Published in final edited form as:

Ann Nucl Med. 2012 November ; 26(9): 757–763. doi:10.1007/s12149-012-0636-x.

[¹⁸F]FLT-PET to predict pharmacodynamic and clinical response to cetuximab therapy in Ménétrier's disease

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Abstract

Molecular imaging biomarkers of proliferation hold great promise for quantifying response to personalized medicine. One such approach utilizes the positron emission tomography (PET) tracer 3'-deoxy-3' [¹⁸F]-fluorothymidine ([¹⁸F]FLT), an investigational agent whose uptake reflects thymidine salvage-dependent DNA synthesis. The goal of this study was to evaluate [¹⁸F]FLT-PET in the setting of Ménétrier's disease (MD), a rare, premalignant hyperproliferative disorder of the stomach treatable with cetuximab therapy. Over 15 months, a patient with confirmed MD underwent cetuximab therapy and was followed with sequential [¹⁸F]FLT-PET. For comparison to MD, an [¹⁸F]FLT-PET study was conducted in another patient to quantify uptake in a normal stomach. Prior to cetuximab therapy, stomach tissue in MD was easily visualized with [¹⁸F]FLT-PET, with pre-treatment uptake levels exceeding normal stomach uptake by approximately 4-fold. Diminished [¹⁸F]FLT-PET in MD was observed following the initial and subsequent doses of cetuximab and correlated with clinical resolution of the disease. To our knowledge, this study reports the first clinical use of [¹⁸F]FLT-PET to assess proliferation in a premalignant disorder. We illustrate that the extent of MD involvement throughout the stomach could be easily visualized using [¹⁸F]FLT-PET, and that response to cetuximab could be followed quantitatively and non-invasively in sequential [¹⁸F]FLT-PET studies. Thus, [¹⁸F]FLT-PET appears to have potential to monitor response to treatment in this and potentially other hyperproliferative disorders.

Keywords

FLT; proliferation; treatment response; EGFR; Ménétrier's disease

INTRODUCTION

Non-invasive molecular imaging offers great promise to assess response to conventional and molecularly targeted therapy [1–3]. Conventional methods to assess proliferation rely on random sampling of tissue by biopsy and subsequent histological assessment of proliferative markers. In addition to potential complications from biopsy, information gleaned by this analysis may be misleading because it does not reflect tissue heterogeneity. The positron emission tomography (PET) tracer 3'-deoxy-3' [^{18}F]-fluorothymidine ([^{18}F]FLT) is commonly used as an imaging biomarker of proliferation in oncology, especially for monitoring response in interventional studies [4–6]. [^{18}F]FLT is transported across cell membranes by nucleoside transporters and then phosphorylated by the enzyme thymidine kinase 1 (TK1). Following phosphorylation, [^{18}F]FLT-monophosphate is trapped and accumulates within the cell without being incorporated into DNA [7, 8]. TK1 is predominantly expressed during the DNA synthesis (S) phase of the cell cycle, but is virtually absent in quiescent cells, forming the basis of [^{18}F]FLT as a proliferation tracer [7–9]. Many groups have evaluated correlation between [^{18}F]FLT accumulation in proliferative tissues and cellular proliferation as assessed by immunohistochemical staining [7], yet clinical evaluation of [^{18}F]FLT as a biomarker of proliferation in disease settings outside of oncology has not been reported.

Ménétrier's disease (MD) is a rare, hyperproliferative disorder of the stomach that has been linked to increased levels of the epidermal growth factor receptor (EGFR) ligand transforming growth factor alpha (TGF- α) and heightened EGFR activity in the gastric mucosa [10–13]. This condition is thought to push differentiating epithelial cells of the gastric unit down the surface mucous cell (pit cell) lineage at the expense of the glandular (parietal and chief cell) lineage. In turn, the allocation of cells is shifted towards the pit such that the normal pit/gland ratio (1:4) is not observed and is frequently inverted. The resulting histological appearance is termed foveolar hyperplasia and is an essential characteristic of MD. Historically, gastrectomy has been considered the only effective treatment option until recent results illustrated the efficacious use of the EGFR neutralizing monoclonal antibody cetuximab [14–16]. A biomarker of response to cetuximab therapy as shown in these studies was reduced proliferation in stomach tissue as measured by Ki67 immunohistochemistry. We subsequently hypothesized that [^{18}F]FLT-PET could serve as a non-invasive biomarker of response to EGFR blockade in MD. Therefore, the goal of this study was to evaluate the relationship between [^{18}F]FLT-PET and both pharmacodynamic and clinical response to cetuximab in a patient with MD.

