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

Hiel, B. van der

SECTION IV

Conclusions

CHAPTER 9

Summary, Discussion and Future Perspectives

SUMMARY

This thesis examines the value of PET/CT in optimizing treatment strategies for stage III and IV melanoma skin cancer. For this, the widely adopted radiopharmaceutical [^{18}F]FDG is used, reflecting glucose metabolism, and in an exploratory context the less common tracer [^{18}F]FLT, visualizing cell proliferation.

Integration of [^{18}F]FDG PET/CT in neoadjuvant and postoperative settings

The understanding of melanoma's high glucose metabolism, its high recurrence rate following complete resection of macroscopic stage III disease, and the proven utility of [^{18}F]FDG PET/CT in restaging has motivated the investigation described in **Chapter 2**. In this retrospective study, two cohorts were analyzed to evaluate whether [^{18}F]FDG PET/CT can detect (early) recurrences following complete resection of advanced stage III melanoma. In cohort 1, 35 asymptomatic stage IIIB/C patients received serial [^{18}F]FDG PET/CT scans every 6 months after resection. Recurrence occurred in 12 patients (34.3%), with seven (20.0%) detected by the first scan at 6 months. PET/CT showed high sensitivity (92.3%) and specificity (100%), and early detection led to treatment changes in six (17.2%) patients, resulting in complete response (CR) or no evidence of disease (NED). In cohort 2, 42 patients received a single [^{18}F]FDG PET/CT before starting adjuvant therapy. Recurrence was suspected in nine (21.4%) patients and confirmed in four (9.5%), influencing treatment decisions in two. These results support the use of [^{18}F]FDG PET/CT for early detection of recurrence post-surgery and before adjuvant therapy.

Given the remarkable tumor shrinkage induced by BRAF/MEKi, the Reductor trial investigated whether R0 resection could be achieved in patients with previously unresectable stage III melanoma (1). A side study (**Chapter 3**) explored the role of [^{18}F]FDG PET/CT in predicting histopathological response, recurrence, and early recurrence detection. In 20 patients receiving neoadjuvant BRAF/MEKi for initially unresectable stage III melanoma, [^{18}F]FDG PET/CT was performed at baseline, 2 weeks, and 8 weeks. Among 18 patients assessed for pathologic response, pathologic complete- or near-complete response (pCR/nearCR) occurred in nine (50%) of patients and nine (50%) patients had pathologic partial- or no response (pPR/pNR). However, PET-based response criteria (EORTC/PERCIST) did not predict pathologic outcome. Also, neither baseline total tumor burden maximum and peak standardized uptake value (SUV), metabolic tumor volume (MTV), and total lesion glycolysis (TLG) nor early changes could predict recurrence, although all eight recurrences during follow-up were successfully detected by [^{18}F]FDG PET/CT, including two (25%) within 3 months post-surgery. These results indicate that while [^{18}F]FDG PET/CT is highly effective for early detection of recurrence following neoadjuvant BRAF/MEKi and surgery, its value as a predictive tool for histopathologic response or recurrence risk remains limited.

Response monitoring and resistance prediction to BRAF/MEKi treatment

Though targeted therapy with BRAF/MEKi initially reduces tumor burden in patients with advanced BRAF-mutated melanoma, resistance inevitably develops in most patients, often accelerating disease progression. Early detection of resistance could help avoid ineffective, costly therapy and unnecessary toxicity. To explore the molecular mechanisms underlying resistance and the potential of PET/CT in prediction, we conducted the REPOSIT study, which is described in **Chapter 4**.

In **Chapter 5**, we present the [^{18}F]FDG PET/CT imaging outcomes of the REPOSIT study, comparing Response Evaluation Criteria in Solid Tumours (RECIST)1.1 and PET Response Criteria in Solid Tumors (PERCIST) for predicting progression-free survival (PFS). Among the 70 patients included, RECIST1.1 responses did not correlate with PFS, but PERCIST-defined complete metabolic response (CMR) at week 7 was linked to significantly longer PFS (median 16.7 vs. 8.5 months, P=0.0003), even after adjusting for LDH. [^{18}F]FDG PET/CT was false-positive in all four (6%) patients with new [^{18}F]FDG-avid lesions but CMR of known metastases at week 7. In addition, a lesion-based visual comparison of 22 patients with confirmed progression revealed that progression of known metastases was the predominant pattern, with no cases of isolated new lesions (outside the brain). This suggests that new [^{18}F]FDG-avid lesions during BRAF/MEKi do not necessarily reflect recurrent disease. Although this study demonstrates that PERCIST response assessment at week 7 is predictive for PFS, independent of LDH, further investigation of different PET parameters is warranted to assess the added value of early [^{18}F]FDG PET/CT.

