Published in final edited form as:

Ann Nucl Med. 2015 July ; 29(6): 535–542. doi:10.1007/s12149-015-0974-6.

[¹⁸F]-FLT PET to predict early response to neoadjuvant therapy in *KRAS* wild-type rectal cancer: a pilot study

Eliot T. McKinley^{1,2}, Jennifer M. Watchmaker^{1,3}, A. Bapsi Chakravarthy^{4,5}, Jeffrey A. Meyerhardt⁶, Jeffrey A. Engelman⁶, Ronald C. Walker^{1,4,7,8}, M. Kay Washington^{4,9}, Robert J. Coffey^{2,4}, and H. Charles Manning^{1,3,7,11,12}

¹Vanderbilt Institute of Imaging Science (VUIIS), Vanderbilt University Medical School, Nashville, TN

²Department of Medicine, Vanderbilt University Medical School, Nashville, TN

³Program in Chemical and Physical Biology, Vanderbilt University, Nashville, TN

⁴Vanderbilt Ingram Cancer Center, Vanderbilt University Medical School, Nashville, TN

⁵ Department of Radiation Oncology, Vanderbilt University Medical School, Nashville, TN

⁶Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

⁷ Department of Radiology and Radiological Sciences, Vanderbilt University Medical School, Nashville, TN

⁸Radiology Service, Tennessee Valley VA Healthcare System, Nashville, TN

⁹ Department of Pathology, Vanderbilt University Medical School, Nashville, TN

¹¹Department of Neurosurgery, Vanderbilt University Medical School, Nashville, TN

¹²Department of Biomedical Engineering, Vanderbilt University, Nashville, TN

Abstract

Object—This pilot study evaluated the utility of 3'-deoxy-3' [¹⁸F]-fluorothymidine ([¹⁸F]-FLT) positron emission tomography (PET) to predict response to neoadjuvant therapy that included cetuximab in patients with wild-type *KRAS* rectal cancers.

Methods—Baseline [¹⁸F]-FLT PET was collected prior to treatment initiation. Follow-up [¹⁸F]-FLT was collected after three weekly infusions of cetuximab, and following a combined regimen of cetuximab, 5-FU, and radiation. Imaging-matched biopsies were collected with each PET study.

Results—Diminished [¹⁸F]-FLT PET was observed in 3/4 of patients following cetuximab treatment alone and in all patients following combination therapy. Reduced [¹⁸F]-FLT PET following combination therapy predicted disease-free status at surgery. Overall, [¹⁸F]-FLT PET agreed with Ki67 immunoreactivity from biopsy samples and surgically resected tissue and was predictive of treatment-induced rise in p27 levels.

Conclusion—These results suggest that [^{18}F]-FLT PET is a promising imaging biomarker to predict response to neoadjuvant therapy that included EGFR blockade with cetuximab in patients with rectal cancer.

Introduction

Standard imaging criteria for evaluating therapeutic response are based upon anatomical information according to Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [1]. These criteria, which are based solely on a reduction in tumor size, do not take advantage of cellular and molecular information now available through contemporary imaging methodologies. Importantly, since relevant cellular and molecular changes may precede changes in size and occur within hours of treatment, RECIST criteria and conventional imaging methods are frequently inadequate for assessing early tumor response. These limitations, coupled with the increasing clinical relevance of employing complex, molecularly targeted therapeutic regimens to treat cancer, highlight a critical need to accelerate the translation of novel imaging approaches that are capable of reporting cellular and molecular responses of tumor cells to therapy.

The widely used positron emission tomography (PET) tracer 2-deoxy-2-([^{18}F])fluoro-D-glucose ([^{18}F]-FDG) is an important tool for cancer diagnosis and staging. [^{18}F]-FDG uptake and image contrast is predicated on increased glucose metabolism in neoplastic tissues as compared to normal tissue. However, [^{18}F]-FDG tissue uptake broadly reflects a host of metabolic processes, highlighting an unmet clinical need for imaging methods that more directly measure proliferation.

The PET tracer 3'-deoxy-3'([^{18}F])fluorothymidine ([^{18}F]-FLT) has been proposed as a potential imaging biomarker of proliferation in oncology, especially to predict response to therapy in clinical trials and drug development [2, 3]. [^{18}F]-FLT PET serves as a marker of proliferation by reporting on the activity of the thymidine salvage pathway. Upon cellular internalization by nucleoside transporters, [^{18}F]-FLT is phosphorylated by thymidine kinase 1 (TK1). [^{18}F]-FLT-monophosphate is trapped and accumulates in the cell resulting in imaging contrast. However, unlike thymidine, [^{18}F]-FLT is not incorporated into the DNA. TK1 is primarily expressed during DNA synthesis (S-phase) and is diminished in quiescent cells, forming the basis of the use of [^{18}F]-FLT PET as a proliferation marker. [^{18}F]-FLT PET has been evaluated in treatment response studies in pre-clinical [4-6] and clinical [7-10] studies. As [^{18}F]-FLT PET measures activity of the thymidine salvage pathway, it may reflect proliferative indices to variable extents, especially when cells utilize *de novo* thymidine synthesis mechanisms. Therefore, [^{18}F]-FLT PET should not be universally considered a surrogate of proliferative index [11, 12]. Nonetheless, [^{18}F]-FLT PET may reflect important cellular and molecular events associated with response to therapy, such as elevated p27, a critical cell cycle inhibitor [13, 14].

