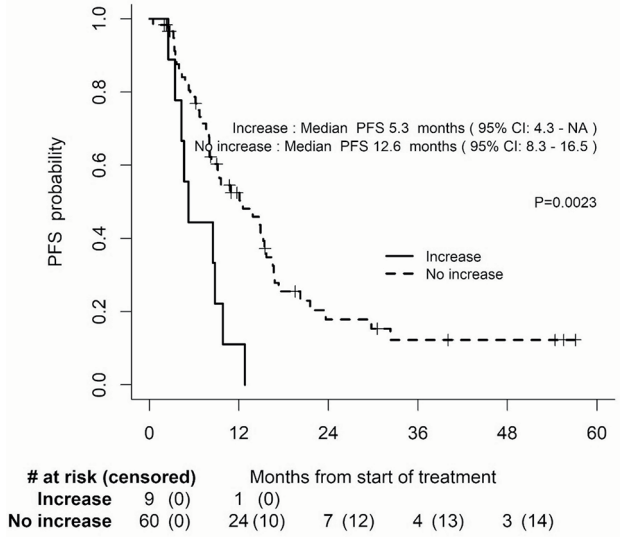

Fig. 7 Progression-free survival of patients with increased MTV during treatment. In nine patients an increase of MTV occurred on PET at week-7 compared to week-2. No patients had higher MTV at week-2 compared to baseline. MTV = metabolic tumor volume; NA = not available

burden and metabolism, with patients achieving substantial decreases in MTV and SUVmax. While baseline tumor burden did not correlate with metabolic response, baseline MTV was a predictor of overall survival (OS), with lower baseline values linked to longer survival. In a retrospective cohort of 57 metastatic melanoma patients treated with BRAF/MEK inhibitors, Annovazzi et al. revealed that a total metabolic tumor volume (TMTV), i.e. the sum of metastases with a SUVmax > 2 and with a volume > 0.5mL, of over 56mL at baseline [^{18}F]FDG PET/CT and the presence of more than two metastatic organ sites were significantly correlated with shorter PFS and OS, with TMTV being the only independent predictor in multivariate analysis [28]. Noteworthy, this cutoff is almost similar to the optimal cutoff of 45mL found in our study, where the minimal differences might be explained by the different threshold for automatic delineation in our study (SUVmax > 4 and volume of > 1mL). These findings underscore that baseline MTV is a valuable predictive indicator for survival in advanced melanoma patients treated with BRAF/MEKi.

Early changes of metabolic parameters during BRAF/MEKi treatment

We compared baseline PET parameters with early metabolic changes to assess their predictive value for progression. While baseline MTV demonstrated the highest predictive performance among baseline PET metrics, early changes in MTV, TLG, and SUVpeak at both week-2 and week-7 showed similar AUCs. Notably, ΔSUVpeak% at week-7 outperformed baseline SUVpeak significantly, suggesting that early changes may better reflect treatment response than baseline values. However, our analyses were limited to patients with quantifiable follow-up scans, introducing possible selection bias. Still, our results highlight the complementary value for predicting PFS based on baseline and early treatment response.

Percentage changes in SUVpeak

At week-2, percentage changes in SUVpeak, when stratified above or below the median, were not predictive of PFS. Patients with not quantifiable lesions on PET (i.e. no metastases above the SUV threshold of 4) had the best PFS, with the hazard for an event significantly

Table 4 Univariable and multivariable Cox regression results for progression-free survival CoV at baseline and on treatment

	No. patients	Univariable			Multivariable		
		HR	95% CI	$P^{\#}$	HR	95% CI	$P^{\#\#}$
Baseline							
CoV using median							
> 0.40	31	1.0 (ref.)			1.0 (ref.)		
≤ 0.40	38	0.63	0.36–1.10	0.106	0.73	0.40–1.36	0.32
On treatment Baseline – 2 weeks							
CoV % difference using median							
> 45.3	23	1.0 (ref.)			1.0 (ref.)		
≤ 45.3	23	2.19	1.11–4.33	0.021*	2.88	1.27–6.56	0.012*
On treatment Baseline – 7 weeks							
CoV % difference using median							
> 48.7	20	1.0 (ref.)			1.0 (ref.)		
≤ 48.7	20	1.79	0.86–3.71	0.11	1.66	0.75–3.69	9.21

The coefficient of variation of SUVpeak values across all evaluable lesions per patient was used as a proxy for patient heterogeneity