Therefore, in **Chapter 6** we investigated the predictive value of various metabolic PET parameters in the same patient cohort to assess their potential as indicators of PFS. Baseline MTV was the strongest predictor of PFS ($AUC_{T=6\ months}$=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 a baseline MTV ‹45.3mL had significantly longer PFS (median PFS 21.6 vs. 8.5 months, P=0.021), whereas those with higher MTV had a threefold increased risk of progression (HR=3.53). These findings remained significant after adjusting for baseline LDH level, Eastern Cooperative Oncology Group (ECOG) performance status and number of metastatic sites at baseline. The results for TLG closely mirrored those of MTV, while baseline SUVpeak showed limited predictive power.

During treatment, 37% (week 2) and 53% (week 7) of patients had a not quantifiable scan (no metastases above the SUV 4) and showed best outcomes. Grouping these with patients with high MTV reduction revealed longer PFS at week 2 (median PFS 13.9 vs. 6.9 months, P=0.012) compared to patients with lower MTV reduction. Lower MTV reduction was associated with an increased risk of progression, even after adjustment (HR=2.36 at week 2; HR=3.28 at week 7). Notably, best PFS was seen in the not quantifiable

group, suggesting absolute SUV rather than MTV as the key outcome predictor. Additionaly, a SUVpeak reduction at week 7, not week 2, was also predictive (median PFS 14.7 vs. 5.0 months, P=0.0002). Patients whose MTV increased between week 2 and week 7 had significantly shorter PFS (5.3 vs. 12.6 months, P=0.0023). Intra-patient metabolic heterogeneity also correlated with outcome: early reductions in variations of SUVpeak between lesions were associated with improved PFS. In conclusion, both baseline and on-treatment MTV and TLG predict PFS, but response during treatment is best indicated by a SUV threshold of 4 rather than MTV. Intra-patient heterogeneity was also predictive for PFS. These findings support the role of [^{18}F]FDG PET/CT for early prediction of treatment response and progression in this patient population.

Exploring [^{18}F]FLT PET in advanced melanoma

With the hypothesis that imaging proliferation might reflect tumor aggressiveness better than glucose metabolism, we investigated the proliferative imaging marker [^{18}F]FLT in 18 patients of the REPOSIT study who underwent [^{18}F]FLT PET/CT at baseline and on Day 14 of BRAF/MEKi treatment. We first determined the influence of BRAF/MEKi on the physiologic distribution of [^{18}F]FLT, further explored in **Chapter 7.** We demonstrated that [^{18}F]FLT accumulation in normal tissues is independent of baseline tumor burden and its response to BRAF/MEKi in patients with advanced melanoma and that interpatient variability of [^{18}F]FLT uptake was relatively low both at baseline and during treatment (Coefficient of Variation [CoV] ‹20%). On treatment, blood pool and muscle SUVmean remained unchanged compared to baseline. However, liver SUVmean increased (P<0.001), while bone marrow SUVmean decreased (P<0.025). These changes were unrelated to liver function or treatment response.

In **Chapter 8**, we explored the potential of [^{18}F]FLT PET/CT imaging to predict resistance to BRAF/MEKi in the same patient population. First, a visual comparison of baseline [^{18}F]FLT and [^{18}F]FDG PET showed shorter PFS (3.5–5.3 months) in the four patients with equal, higher or a heterogeneous [^{18}F]FLT uptake pattern compared to patients with consistently lower [^{18}F]FLT than [^{18}F]FDG uptake (median 9.6 months, range 3.4–32.3). Second, no significant association was found between baseline [^{18}F]FLT uptake and Ki-67 (r=-0.17, P=0.601), nor was Ki-67 expression linked to PFS (P=0.39). Last, baseline [^{18}F]FLT uptake was not predictive for PFS (P=0.601). However, a greater percentage change in [^{18}F]FLT uptake by Day 14 was associated with longer PFS compared to those with a smaller change (median PFS 13.9 months vs. 4.3 months, P=0.005). These findings suggest that early changes in [^{18}F]FLT uptake may provide insight into treatment response, whereas baseline [^{18}F]FLT PET and Ki-67 expression are not predictive of PFS.

