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

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SECTION III

Exploring [^{18}F]FLT PET in Advanced Melanoma

CHAPTER 7

Baseline and on Treatment Biodistribution Variability of [^{18}F]FLT Uptake in Patients with Advanced Melanoma: Brief Communication

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ABSTRACT

Purpose

This prospective study evaluates the biodistribution of 3'-deoxy-3'-18F-fluorothymidine ([^{18}F]FLT) in patients with advanced melanoma before and after treatment with BRAF/MEK inhibitors.

Methods

Eighteen BRAF-positive unresectable stage IIIc or IV melanoma patients referred for [^{18}F]FLT PET/CT before (BL) and during (D14) BRAF/MEK inhibition were included. [^{18}F]FLT accumulation in liver, bone marrow, blood, and muscle were quantified.

Results

Baseline interpatient [^{18}F]FLT uptake had a coefficient of variation between 17.5-21.5%. During treatment, liver uptake increased ($SUVmean_{BL}$=4.86±0.98, $SUVmean_{D14}$=6.31±1.36, P<0.001) and bone marrow uptake decreased ($SUVmean_{BL}$= 7.67±1.65, $SUVmean_{D14}$=6.78±1.19, P<0.025). Both changes were unrelated to baseline MTV or tumor response.

Conclusion

To assess [^{18}F]FLT PET both liver and bone marrow uptake may be used as normal tissue references at baseline, but [^{18}F]FLT biodistribution significantly changes in longitudinal response studies when treated with BRAF/MEK inhibitors.

BACKGROUND

Patients with advanced BRAF-mutated melanoma often present with high tumor burden for which targeted therapy with combined BRAF- and MEK inhibitors (BRAF/MEKi) is given to reduce tumor load. When treated with BRAF/MEKi, metastases show a rapid decrease in both lesion size and glucose metabolism, even within 2 weeks after initiation (1). Therefore, in-vivo visualization of changes in tumor proliferation could be a powerful addition to routine imaging, in providing information for predicting prognosis and response monitoring during BRAF/MEKi therapy (2).

Positron emission tomography with 3'-deoxy-3'-^{18}F-fluorothymidine ([^{18}F]FLT PET) has the potential to visualize active cellular proliferation in proportion to the DNA synthesis rate (3). [^{18}F]FLT is a structural surrogate of the nucleoside thymidine that is not incorporated into the DNA but is trapped in cells due to its phosphorylation by thymidine kinase 1 (TK-1). For accurate interpretation and quantitative analysis of [^{18}F]FLT PET, adequate knowledge of normal tissue distribution is essential since high variability of normal tissue uptake may lead to unreliable quantitative PET parameters. However, neither the effects of tumor burden on the biodistribution of [^{18}F]FLT in healthy tissue nor its rapid changes in volume and metabolism when treated with BRAF/MEKi are known. So, the aim of this study was to evaluate the biodistribution of [^{18}F]FLT in relevant normal tissues in patients with advanced melanoma and to investigate whether treatment with BRAF/MEKi effects this biodistribution.

PATIENTS AND METHODS

This analysis included patients who underwent [^{18}F]FLT PET/CT (March 2015 to August 2018) as part of a phase II multi-center trial (NCT02414750) (4). In this prospective study, patients with BRAF-mutated unresectable stage IIIc (n=1) or IV (n=17) melanoma were treated with combined BRAF/MEK inhibitor vemurafenib plus cobimetinib until progression or uncontrollable toxicity. The study was approved by the Ethics Committee, written informed consent was obtained from all patients.