CoV = coefficient of variation

$^{\#}$ Log-rank test p-value

$^{\#\#}$ From multivariable Cox regression model adjusted for baseline CoV, LDH, ECOG performance status and the number of metastatic organs at baseline

* p-value < 0.05

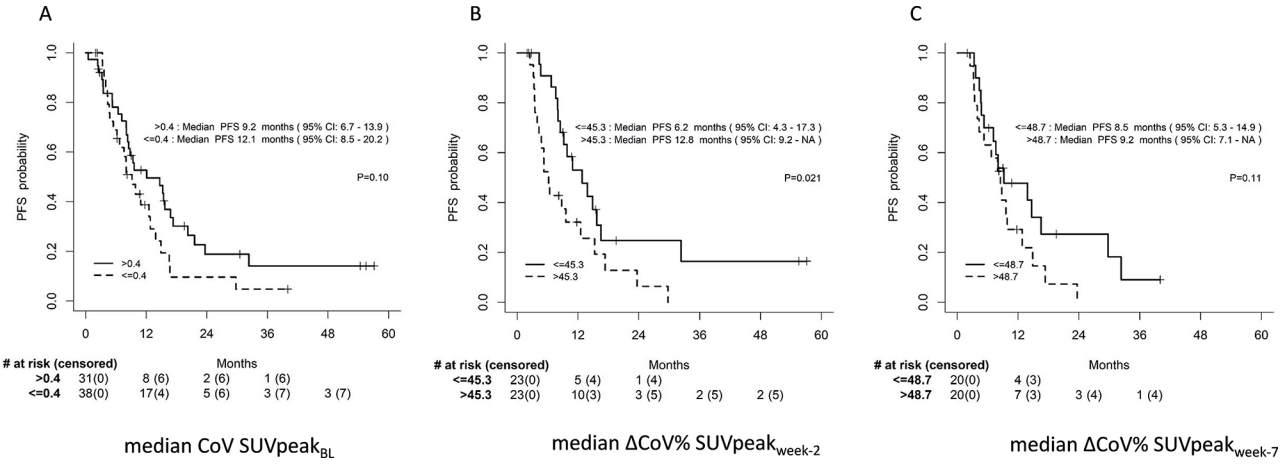

Fig. 8 Kaplan-Meier progression-free survival by baseline and on-treatment intra-patient heterogeneity. Kaplan-Meier progression-free survival curves by baseline median CoV of SUVpeak across evaluable lesions per patient (**A**), median ΔCoV% between baseline and week-2 (**B**) and median ΔCoV% between baseline and week-7 (**C**). CoV = coefficient of variation; ΔCoV%= percentage difference in COV; BL = baseline

higher for patients with a percentage change in SUVpeak above or below the median. These results indicate that early response prediction for determining the best PFS is more accurately associated with an absolute SUV threshold rather than mean percentage differences. In contrast, percentage changes in SUVpeak became predictive at week-7, where a shorter PFS was seen for patients with percentage changes in SUVpeak below the median compared to patients with changes above the median or the not quantifiable patients. Therefore, in our study no incremental predictive benefit was observed using percentage changes of SUVpeak on early [^{18}F]FDG PET/CT at week-2.

Only one study investigated the correlation between SUV on early [^{18}F]FDG PET/CT response and survival during BRAF/MEKi treatment of melanoma patients [15]. In this study by Schmitt et al., changes in SUVmax of the hottest lesion and of the least responsive tumor on follow-up [^{18}F]FDG PET/CT were calculated in 24 patients and correlated to PFS and OS. Mean time from baseline to follow-up [^{18}F]FDG PET/CT was 26 days, being approximately double the duration compared to our study. They observed a significant association between the smallest change in SUVmax and progression-free survival ($P = 0.01$), but not overall survival ($P = 0.52$). Though our results indicate that percentage change SUVpeak might be predictive at week-7, it might already be at an earlier time-point of four weeks, as was observed by Schmitt et al.

While the predictive value of [^{18}F]FDG PET/CT in melanoma patients treated with BRAF/MEKi has been scarcely investigated, early response assessment has been more extensively studied in patients receiving ICI [17–26]. These studies have typically imaged patients after 3–4 weeks of therapy, and more commonly beyond 6 weeks. In line with our findings, they consistently demonstrate the predictive value of early metabolic changes on [^{18}F]FDG PET/CT. Notably, Cho et al. showed that changes in metabolic activity as early as 2 weeks after ICI initiation were predictive of response and survival [17]. More recently, Anderson et al. demonstrated that [^{18}F]FDG PET/CT imaging just one week after a single pembrolizumab dose could already predict treatment response, with decreased uptake correlating with improved outcomes [18]. Similarly, Hribernik et al. confirmed the prognostic value of [^{18}F]FDG PET/CT at 2–4 weeks in melanoma patients receiving ICI [19].