GENERAL DISCUSSION

Detection and prediction of early recurrence or pathologic response

[^{18}F]FDG PET/CT for early recurrence detection

Across different cohorts [^{18}F]FDG PET/CT has demonstrated significant value in detecting recurrence in patients with advanced stage III melanoma after R0 resection.

In the surveillance cohort (**Chapter 2**, Cohort 1), routine 6-monthly [^{18}F]FDG PET/CT scans after R0 resection of stage III effectively detected asymptomatic recurrences, with 7/12 (58.3%) recurrences identified on the first scan. This aligns with previous literature reporting that the majority of recurrences in high-risk melanoma occur within the first 2 years after surgery (2). Given that many of our excluded patients experienced symptomatic recurrence before their first scheduled scan, earlier surveillance imaging at 3–4 months postoperatively may be beneficial.

In addition, [^{18}F]FDG PET/CT prior to adjuvant therapy (**Chapter 2**, Cohort 2) identified recurrence in 9.5% of patients, emphasizing its utility in screening before systemic treatment initiation, particularly in cases where delays in adjuvant therapy may increase the risk of disease progression. Similarly, in our cohort of patients with previously unresectable stage III melanoma treated with neoadjuvant BRAF/MEK inhibitors (**Chapter 3**), [^{18}F]FDG PET/CT effectively detected recurrence during follow-up, with some recurrences identified as early as 3 months postoperatively. Another potential interesting finding in this study was that in patients with a large tumor burden, an MTV higher than 150cc, no pCR/nearCR was achieved. Although the sample size was too limited to draw definitive conclusions, the findings may suggest the existence of a certain MTV threshold above which pCR/nearCR is unlikely to be achieved within 8 weeks of BRAF/MEKi therapy. However, it cannot be excluded that prolonged treatment duration might still result in such responses.

From adjuvant toward neoadjuvant ICI therapy

Nevertheless, the above findings must be considered in the context of the evolving treatment landscape for stage III melanoma. Although adjuvant BRAF/MEK inhibition improves recurrence-free survival (RFS), recent long-term analyses failed to demonstrate a statistically significant improvement in overall survival (OS), as reported by Long et al. (3). Additionally, the treatment paradigm for resectable stage III melanoma is shifting from adjuvant toward neoadjuvant ICI therapy. Recent trials have demonstrated superior event-free survival (EFS) with neoadjuvant plus adjuvant ICIs compared to adjuvant-only strategies, with higher rates of major pathologic response and potential for reduced treatment duration post-surgery (4, 5). In a prospective phase 2 trial, patients treated with neoadjuvant plus adjuvant pembrolizumab had significantly longer EFS than those receiving adjuvant-only treatment (P=0.004), with

a 2-year landmark analysis EFS of 72% versus 49%, respectively (4). Similarly, the NADINA trial reported an estimated 12-month EFS of 83.7% in patients treated with neoadjuvant ipilimumab plus nivolumab, compared to 57.2% in the adjuvant-only group (5). In this study, only patients in the neoadjuvant group with a pathologic PR (8.0%) or NR (26.4%) received adjuvant treatment.

[^{18}F]FDG PET/CT to predict pathologic response

In our study, [^{18}F]FDG PET/CT was unable to predict pathologic response in patients treated with neoadjuvant BRAF/MEK inhibitors. However, this analysis was conducted in a cohort of patients with previously unresectable stage III melanoma, who had a relatively high tumor burden. This contrasts with patients with resectable stage III melanoma, for whom neoadjuvant ICI therapy is now the standard approach, and who generally present with a lower tumor burden. In our preliminary (unpublished) data, achieving a CMR on [^{18}F]FDG PET after two cycles of neoadjuvant pembrolizumab may be predictive of a pathologic CR, though further data are needed to confirm this finding.

In addition, in the neoadjuvant-group of the NADINA trial, the decision to administer adjuvant ICIs was guided by the pathologic response to neoadjuvant ICIs (5). In this group, the estimated 12-month RFS was 95.1% in patients with a major pathologic response, 76.1% in patients with a partial response, and 57.0% in patients with a pathologic no response. In addition to our findings that [^{18}F]FDG PET/CT is a highly effective imaging modality for detecting recurrence in high-risk melanoma patients following complete surgical resection, the findings from the NADINA trial may help refine patient selection and optimize the timing of [^{18}F]FDG PET/CT for detecting early recurrence in these patients. Furthermore, [^{18}F]FDG PET/CT might even support postponing or omitting surgery altogether in cases of CMR prior to surgery.