[^{18}F]FLT PET/CTs were performed within 1 month before start BRAF/MEKi (BL) and on treatment at day 14 of cycle 1 (D14). [^{18}F]FLT PET/CTs were acquired from skull base to thighs at 3-4 min per bed position approximately 60 minutes after intravenous administration of 4MBq/kg [^{18}F]FLT (max. activity of 450MBq). [^{18}F]FDG PET/CTs were performed according to EARL 1 day after [^{18}F]FLT PET/CT. Scans were acquired at four hospitals on a Gemini TF PET/CT, TF Big Bore PET/CT, Ingenuity TF PET/CT (all Philips Medical Systems, Netherlands), and Biograph mCT PET/CT (Siemens, Germany). For each patient, all PET/CT scans were performed on the same scanner, with a maximum activity difference of 10%.

On [18F]FLT PET/CTs, SUVmean and SUVpeak were determined in four tissues; 1) the liver using a 3-cm spherical volume of interest (VOI) placed in the right lobe, avoiding metastases (5), 2) the blood pool using a 1-cm spherical VOI in the descending thoracic aorta, without involving the vessel wall, 3) the red bone marrow using a 2-cm spherical VOI placed in three thoracic vertebrae (6), and 4) in muscle using a 2-cm VOI placed in the m. erector spinae. On [18F]FDG PET/CTs metabolic tumor volume (MTV) was calculated by adding up the volumes of all metastases with a SUV>4 and volume >1mL.

For descriptive statistics, quantitative values were expressed as mean plus range and categorical values as numbers plus percentage. All quantitative PET data was normally distributed (Shapiro-Wilk test), therefore parameters were compared using paired T-tests and variability between patients was assessed with the coefficient of variation (CoV). A p-value ‹0.05 was considered statistically significant. Pearson correlation was used to measure the strength of a linear relationship between two variables. Statistical analyses were performed by using SPSS (IBM, v.22.0, NY, USA).

RESULTS

This study included 18 patients, see **Table 1** for patient demographics. Seventeen patients underwent [18F]FLT PET/CT both at baseline and Day 14, one patient underwent [18F]FLT PET/CT imaging only at baseline. All patients underwent [18F]FDG PET/CT at both visits.

SUVpeak and SUVmean of the tissues are displayed in **Table 2**. CoVs for both SUVmean and SUVpeak proved comparable at both baseline and Day 14. Hence, we further focused on SUVmean, being more commonly used for analysis of biodistribution in healthy tissues. Liver SUVmean during treatment was significantly higher compared to baseline ($SUVmean_{BL}$=4.86±0.98, $SUVmean_{D14}$=6.31±1.36, P<0.001), whereas bone marrow SUVmean was significantly lower during treatment ($SUVmean_{BL}$=7.67±1.65, $SUVmean_{D14}$=6.78± 1.19, P<0.025). SUVmean of blood pool and muscle were not significantly different between the two visits. The distribution of SUVmean is illustrated in **Figure 1**.

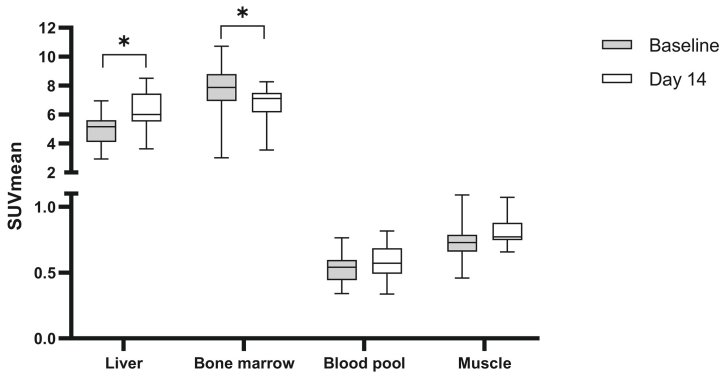

***Figure 1**. Boxplots of baseline and Day 14 to illustrate the distribution of SUVmean as a function of the different tissue types. *=P<0.05.*

Table 1. Patient demographics.