We illustrate that the extent of MD involvement throughout the stomach could easily be visualized using [^{18}F]FLT-PET, and that response to cetuximab could be followed quantitatively and non-invasively in sequential [^{18}F]FLT-PET studies. Thus, [^{18}F]FLT-PET appears to have potential to monitor response to treatment in this and potentially other hyperproliferative disorders.

MATERIALS AND METHODS

Patients

All studies were approved by the Vanderbilt Institutional Review Board. Written informed consent was received from the patient prior to study enrollment. A 48-year old caucasian female with MD was enrolled in a clinical trial investigating cetuximab for the treatment of refractory MD. The patient was treated with an initial loading dose of cetuximab (400 mg/m²) at week 1 followed by additional treatments (250 mg/m²) at weeks 2, 3, and 4. The patient continued with bi-monthly cetuximab treatments for a total of 15 months. [^{18}F]FLT uptake in normal stomach was quantified in a 47 year old female with rectal cancer but an

otherwise stomach who was enrolled in an unrelated [^{18}F]FLT PET study of patients with colorectal cancer.

Gastric Biopsy—Pinch biopsies of the gastric fundus in the MD patient was performed at multiple time points throughout the study (1 day before treatment, 1 day after treatment, 1 month after treatment, 15 months after treatment). Samples were fixed in buffered formalin overnight and then transferred to 70% ethanol prior to immunohistochemical analysis.

Radiopharmaceutical Synthesis—[^{18}F]FLT (3'-[^{18}F]fluoro-3'-deoxythymidine) was prepared as described previously [17, 18]. [^{18}F]FLT was obtained with average radiochemical purity of 98.3% and specific activity of 128.76 TBq/mmol.

Imaging Study Protocol—Patients received an intravenous injection of 185 MBq of [^{18}F]FLT and then rested for 60 minutes before imaging. Immediately prior to imaging, the MD patient was administered an oral contrast agent (VoLumenTM, Bracco Diagnostics, Inc.). Imaging was performed with an integrated PET/CT scanner (Discovery LS, GE Healthcare), consisting of a combined full-ring PET scanner (Advance NXi) and an eight-section helical CT scanner (Light Speed Plus). The PET/CT scan was performed from the lower chest to the iliac crests. The CT was performed with 140 kV, 80 mA, a tube-rotation time of 0.8 seconds per rotation, a pitch of 1.675 mm/rotation, with CT slice thickness of 3.75 mm at intervals of 3.27 mm and a matrix of 512 by 512 pixels. The CT data were reconstructed with a slice thickness of 5.0 mm and a matrix of 512 by 512 pixels. Patients were allowed normal, quiet breathing during imaging. PET imaging was performed with 5 minutes per table position with two positions acquired. PET data sets were reconstructed iteratively using the ordered subsets expectation maximization (OSEM) algorithm with segmented attenuation correction (two iterations). PET images were up-interpolated by vendor-provided software to match the CT matrix for image fusion. Independent as well as co-registered images were displayed by using a vendor provided workstation (Xeleris; GE Medical Systems). As there is no focal lesion in MD, the maximum standardized uptake value (SUV_{max}) was determined across the entire stomach. The rugal fold thickness along the greater curve of the stomach was measured using the CT images.

Histological Validation—Biopsy samples of the gastric fundus were blocked in paraffin and sectioned prior to immunostaining for proliferation markers including Ki67 (G1 through M-phase marker), proliferating cell nuclear antigen (PCNA, S-phase marker), DNA replication licensing factor MCM6 (MCM6, early G1 through M-phase marker), and p-Histone H3 (M-phase marker), as well as with Periodic-acid Schiff (PAS) stain. Serial sections were incubated with primary monoclonal antibodies for Ki67 (Dako #M7240, 1:100 dilution), PCNA (Biomedex #V1006, 1:1000 dilution), MCM6 (Santa Cruz #sc-9843, 1:1000 dilution) or p-Histone H3 (Cell Signaling #9701, 1:100 dilution). PAS-stained samples were incubated with 0.5% periodic acid followed by Schiff reagent, and then counterstained with hematoxylin. Stained samples were imaged at high magnification and analyzed by a gastrointestinal pathologist (MKW). The proliferative index for each marker was expressed as the average number of positive cells per gland based on counting at least 10 well-oriented glands per sample.