A relevant observation across both studies is the high sensitivity of [^{18}F]FDG PET/CT in detecting distant metastases, and its crucial role for guiding treatment decisions. In addition, [^{18}F]FDG PET/CT has proven invaluable for restaging stage III melanoma, often leading to modifications in treatment strategy (6-9). Taken together, these findings emphasize that the key question is not whether [^{18}F]FDG PET/CT plays a role in early recurrence detection, but rather how to determine the optimal timing for its use.

[^{18}F]FDG as a predictive biomarker in BRAF-mutated stage III or IV melanoma

The REPOSIT study: insights into tumor behavior on BRAF/MEKi until resistance

Despite the impressive initial responses on BRAF/MEKi, nearly all patients ultimately develop resistance, the timing of which remains unpredictable. Once resistance emerges, disease progression can be rapid, leaving limited or no further treatment options. Given these challenges, we developed the REPOSIT study (**Chapter 4**). The study initially aimed to include 90 patients. However, recruitment was discontinued

after enrolling 75 patients due to a slow accrual rate, largely driven by shifts in the treatment landscape. With emerging evidence supporting a more adaptive treatment approach, patients were no longer treated with BRAF/MEKi until disease progression. Instead, they were transitioned to ICIs once sufficient tumor shrinkage was achieved, given the longer duration of response associated with ICI therapy. When comparing our study with literature, however, 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) until progression and in which [^{18}F]FDG PET/CT was performed at consistent time points. Therefore, although the REPOSIT study did not reach the intended sample size, the cohort of 75 patients remains unique, providing valuable insights into tumor behavior on BRAF/MEKi until resistance emerged.

Baseline [^{18}F]FDG PET/CT for predicting progression-free survival

At baseline, we evaluated [^{18}F]FDG SUVpeak, MTV and TLG, which were obtained from an automatically delineated ROI of the summed lesions with a fixed threshold of 4.0 and a volume of >1mL (**Chapter 6**). MTV demonstrated the strongest predictive value, with patients having a baseline MTV ≤45.3mL experiencing significantly longer PFS (21.6 vs. 8.5 months, P=0.021). Higher MTV was associated with a threefold increased risk of progression (HR=3.53). Previous studies on [^{18}F]FDG PET in advanced melanoma similarly identified baseline MTV as a key predictor of survival in patients receiving BRAF/MEKi (10, 11). Notably, the optimal MTV cutoff in our study closely aligned with prior findings, despite differences in delineation thresholds (11). These findings support the predictive value of baseline MTV in BRAF/MEKi-treated melanoma patients and could aid in guiding BRAF/MEKi-ICI treatment strategy.

Early and standard response assessment to predict progression-free survival

Using standard response assessment with PERCIST, we found that in a multivariable analysis including LDH, PERCIST at 2 weeks was not predictive for PFS, but became predictive at 7 weeks (**Chapter 5**). Patients achieving CMR at 7 weeks were significantly more likely to have a durable response, thus suggesting effective inhibition of the MAPK/ERK-pathway. Our findings align with precious studies (10-13). However, the timing of response imaging in literature varies widely, ranging from 13 days to 6 months, with no consistent assessment intervals. While our results could not predict resistance in individual patients, we revealed that awareness of early development of resistance is warranted in patients who do not achieve CMR early or 7 weeks after treatment initiation. Based on our findings, we propose that 7 weeks is an appropriate time point for early response prediction. In contrast, RECIST1.1 response assessment could not predict PFS.

Similar to PERCIST, also percentage changes in SUVpeak were not predictive of PFS at 2 weeks, but became predictive at 7 weeks (**Chapter 6**). Patients showing smaller

SUVpeak reductions demonstrated shorter PFS compared to those with greater reductions or not quantifiable lesions (i.e. no metastases exceeding an SUV threshold of 4). These findings align with prior research by Schmitt et al., which reported that changes in SUVmax at approximately 4 weeks (closer to our 7-week time point) correlated with PFS (13). Though for MTV and TLG, already at week 2 reductions were significantly associated with longer PFS, for all three PET parameters patients with not quantifiable lesions had the best PFS, suggesting that an absolute SUV threshold may be more relevant for early response prediction than percentage changes.