	n=18
Sex	
male	15 (83.4%)
female	3 (16.6%)
Age	58 (33-88)
ECOG performance status	
0	9 (50%)
1	9 (50%)
LDH >ULN (n=16)	10 (62.5%)
Liver function	
ALT >ULN	4 (22.2%)
AST >ULN	3 (16.7%)
serum Albumin <ULN	4 (22.2%)
Number of metastases	37.5 (3-112)
Metastatic sites	
lymph node	12 (66.7%)
lung	9 (50%)
liver	10 (55.6%)
bone	13 (72.3%)
(sub)cutaneous	10 (55.6%)
intramuscular	7 (38.9%)
other*	12 (66.7%)
Metabolic tumor volume (cm^3)	
baseline	438.44 (22.08-1897.15)
on treatment	18.01 (0-137.73)
% reduction MTV	95% (73-100%)

Continuous variables are shown as mean (range); categorical variables as numbers (percentages). ECOG=Eastern Cooperative Oncology Group; LDH=lactate dehydrogenase; AST=aspartate aminotransferase; ALT=alanine aminotransferase; *Other: peritoneum (n=6), intestine (n=4), adrenal gland (n=1), thyroid (n=1), pleura (n=2), pancreas (n=1), spleen (n=2).

Table 2. SUVpeak and SUVmean of reference tissues at baseline and on treatment (Day 14).

SUVpeak	**Baseline**	**CoV%**	**Day 14**	**CoV%**	**Difference#**	**P**
liver	5.25±1.01	19.2%	6.66±1.40	21.0%	1.41±0.81	<0.001*
bone marrow	8.76±1.80	20.5%	7.98±1.30	16.3%	-0.78±1.61	0.063
blood pool	0.63±0.11	17.5%	0.65±0.15	23.1%	0.019±0.11	0.461
muscle	0.83±0.16	19.3%	0.88±0.13	14.8%	0.053±0.21	0.318
SUVmean	**Baseline**	**CoV%**	**Day 14**	**CoV%**	**Difference#**	**P**
liver	4.86±0.98	20.2%	6.31±1.36	21.6%	1.45±0.81	<0.001*
bone marrow	7.67±1.65	21.5%	6.78±1.19	17.6%	-0.88±1.47	0.025*
blood pool	0.54±0.11	20.4%	0.58±0.13	22.4%	0.036±0.11	0.194
muscle	0.72±0.15	20.8%	0.81±0.11	13.6%	0.088±0.17	0.054

CoV=coefficient of variation; #Relative difference using paired samples T-test. *=P<0.05.

The mean MTV at baseline [^{18}F]FDG PET/CT was 438.44cm^3 (range 22.08-1897.15cm^3). At Day 14, MTV had decreased in all patients (mean 18.01cm^3, range 0-137.73cm^3). There was no correlation between baseline MTV and SUVmean (**Figure 2**). To exemplify this marked reduction, a representative case is shown in **Figure 3**.

The increase in liver SUVmean and decrease in bone marrow SUVmean did not correlate with the decrease of MTV (liver r=0.029, P=0.911; bone marrow r=0.318, P=0.214). When assessing liver function at baseline, four (22%) patients showed signs of impaired liver function (**Table 1**). However, the biochemical parameters for liver function did not correlate with SUVmean [^{18}F]FLT uptake (AST_{BL} r=0.023, P=0.928; AST_{D14} r=-0.291, P=0.313; ALT_{BL} r=0.119, P=0.638; ALT_{D14} r=0.316, P=0.233).