Percentage changes in MTV and TLG

When examining the impact of MTV and TLG during treatment, significant reductions in MTV and TLG at both week-2 and week-7 follow-up scans were associated with improved PFS. Patients who demonstrated a ≥ 96% reduction in MTV at week-2 and ≥ 98% reduction at week-7 experienced significantly longer PFS compared to those with lesser reductions. When adjusting for LDH, ECOG performance status and number of metastatic sites, the risk of progression for patients with MTV above this threshold remained significantly increased at both time-points compared to the Grouped MTV (HR = 2.36 week-2 vs. HR = 3.28 week-7). However, at both week-2 and week-7, best PFS was observed in the not quantifiable group, being classified based on a SUV threshold of 4. So, during treatment, patients with the best PFS are determined based on SUV rather than MTV, indicating that metabolic activity, as reflected by changes in SUV, may be a more sensitive and reliable predictor of treatment response and long-term outcomes than the overall tumor burden measured by MTV. However, these findings do emphasize the potential of MTV and TLG as early markers of treatment efficacy, with the possibility of identifying patients at risk of early progression

even before clinical or radiographic evidence of disease worsening. Interestingly, patients whose MTV increased between week-2 and week-7 had a median PFS of only 5.3 months, compared to 12.6 months for patients with continued MTV reduction. This suggests that any increase in MTV during treatment may be an early indicator of resistance to BRAF/MEKi therapy. Such findings underscore the importance of serial [^{18}F]FDG PET/CT imaging in monitoring treatment response, as changes in MTV could provide critical insights into disease dynamics, allowing for timely modifications to treatment strategies. In this context, [^{18}F]FDG PET/CT might be a valuable tool for patients who are too frail to initiate treatment with first line ICI, but for whom a switch to immunotherapy is being considered later following BRAF/MEKi therapy.

Pharmacological effect of TKI on glucose metabolism

An important consideration is whether the observed early declines in [^{18}F]FDG uptake following BRAF/MEKi initiation reflect true anti-tumor activity or may partly result from direct pharmacologic effects on glucose metabolism. Preclinical studies have demonstrated that BRAF and MEK inhibitors can modulate glucose transporters and glycolytic pathways, potentially reducing [^{18}F]FDG uptake independently of cell viability [48, 49]. However, in our study, metabolic responses on PET were accompanied by significant tumor shrinkage on CT in most patients, supporting the interpretation that [^{18}F]FDG decline primarily reflects actual tumor response. Nonetheless, we cannot fully exclude that early changes in glucose metabolism due to direct drug effects also contribute to the observed reductions in [^{18}F]FDG uptake. As such, the extent to which PET changes represent tumor killing versus metabolic reprogramming remains uncertain, particularly at early time points.

Intra-patient heterogeneity

Our exploratory analysis on intra-patient metabolic heterogeneity supports the hypothesis that non-uniform response among lesions may reflect treatment resistance. We found that a smaller decrease in the coefficient of variation (CoV) of SUVpeak values after 2 weeks of therapy was significantly associated with shorter PFS, suggesting that persistent or emerging heterogeneity early during treatment may portend poor outcomes. Although baseline CoV and changes at 7 weeks did not reach statistical significance, the observed trends suggest a potential role of heterogeneity dynamics as an early biomarker of treatment efficacy. These results highlight the importance of lesion-level assessment in addition to global metrics, and warrant further validation in larger datasets.

Study limitations

A key limitation of our study is the relatively small sample size ($n = 69$), which may limit the generalizability of our findings. Additionally, due to the application of the SUV threshold for MTV and TLG analysis during treatment to adequately distinguish tumor from physiologic uptake, the sample size of patients eligible for evaluation of MTV and TLG was further reduced. Nevertheless, to our knowledge, this is the only study to prospectively investigate early [^{18}F]FDG PET/CT during treatment of BRAF/MEK inhibition in advanced BRAF-naïve melanoma patients. Furthermore, the uniqueness of this cohort lies in the fact that patients were treated with BRAF/MEK inhibitors until disease progression, a treatment approach that is less feasible in current clinical practice due to changes in the therapeutic landscape.