Transient early immune responses influencing [^{18}F]FDG uptake

In line with literature (14) and our results from the neoadjuvant Reductor trial (**Chapter 3**), the findings from the REPOSIT study (**Chapter 5** and **Chapter 6**) indicate that the most pronounced reduction in [^{18}F]FDG uptake on PET/CT occurs within the first 2 weeks of BRAF/MEKi treatment, with a median SUVpeak reduction of 61%. By week 7, a further but more modest decline is observed (median SUVpeak reduction of 64% relative to baseline). This pattern suggests that while BRAF/MEKi rapidly suppresses metabolic activity within the first 2 weeks, the treatment effect has not yet reached complete steady state, as a continued, albeit smaller, reduction is seen at week 7. One possible explanation for this delayed continued reduction in [^{18}F]FDG uptake is the transient immunomodulatory response induced by BRAF inhibition. Early after treatment initiation, BRAF inhibitors have been shown to enhance T-cell infiltration within melanoma tumors, temporarily boosting the immune response (15, 16). This immune-related metabolic activity, visible as increased [^{18}F]FDG uptake on PET/CT, may confound the assessment of true tumor response. However, these effects appear to be transient, with studies showing that tumor immune infiltration diminishes beyond 2 weeks of therapy (16, 17). This transient immune response may account for the ongoing, albeit reduced, metabolic decline observed at week 7 and could explain why [^{18}F]FDG PET imaging emerged as a stronger predictor of treatment response at this time point compared to week 2.

Early [^{18}F]FDG PET/CT to identify high-risk patients

While [^{18}F]FDG PET/CT at 7 weeks provides the most robust predictive value for long-term response, early [^{18}F]FDG PET/CT at 2 weeks may serve as a tool for identifying high-risk patients. Our findings demonstrate that at this early time point, [^{18}F]FDG PET/CT enhances the ability identifying patients at risk for early progression, enabling the identification of those who may experience resistance even before clinical or radiographic evidence of disease worsening (**Chapter 6**). Notably, 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 those with continued MTV reduction. This suggests that increase in MTV during treatment may serve as an early indicator of resistance to BRAF/MEKi therapy, allowing for timely adjustments to treatment strategies.

Overall, early PET imaging at week 2 can help identify patients at risk and potentially guide treatment decisions, especially for those who may be too frail to initiate first-line immunotherapy. Additionally, no patients at week 2 and only one patient at week 7 demonstrated PMD (**Chapter 5**). This finding is highly relevant, as BRAF/MEKi is sometimes given as bridging treatment (typically for 8 weeks) to reduce tumor load before switching to ICI. Thus, short-term induction treatment with BRAF/MEKi is safe for achieving a rapid and effective response in this context.

Valuable insights into dissemination patterns and tumor heterogeneity

With this unique cohort of patients receiving continuous BRAF/MEKi until progression, the REPOSIT study provided valuable insights into dissemination patterns at disease progression (**Chapter 5**). The lesion-based evaluation of 22 patients with confirmed progression demonstrated that the predominant pattern was the progression of known metastases, with no patients presenting with solely new lesions outside the brain. All false-positive new lesions identified in our study were [^{18}F]FDG-avid lymph nodes. Similar to other ICIs, these false-positive nodes likely represent reactive lymph nodes within the lymphatic drainage basin of metastatic sites, triggered by immune response activation (16, 18). On [^{18}F]FDG PET/CT, these reactive lymph nodes often exhibit increased [^{18}F]FDG uptake and are typically observed within the first few weeks following the initiation BRAF/MEKi therapy (16, 17). Thus, our findings indicate that new [^{18}F]FDG avid lesions, particularly lymph nodes, during BRAF/MEKi treatment should not be automatically interpreted as disease progression, emphasizing the importance of evaluating imaging findings within the appropriate clinical context.

Our exploratory analysis of intra-patient metabolic heterogeneity supports the hypothesis that a non-uniform response among individual lesions may indicate early treatment resistance (**Chapter 6**). Specifically, a smaller reduction in the coefficient of variation (CoV) of SUVpeak values after 2 weeks of BRAF/MEKi therapy was significantly associated with shorter progression-free survival (PFS), suggesting that persistent or emerging metabolic heterogeneity early in the treatment course may be prognostically unfavorable. While baseline CoV and changes at week 7 did not reach statistical significance, the observed trends point toward a potential role for heterogeneity dynamics as an early biomarker of therapeutic efficacy. These findings underscore the added value of lesion-level analysis in complementing global PET metrics and highlight the need for further validation in larger, prospective cohorts.

Exploring [^{18}F]FLT PET in advanced melanoma

[^{18}F]FLT normal tissue distribution

For accurate interpretation and quantitative analysis of [^{18}F]FLT PET, we first evaluated the biodistribution of [^{18}F]FLT in relevant normal tissues and found that [^{18}F]FLT uptake is not influenced by baseline tumor burden or its response to BRAF/MEKi (**Chapter 7**).