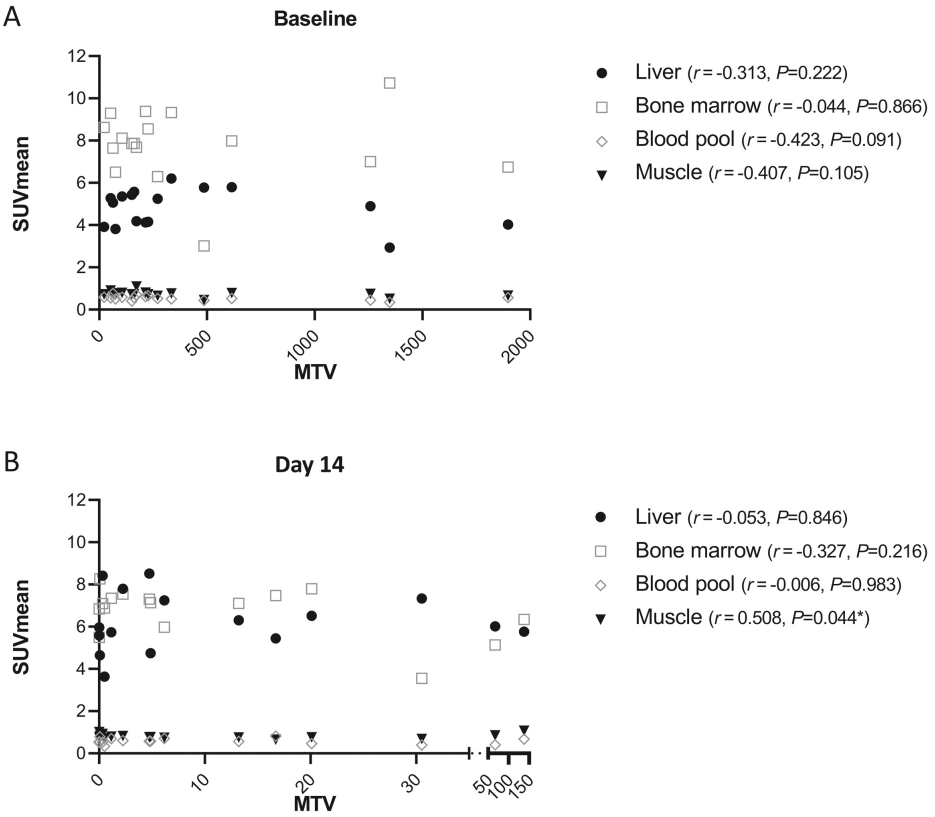

***Figure 2**. SUVmean in liver, bone marrow, blood pool and muscle as a function of MTV at baseline (A) and Day 14 (B); SUV=standardized uptake value.*