Conclusions

In conclusion, this study highlights the predictive value of [^{18}F]FDG PET/CT in assessing BRAF/MEKi treatment response in advanced BRAF-mutated melanoma. Baseline metabolic tumor volume (MTV) was the strongest predictive indicator, with lower MTV linked to longer PFS, while early changes in MTV, TLG, and especially week-7 ΔSUVpeak% showed similar or improved performance. During treatment, percentage changes in MTV and TLG all correlated with improved PFS already on early imaging and additional SUVpeak at week-7, with treatment response best predicted by an absolute SUV threshold of 4. Increase of MTV on serial [^{18}F]FDG PET/CT from week-2 to week-7 can identify early resistance. Additionally, intra-patient metabolic heterogeneity was associated with outcome, with early reductions in SUVpeak variation between lesions correlating with better PFS. These findings support the role of [^{18}F]FDG PET/CT for early prediction of treatment response and progression in this patient population.

Abbreviations

[^{18}F]FDG	18F-Fluorodeoxyglucose
AUC	Area under the curve
CI	Confidence interval
CR	Complete response
CT	Computed tomography
EANM	European Association of Nuclear Medicine
EARL	EANM Research Ltd
ECOG	Eastern Cooperative Oncology Group
HR	Hazard ratio
ICI	Immune checkpoint inhibitors
IQR	Interquartile range
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinase
MTV	Metabolic tumor volume
NA	Not available
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PR	Partial response
RECIST	Response evaluation criteria in solid tumors

ROC	Receiver-operating characteristic
ROI	Region of interest
SD	Stable disease
SUV	Standardized uptake value
TF	Time of flight
TLG	Total lesion glycolysis
TTB	Total tumor burden

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions
BvdH, LdW, AvdE, MS, RB and JH contributed to the conception and design of the study. Data collection, including patient-related activities was done by BvdH, AvdE, JH, EK, GH, MA, FdV, MB, AvdV and JWdG. Data analysis, statistics and interpretation of data was performed by BvdH, MS, ML, RB, LdW and WV.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The Medical Ethical Committee of the Netherlands Cancer Institute approved the study for all participating centers. Informed consent was obtained from all participants before entering the study.

Consent for publication
The authors affirm that human research participants provided informed consent for publication of the manuscript.

Competing interests
AvdE: Study grant: Roche, Idera, Travel expenses: Ipsen Advisory Board: Bristol-Myers Squibb, MSD Oncology, Ipsen, Janssen Cilag BV, Pierre Fabre EK: consultancy/advisory relationships with Bristol Myers Squibb, Novartis, Merck, Pierre Fabre, Lilly and Bayer, all paid to institute, received research grants not related to this paper from Bristol Myers Squibb, Delcath, Novartis and Pierre Fabre. GH: consultancy/advisory relationships with Amgen, Bristol-Myers Squibb, Roche, MSD, Pfizer, Novartis, Sanofi, Pierre Fabre, all paid to institute, received research grants from Bristol-Myers Squibb, Seerave, all paid to institute. MA: advisory board / consultancy honoraria from Amgen, Bristol Myers Squibb, Novartis, MSD-Merck, Merck-Pfizer, Pierre Fabre, Sanofi, Astellas, Bayer. Research grants Merck-Pfizer, all paid to institute and not related to current work. FdV: received research grant from Foundation STOPBraintumors.org, BMS, Novartis, Servier, CureVac, EORTC, all paid to institute. AvdV: consultancy roles (all paid to the institute) for BMS, MSD, Roche, Sanofi, Novartis, Pierre Fabre, Merck, Ipsen, Eisai, Pfizer, all paid to the institute. JH: advisory roles for BMS, CureVac, GSK, Ipsen, Iovance Biotherapeutics, Imcyse, Merck Serono, Molecular Partners, MSD, Novartis, Pfizer, Roche, Sanofi, Third Rock Ventures, member of SAB of Achilles Tx, BioNTech, Gadeta, Immunocore, Instil Bio, PokeAcell, Scenic, T-Knife and Neogene Tx, all paid to institute except Neogene Tx and Scenic, received grant support from Amgen, Asher Bio, BioNTech, BMS, MSD, Novartis, and Sastra Cell Therapy, all paid to institute. The other authors declare no conflict of interest.

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