Furthermore, interpatient variability of [^{18}F]FLT uptake proved relatively low both at baseline and on treatment (CoV<20%) for all measured normal tissues, which is in line with previous studies (19-21). However, during BRAF/MEKi [^{18}F]FLT uptake in bone marrow significantly decreased, most likely explained by actual inhibition of proliferation. In addition, [^{18}F]FLT liver uptake significantly increased during BRAF/MEKi treatment, which was not related to on-treatment changes in liver function nor with response to treatment. Oncological therapies, including tyrosine kinase inhibitors (TKIs) like vemurafenib, can alter [^{18}F]FLT metabolism by affecting UDP-glucuronosyltransferase enzymes, increasing FLT bioavailability and cellular accumulation (22). Although we did not measure FLT-glucuronide levels, also no relevant increase in [^{18}F]FLT-signal in the blood pool was observed. Another possible explanation for lower baseline liver uptake is the so-called 'tumor sink effect', where high tumor burden reduces tracer availability for normal tissues. Given melanoma's high proliferation rate and large tumor volumes, this effect could limit [^{18}F]FLT bioavailability pre-treatment. However, despite substantial tumor shrinkage (~95%) during therapy, no correlation was found between tumor volume and [^{18}F]FLT uptake, suggesting the tumor sink effect did not fully explain the increased liver uptake.

[^{18}F]FLT PET/CT for predicting treatment resistance to BRAF/MEKi

Baseline visual comparisons revealed that most metastatic lesions exhibited lower [^{18}F]FLT uptake than [^{18}F]FDG uptake (**Chapter 8**). This aligns with the understanding that glucose uptake primarily reflects tumor metabolism via glycolysis, whereas thymidine ([^{18}F]FLT) uptake is more specifically associated with cellular proliferation and is restricted to actively cycling cells (S-phase) (23, 24). We also demonstrated that patients with either heterogeneous uptake patterns or consistently equal to or higher [^{18}F]FLT uptake compared to [^{18}F]FDG demonstrated a tendency toward shorter PFS, suggesting that increased [^{18}F]FLT uptake may identify more aggressive, treatment-resistant disease phenotypes. These findings highlight the complementary role of [^{18}F]FLT PET in assessing tumor biology beyond metabolic activity.

Previous studies have identified [^{18}F]FLT as a surrogate marker for proliferation, correlating with Ki-67 expression (25). However, our study found no significant association between [^{18}F]FLT uptake and Ki-67, likely due to sampling limitations, tumor heterogeneity, or factors such as hypoxia or necrosis. As [^{18}F]FLT uptake can be heterogeneous within metastases, biopsies may not always reflect areas of highest tracer accumulation. While Ki-67 has prognostic value in primary melanoma (26, 27), its predictive power for PFS in metastatic lesions appears limited. This aligns with findings from a meta-analysis by Liu et al., which also reported no correlation between Ki-67 expression and PFS, though it was linked to poorer OS (28).

The semi-quantitative analysis of early [^{18}F]FLT uptake reductions 2 weeks after initiation of BRAF/MEKi strongly correlated with PFS, suggesting its potential as an

early biomarker of treatment efficacy. This contrasts with findings from the REPOSIT study, where early [^{18}F]FDG PET/CT failed to predict PFS (**Chapter 7** and **Chapter 8**). Predicting resistance at an early stage could guide timely treatment modifications, such as switching to immune checkpoint inhibitors. While [^{18}F]FLT PET has been extensively studied in other cancers, particularly lung and breast cancer, its role in melanoma remains underexplored. Prior studies in melanoma have primarily focused on [^{18}F]FLT's role in monitoring immunotherapy or distinguishing immune responses from true progression (29-31). Although findings in other malignancies suggest promising predictive value (32-34), small sample sizes limit definitive conclusions, emphasizing the need for further research.