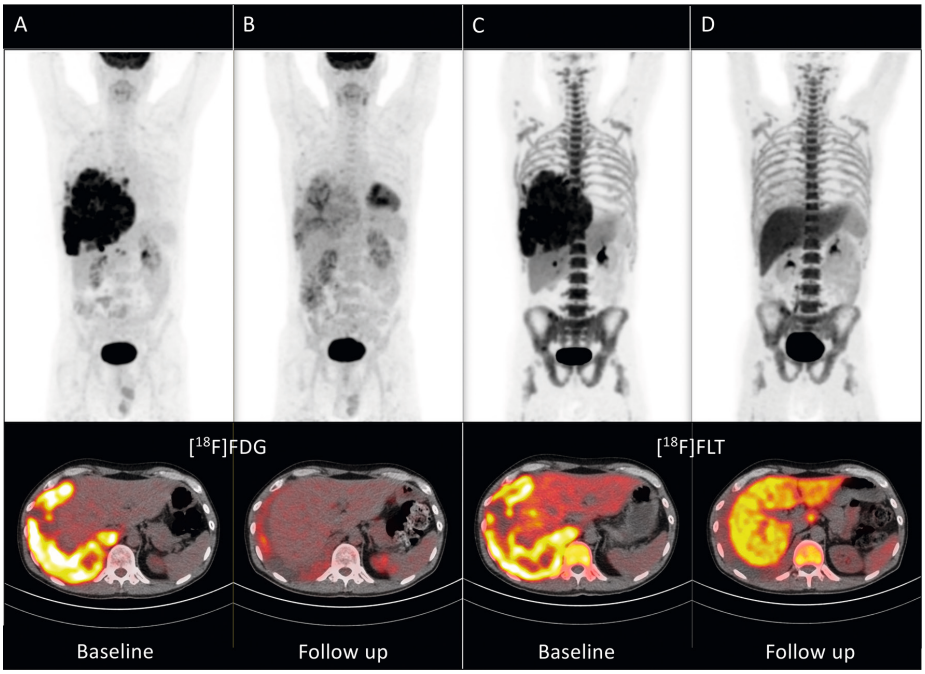

***Figure 3**. [18F]FDG and [18F]FLT PET images of a 48-year-old male with pleural melanoma metastases demonstrating high MTV at baseline with a significant response early after start BRAF/MEKi. MTV on [18F]FDG PET decreased from 1897cm³ at baseline (A) to 67cm³ on treatment (B). [18F]FLT PET at baseline (C) illustrates the high proliferative tumor burden and physiologic accumulation in bone marrow and liver. On treatment (D), [18F]FLT uptake in healthy liver has increased (SUVpeak from 4.0 to 6.0). Decrease of [18F]FLT accumulation in bone marrow was less pronounced (SUVpeak from 6.7 to 5.1).*

DISCUSSION

In present study, it is shown that [18F]FLT accumulation in normal tissue is independent of baseline lumped tumor burden or its response to BRAF/MEKi in patients with advanced melanoma. Interpatient variability of [18F]FLT accumulation proved relatively low both at baseline and on treatment (CoV<20%). However, the accumulation in liver and bone marrow significantly changed during BRAF/MEKi treatment, which was not related to on-treatment changes in liver function nor with response to treatment.

The normal biodistribution of FLT includes increased uptake in organs such as kidneys, bladder, red bone marrow, spleen, and liver (7). Kidney and bladder accumulation are explained by its clearance and excretion. The liver uptake is related to FLT being broken-down into plasma-bound metabolites, whereas the red marrow and spleen uptake represents actual cell proliferation (8, 9). The content of 18F in the circulation is generally low and will contain both intact [18F]FLT and its metabolites after administration. Our findings for baseline FLT biodistribution and interpatient variability are in line with previous studies (3, 6, 10). Cysouw et al. recommend liver and bone marrow to be used

as normal tissue references to assess comparability in biodistribution for repeated [^{18}F]FLT PET scans, because of their generally low intrapatient variability (6).

This 'normal' distribution and metabolism of [^{18}F]FLT is known to be altered by oncological therapies including specific chemotherapies and pain medication. Like in our study, Cysouw et al. also observed a significant increase of [^{18}F]FLT uptake in the liver during treatment with tyrosine-kinase inhibitors (TKI's). Vemurafenib is a small molecule TKI, which like all TKI's, influences the activity of UDP-glucuronosyltransferase enzymes, which metabolize FLT to FLT-glucoronide. As a result, the bioavailability of [^{18}F]FLT increases, favoring an increase in FLT accumulation in the cells (11). Though we did not measure FLT-glucuronide levels to determine enzymatic activity during this study, we also did not observe a relevant increase in ^{18}F-signal from the aorta before compared to on-treatment. The decrease in bone marrow uptake is most likely explained by actual inhibition of proliferation.

Another potential explanation for the lower [^{18}F]FLT liver uptake at baseline compared to on-treatment could be the so-called 'tumor sink effect', defined as decreased tracer uptake in healthy tissue due to sequestration by a large tumor volume (12). Melanoma is not only a highly proliferative tumor-type, but patients with advanced melanoma often present with high tumor volumes. Therefore, the suggested tumor sink effect on baseline PET could negatively affect bioavailability of the tracer for normal tissues. An impressive tumor shrinkage of approximately 95% was seen in all patients during treatment, resulting in a decreased tumor sequestration and hence a higher degree of bioavailability for other tissues. Though this principle is plausible, there was no correlation found between tumor volume and [^{18}F]FLT uptake at baseline nor on treatment, suggesting that the increase in liver uptake was not explained by this tumor sink effect. However, the latter statement could not be fully proven due to the relatively small population of 18 patients.