RESULTS

PET/CT imaging

Consistent with previous findings in MD patients [10], abdominal CT images collected one day prior to administration of cetuximab revealed enlargement of rugal folds throughout the stomach (Fig. 1a, Table 1). Subsequent [^{18}F]FLT PET images revealed the physiological

distribution of the tracer in normal liver and bone marrow, as well as significant, focal uptake in stomach tissue (SUV_{max} 5.3; Fig. 1b). Within the stomach itself, tracer accumulation was highest in the fundus and antrum and somewhat less along the greater curvature. Following the imaging session, the patient was administered cetuximab and returned for imaging 24 hr following treatment. One day following the initial treatment with cetuximab, no significant changes were observed in stomach distention or rugal fold thickness by CT imaging (Fig. 1c). However, there was significantly reduced [^{18}F]FLT-PET tracer uptake throughout the stomach (SUV_{max} 1.6; Fig. 1d). Reduced [^{18}F]FLT uptake was also observed after 1 month of treatment (SUV_{max} 2.3; Fig. 1f) and after 15 months of treatment (SUV_{max} 2.4; Fig. 1h). Compared to 1 day post-treatment, [^{18}F]FLT-PET at 1 month and 15 months was modestly increased and appeared to correlate with remodeling of the gastric mucosa and a return to normal pathology. At 1 month, we did not observe significant changes in stomach distention or rugal fold thickness (Fig. 1e). However stomach distention and rugal fold thickness were both markedly reduced at 15 months (Fig. 1g).

In the patient presenting with a normal stomach, CT imaging of the stomach was unremarkable with a normal fasting appearance with normal appearing mucosa and gastric wall (Fig. 2a). Modest [^{18}F]FLT uptake was observed in the stomach (SUV_{max} 1.2; Fig. 2b, Table 1) as well as characteristic uptake in the liver and bone marrow.

Histology

Prior to treatment, histological samples from the MD patient revealed significant foveolar hyperplasia, with marked thickening of the gastric mucosa and an inverted pit/gland ratio (Fig. 3), which were both distinct from normal stomach tissue (Table 1). Gastric biopsy samples in MD showed elevated levels of proliferation markers Ki67, PCNA, MCM6, and p-Histone H3 (Fig. 4, Table 1) compared to normal tissue. In agreement with the measured decrease in post-treatment [^{18}F]FLT uptake, all histological metrics ultimately showed a marked reduction over the course of treatment (Table 1). However, like [^{18}F]FLT-PET, all markers except PCNA remained somewhat elevated following 15 months of treatment when compared to normal stomach tissue. In evaluating pharmacodynamic response (1 day following cetuximab), Ki67, MCM6, and p-Histone H3 appeared to correlate well with decreased [^{18}F]FLT-PET. Interestingly, PCNA and p-Histone H3 appeared to exhibit better correlations with [^{18}F]FLT-PET than Ki67 or MCM6 when comparing pre-treatment levels with measurements taken at steady-state cetuximab levels (e.g., 1 month and 15 months post-treatment). Histological normalization of stomach architecture, with near complete resolution of foveolar hyperplasia, decreased thickness of the gastric mucosa, and decreased pit/gland ratio was observed at 15 months post-treatment (Fig. 3, Table 1).

DISCUSSION

To our knowledge, this study reports the first clinical use of [^{18}F]FLT-PET to assess proliferation in a non-malignant disorder. This study suggests that pharmacodynamic response to EGFR blockade and clinical resolution of MD can be predicted by [^{18}F]FLT-PET. This finding is not unexpected given that EGFR inhibition and attendant downstream mitogen-activated protein kinase (MAPK) pathway inhibition can result in reduced cellular proliferation in a cyclin D1-CDK4/6-pRb-E2F-TK1 dependent fashion [19]. Signaling cascades involved in MD are incompletely described, although it has been suggested that downstream signaling in MD could proceed through EGFR-HER3 heterodimeric activation of the phosphatidylinositol 3-kinase (PI3K) pathway [10]. Activation of PI3K can affect cell cycle in complex ways including targeted degradation of cell cycle inhibitors (e.g., p27) and mammalian target of rapamycin (mTOR) -mediated activation of pro-translational molecules at the ribosomal cap (e.g., eIF4E) [15]. Importantly for early prediction of response associated with pharmacodynamic target inhibition, reduced [^{18}F]FLT uptake was observed