Limitations of [^{18}F]FLT PET/CT in metastatic melanoma treated with BRAF/MEKi

To our knowledge, no previous studies have investigated the correlation between differences in [^{18}F]FLT and [^{18}F]FDG uptake distribution patterns and PFS and our study is the first prospective analysis of [^{18}F]FLT PET/CT for predicting PFS in melanoma patients on targeted therapy. While [^{18}F]FLT PET/CT offers unique insights into tumor proliferation, its clinical utility in metastatic melanoma treated with BRAF/MEKi is limited by several factors. As demonstrated in our study, [^{18}F]FLT uptake is typically lower than [^{18}F]FDG uptake, potentially leading to underestimation of tumor burden. We also demonstrated the influence of BRAF/MEKi on FLT metabolism, especially in normal liver tissue. This pharmacokinetic variability potentially introduces challenges in response assessment, particularly given the lack of standardized criteria for [^{18}F]FLT PET/CT interpretation in melanoma. Additionally, physiological high [^{18}F]FLT uptake in bone marrow and liver complicates lesion detection in these sites, which are common metastatic locations in melanoma, reducing image contrast and diagnostic accuracy. Moreover, [^{18}F]FLT PET/CT remains costly and the tracer less widely available than [^{18}F]FDG, limiting its integration into routine clinical practice. Due to these challenges, [^{18}F]FLT PET/CT is currently more applicable in research settings rather than standard oncologic imaging for melanoma patients undergoing targeted therapy.

In conclusion, though we demonstrated that [^{18}F]FLT PET/CT provides unique tumor biology insights and shows potential as a complementary tool that may help identify patients at higher risk of resistance to BRAF/MEKi therapy, the small sample size and exploratory nature of this study warrant cautious interpretation. Together with the previous mentioned limitations, integrating [^{18}F]FLT PET/CT into clinical practice will require further refinement of its role relative to existing imaging standards.

FUTURE PERSPECTIVES

Managing patient care

As the number of newly diagnosed cancer patients and long-term cancer survivors continues to rise, it is crucial to ensure that healthcare remains sustainable in terms of costs and capacity, guaranteeing ongoing access to treatment for all patients in need. To achieve this, optimizing the patient journey is essential. Key components include: (a) early cancer detection to facilitate minimally invasive treatment, reduce recurrence rates, and prevent costly interventions; (b) accurate patient selection for therapy to ensure that only those likely to benefit receive treatment, thereby avoiding unnecessary procedures that contribute to financial burdens and toxicity; and (c) effective follow-up strategies to monitor disease progression, detect recurrences, and adjust management as needed.

This thesis points out relevant aspects of these key components, such as the critical role of [^{18}F]FDG PET/CT in detecting recurrences in high-risk melanoma patients after surgical resection and systemic therapy. These findings emphasize the importance of effectively managing patient care in the evolving oncology landscape. The ability of [^{18}F]FDG PET/CT to identify asymptomatic recurrences early supports the need to refine imaging schedules to balance clinical benefits with resource utilization. Optimizing the frequency of surveillance imaging—potentially shifting toward earlier postoperative scans—could further enhance patient outcomes while maintaining manageable healthcare capacity.

PET imaging in adaptive treatment strategies

The evolving melanoma treatment paradigm has moved away from continuous BRAF/MEKi until disease progression. Instead, there is a focus on transitioning to ICIs following sufficient tumor shrinkage. The transition from adjuvant BRAF/MEK inhibitors to ICIs has significant implications for patient management, as ICIs demonstrate superior EFS and durable responses. Furthermore, increasing evidence supports the use of neoadjuvant ICIs in resectable stage III melanoma, underscoring the need for effective imaging biomarkers to inform treatment decisions.

Although [^{18}F]FDG PET/CT has not consistently predicted the pathologic response to neoadjuvant BRAF/MEK inhibition in previously unresectable melanoma, preliminary data indicate that metabolic response assessments during neoadjuvant ICIs in resectable stage III melanoma may correlate with pathologic outcomes. If validated, this could facilitate early treatment adaptations, minimizing unnecessary toxicity and refining patient selection for adjuvant therapy based on response stratification. Furthermore, our findings suggest that early [^{18}F]FDG PET/CT imaging at 2 weeks can identify patients at risk for rapid progression, thereby guiding early treatment modifications. These insights provide a rationale for integrating [^{18}F]FDG PET/CT imaging

into adaptive treatment approaches, ensuring patients receive the most appropriate therapy at the right time.

Our findings reinforce the prognostic utility of baseline metabolic tumor volume (MTV) and total lesion glycolysis (TLG) over SUVpeak in predicting PFS in unresectable stage III and IV melanoma treated with BRAF/MEKi, aligning with prior studies. Since volumetric PET parameters provide a comprehensive assessment of disease burden, future research should explore their integration into routine clinical practice for risk stratification and treatment monitoring.

From [^{18}F]FLT PET to immunoPET in the era of immunotherapy

[^{18}F]FLT PET may serve as a complementary tool in cases where [^{18}F]FDG PET is inconclusive, particularly for the early identification of treatment-resistant disease. Given its ability to distinguish proliferative activity from metabolic changes, [^{18}F]FLT PET could refine patient selection for alternative therapeutic strategies. However, its clinical utility remains limited due to lower tumor uptake, variability in normal tissue distribution, and the pharmacokinetic effects of BRAF/MEKi.