CONCLUSION

In conclusion, we demonstrate that the high tumor volumes and proliferative character of advanced melanoma do not influence the [^{18}F]FLT biodistribution in healthy tissue. Interpatient variability of [^{18}F]FLT accumulation in liver and bone marrow are relatively low, indicating that these tissues can be used as reference tissues to assess comparability for response monitoring. Caution is advised when patients are treated with TKI's as these drugs will influence tracer [^{18}F]FLT uptake in liver and bone marrow.

REFERENCES

1. McArthur GA, Puzanov I, Amaravadi R, Ribas A, Chapman P, Kim KB, et al. Marked, homogeneous, and early [18F]fluorodeoxyglucose-positron emission tomography responses to vemurafenib in BRAF-Mutant advanced melanoma. J Clin Oncol. 2012;30(14):1628-34.
2. Bollineni VR, Kramer GM, Jansma EP, Liu Y, Oyen WJ. A systematic review on [(18)F]FLT-PET uptake as a measure of treatment response in cancer patients. Eur J Cancer. 2016;55:81-97.
3. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, et al. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. Nat Med. 1998;4(11):1334-6.
4. van der Hiel B, Haanen J, Stokkel MPM, Peeper DS, Jimenez CR, Beijnen JH, et al. Vemurafenib plus cobimetinib in unresectable stage IIIc or stage IV melanoma: response monitoring and resistance prediction with positron emission tomography and tumor characteristics (REPOSIT): study protocol of a phase II, open-label, multicenter study. BMC Cancer. 2017;17(1):649.
5. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. J Nucl Med. 2009;50(Suppl 1):122S-50S.
6. Cysouw MCF, Kramer GM, Frings V, De Langen AJ, Wondergem MJ, Kenny LM, et al. Baseline and longitudinal variability of normal tissue uptake values of [18F]-fluorothymidine-PET images. Nucl Med Biol. 2017;51:18-24.
7. Annovazzi A, Ferraresi V, Rea S, Russillo M, Renna D, Carpano S, Sciuto R. Prognostic value of total metabolic tumour volume and therapy-response assessment by [(18)F]FDG PET/CT in patients with metastatic melanoma treated with BRAF/MEK inhibitors. Eur Radiol. 2022;32(5):3398-407.
8. Nicolas F, De Sousa G, Thomas P, Placidi M, Lorenzon G, Rahmani R. Comparative metabolism of 3'-azido-3'-deoxythymidine in cultured hepatocytes from rats, dogs, monkeys, and humans. Drug Metab Dispos. 1995;23(3):308-13.
9. Muzi M, Mankoff DA, Grierson JR, Wells JM, Vesselle H, Krohn KA. Kinetic modeling of 3'-deoxy-3'-fluorothymidine in somatic tumors: mathematical studies. J Nucl Med. 2005;46(2):371-80.
10. Lovinfosse P, Rousseau C, Pierga JY, Bouchet F, Cochet A, Alberini JL, et al. Dual time point [(18)F]FLT-PET for differentiating proliferating tissues vs non-proliferating tissues. EJNMMI Res. 2019;9(1):109.
11. Yin H, Wang Z, Wang X, Lv X, Fan X, Yan M, et al. Inhibition of human UDP-glucuronosyltransferase enzyme by Dabrafenib: Implications for drug-drug interactions. Biomed Chromatogr. 2021;35(11):e5205.
12. Viglianti BL, Wale DJ, Wong KK, Johnson TD, Ky C, Frey KA, Gross MD. Effects of Tumor Burden on Reference Tissue Standardized Uptake for PET Imaging: Modification of PERCIST Criteria. Radiology. 2018;287(3):993-1002.