as early as 24 hr following initiation of cetuximab therapy, somewhat earlier than a portion of the tissue-based markers evaluated in this study. Post-treatment [^{18}F]FLT-PET collected 1 day following the first administration of cetuximab predicted the ultimate resolution of foveolar hyperplasia and normalization of the gastric architecture in this patient. PET also correlated, albeit to varying degrees, with proliferative indices measured throughout the treatment cycle. At the conclusion of therapy (15 months), the patient was asymptomatic and exhibited a nearly histologically normal gastric fundus. At this time, [^{18}F]FLT uptake was reduced by 56% from measurements prior to cetuximab therapy. Over the course of therapy, [^{18}F]FLT-PET was able to provide a more sensitive and global assessment of MD in this patient compared to random biopsy alone.

CONCLUSION

This study suggests that [^{18}F]FLT-PET may improve therapeutic management of patients with MD and accelerate clinical development of therapeutic approaches for these patients and potentially other premalignant, hyperproliferative disorders.

Acknowledgments

Funding:

P50-951903; R01-CA140628; K25-CA127349; RC1-CA145138; R01-CA46413; The Kleberg Foundation.

The authors wish to acknowledge Mr. M. Sib Ansari, Dr. Ronald M. Baldwin and Dr. Michael L. Nickels of the VUIIS Research Radiochemistry Core Facility for production of [^{18}F]FLT, Mr. Frank Revetta of the Vanderbilt University Department of Pathology for histological expertise.

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FIGURE 1. Cetuximab therapy in Ménétrier's disease can be monitored by non-invasive imaging

Coronal CT (left) and [^{18}F]FLT-PET (right) images of a patient with Ménétrier's disease collected one day before (a/b), one day following (c/d), one month following (e/f), and 15 months following (g/h) initiation of cetuximab therapy. [^{18}F]FLT uptake in the stomach (white arrows) is significantly reduced at all imaging time points following treatment.

FIGURE 2. Imaging in normal stomach

Coronal CT (a) and [^{18}F]FLT-PET (b) images of a patient with a normal stomach demonstrate lower [^{18}F]FLT-PET uptake compared to the pre-treatment [^{18}F]FLT-PET scan in the Ménétrier's patient.

FIGURE 3. Treatment with cetuximab results in gradual remodeling and normalization of gastric architecture

(a) Prior to therapy, stomach biopsy revealed marked foveolar hyperplasia with deep, tortuous pits (arrow). (b, c) Samples taken at 1 day and 1 month following therapy show a gradual normalization of pit structure and a resolution of foveolar hyperplasia. (d) At 15 months post-therapy, the stomach has returned to a more histologically normal appearance, with shallow pits (arrow) and a normal pit/gland ratio.

FIGURE 4. Cetuximab therapy results in progressively decreased staining of proliferation markers over the course of treatment

Cetuximab therapy results in progressively decreased staining of proliferation markers over the course of treatment. Prior to therapy, samples exhibit elevated levels of Ki67, PCNA, MCM6, and p-Histone H3. Following initiation of treatment, samples begin to exhibit normalization of the gastric architecture. By 15 months, levels of all proliferation markers are significantly decreased from pre-treatment levels. Corresponding tissue samples are shown for normal stomach tissue. Quantitative results can be seen in Table 1.

Table 1

Quantitative imaging results from [¹⁸F]FLT-PET imaging and CT imaging, and immunohistochemistry results for proliferation markers and anatomical features of the stomach in Ménétrier's disease, and normal stomach.

	MD				Normal
	1 day pre-	1 day post-	1 month post-	15 months post-	
[¹⁸ F]FLT uptake (SUV _{max})	5.3	1.6	2.3	2.4	1.2
Rugal fold thickness (mm)	9	9	9	6	-
Ki67 (% + cells/field)	46.7	37.7	46.2	25.8	6.9
PCNA (% + cells/field)	34.9	42.8	14.9	9.2	10.9
MCM6 (% + cells/field)	62.2	43.5	38.2	4.7	17.7
p-His H3 (% + cells/field)	14.1	8.7	8.5	7.0	3.1
Mucosal thickness (mm)	2.14	1.98	1.60	1.12	0.78
Pit/gland ratio	1.29	1.00	0.88	0.49	0.21