As the therapeutic landscape shifts from BRAF/MEKi to ICIs, there is an increasing need for advanced imaging tracers capable of capturing immune-related tumor characteristics. In this context, immunoPET represents a promising approach for assessing tumor microenvironment dynamics and predicting responses to immunotherapy. By enabling direct visualization of immune-related targets, such as PD-L1 expression and CD8+ T-cell infiltration, PET imaging offers a more specific assessment of tumor biology beyond conventional metabolic imaging. Radiotracers like [^{89}Zr]-labeled antibodies targeting PD-1/PD-L1, are being investigated for their ability to provide real-time information on the tumor immune microenvironment, potentially guiding immunotherapy selecionț and response monitoring. In addition to PD-1/PD-L1 and CD8+ T cell radiotracers, novel radiotracers targeting melanin and fibroblast activation protein (FAP) are emerging as a potential PET biomarkers, though their diagnostic value remains to be fully evaluated.

Advances in PET/CT technology and artificial intelligence

Rapid evolution of PET/CT technology

The field of PET/CT has undergone rapid advancements, evolving from PET-only imaging to hybrid PET/CT, followed by time-of-flight technology, and now culminating in the development of a new generation of Total Body PET/CT scanners. These state-of-the-art systems enable significantly faster acquisitions, require lower radiotracer doses, and, most importantly, offer unparalleled sensitivity in detecting metastatic disease. The resulting high-resolution images not only enhance diagnostic accuracy but also reinforce the critical role of PET/CT in clinical decision-making.

The introduction of long axial field-of-view (LAFOV) PET/CT scanners further enhances these benefits, particularly for tracers with inherently higher radiation burden, such as Zr-89-labeled compounds, by allowing for high-quality imaging with substantially reduced injected doses. Moreover, their capacity for whole body dynamic imaging unlocks new opportunities for pharmacokinetic modeling—potentially eliminating the need for invasive arterial blood sampling—thereby broadening the scope of PET beyond static diagnostics into the realm of quantitative, physiology-based imaging.

Although the initial costs of Total Body PET/CT remain high, the substantial improvement in sensitivity and efficiency suggests that these scanners will become the standard of care in the coming years. Their ability to facilitate precise patient stratification, optimize treatment selection, and reduce unnecessary interventions aligns with the pressing need for cost-effective healthcare strategies, particularly as the oncologic patient population continues to grow. Notably, the increased sensitivity of these scanners allows for the detection of even the smallest metastases in morphologically normal tissue, pushing the clinical utility of PET/CT toward earlier disease detection and intervention. As technological advancements in PET/CT continue to refine oncologic imaging, its integration into earlier stages of the patient journey will further improve outcomes by enabling timely and personalized treatment strategies.

Artificial intelligence in the field of Nuclear Medicine

Artificial intelligence (AI) is playing an increasingly prominent role in medical imaging, offering the potential to automate image analysis, improve lesion detection, and refine risk stratification. For example, AI-enhanced PET/CT interpretation could be particularly valuable in assessing MTV and SUV, as metastatic melanoma patients often present with widespread disease. By leveraging AI to analyze these quantitative parameters, clinicians may achieve more precise and less time-consuming disease burden assessment, enabling better prognostication and treatment monitoring. Machine learning algorithms may enhance PET/CT interpretation by identifying subtle metabolic changes indicative of disease recurrence or treatment response that might be overlooked by conventional analysis. AI-driven predictive models could also support personalized surveillance strategies, optimizing imaging intervals based on individual patient risk profiles. The integration of AI into nuclear medicine workflows holds promise for improving diagnostic accuracy, reducing interobserver variability, and enhancing the efficiency of clinical decision-making.

In conclusion, while [^{18}F]FDG PET/CT remains a cornerstone of melanoma imaging, advancements in radiotracer development, imaging technology, and artificial intelligence are reshaping the field. Future research should focus on optimizing the timing and integration of PET/CT in staging, surveillance, and response assessment, validating novel imaging biomarkers, and leveraging AI-driven approaches to enhance precision oncology. Investigating the role of PET imaging in adaptive treatment

strategies, particularly in the transition from BRAF/MEKi to ICIs, remains a relevant aspect. These developments will ultimately contribute to more effective, personalized, and cost-efficient melanoma care.

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