The epidermal growth factor receptor (EGFR, HER1, ErbB-1) is frequently over-expressed in colorectal cancer (CRC) and, as such, has become an important target for therapy in advanced CRC [15]. A number of small molecule inhibitors of EGFR tyrosine kinase inhibitors have been developed, and have shown promise in many settings such as mutant

EGFR lung cancer [16]. However, trials of EGFR tyrosine kinase inhibitors have not been successful in CRC [17]. Treatment of CRC with monoclonal antibodies, such as cetuximab (Erbix), has shown more promise clinically when used in patients with metastatic disease whose tumors express wild-type *KRAS* [18-20]. We have previously evaluated [^{18}F]-FLT PET to assess treatment response to cetuximab in preclinical [4] and clinical studies [8]. The goal of this pilot study was to evaluate [^{18}F]-FLT PET to predict treatment response in a phase II neoadjuvant clinical trial of cetuximab followed by combined cetuximab and chemoradiotherapy in patients with advanced rectal cancer.

Materials and Methods

Patients

All studies were approved by the Vanderbilt Institutional Review Board (ClinicalTrials Identifier: NCT01207895). Written informed consent was obtained from the patients prior to patient enrollment. Patients with histologically confirmed wild-type *KRAS*-expressing rectal tumors were enrolled in a multi-center clinical trial investigating the benefits of adding cetuximab to standard neoadjuvant chemoradiotherapy (NCT01207895) at Vanderbilt University Medical Center. A summary of patient characteristics can be found in **Table 1**. The study schema is shown in **Fig. 1**. A pre-treatment [^{18}F]-FLT PET/CT study and tissue needle biopsy were obtained immediately prior to treatment with a loading dose of 400 mg/m² cetuximab, followed by weekly infusions of 250 mg/m². A second [^{18}F]-FLT PET/CT study and tissue needle biopsy were obtained after the third cetuximab infusion, and prior to the initiation of chemotherapy and radiation. Cetuximab infusions continued weekly combined with continuous infusion of 225mg/m²/day fluorouracil (5-FU) and radiation therapy to a total dose of 50.4 Gy in 28 fractions. A third [^{18}F]-FLT PET/CT image was obtained prior to surgical resection of the tumor. Additionally, [^{18}F]-FLT PET uptake in the normal rectum was measured in three patients enrolled in a separate imaging study.

Radiochemical synthesis

[^{18}F]-FLT was prepared from [^{18}F]-fluoride in a two-step, one-pot reaction as previously described [4, 21] using a GE TRACERlab FX-FN automated module. Aqueous [^{18}F]-fluoride was eluted with Kryptofix-222 and K₂CO₃ in CH₃CN/H₂O into the reaction vessel. Three sequences of heating (110°C) with He(g) flow resulted in dry [^{18}F]-fluoride/Kryptofix-222/K₂CO₃. The cyclic precursor 2,3'-anhydro-5'-*O*-benzoyl-2'-deoxythymidine (ABX Advanced Biochemical Compounds) was added in DMSO and reacted for 10 min at 160°C. The benzoyl-protecting group was removed from the labeled intermediate by basic hydrolysis (0.25 mol/L NaOH, 50°C, 10 minutes). The reaction mixture was purified on a semi-preparative C-18 high-performance liquid chromatography column eluting with 10% ethanol/10 mmol/L sodium phosphate buffer and sterilized by 0.2 µm membrane filtration. Radiochemical identity, purity, and specific activity were determined by analytic high-performance liquid chromatography. The [^{18}F]-FLT product was obtained with average radiochemical purity of 98.3% and specific activity of 345.5 TBq/mmol.

[¹⁸F]-FLT PET/CT Imaging

Patients received an intravenous injection of 185 MBq of [¹⁸F]-FLT and then rested for 60 minutes prior to imaging. 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 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 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). Images were evaluated by a qualified nuclear medicine physician (RCW).

Immunohistochemistry

Biopsy samples were blocked in paraffin and sectioned prior to immunostaining for proliferation markers including Ki67, TK1, and p27. Five micrometer thick sections were incubated with primary monoclonal antibodies for Ki67 (Dako #M7240, 1:100 dilution), TK1 (Abcam #57757, 1:100 dilution) and p27 (Dako #M7203, 1:100 dilution). Briefly, tissues were deparaffinized, rehydrated, and antigen retrieval was performed using a citrate buffer solution (pH 6.0) applied for 15 minutes at 105° C followed by a 10 minute cool down at room temperature. A solution of 3% H₂O₂ was used to eliminate endogenous peroxidase activity and then blocked with a serum-free protein blocking reagent for 20 minutes. For detection of primary antibodies, tissue sections were incubated for 60 minutes at room temperature at the dilutions noted above. Subsequently, samples were incubated for 30 minutes utilizing the Envision+ System-HRP Labeled Polymer detection method. Staining was completed after incubation with a DAB substrate-chromagen solution. Stained samples were evaluated by a gastrointestinal pathologist (MKW) and imaged at high magnification for reproduction.

Tumor genotyping

DNA was extracted from paraffin-fixed, formalin-embedded samples from each patient enrolled in the study. Tumor genotypes were assayed from 20 ng DNA using the SNaPshot mutational profiling method which consists of multiplexed PCR, multiplexed single-base primer extension, and capillary electrophoresis [22-24]. The current assay was designed to detect 62 point mutations in 7 genes (**Table S1**). Briefly, PCR primers were pooled to amplify the target DNA, and PCR was performed using the following conditions: 95°C (8 min), followed by 40 cycles of 95°C (20 sec), 58°C (30 sec) and 72°C (1 min), and then a final extension of 72°C (3 min). Next, PAGE-purified primers were pooled together and multiplex single-base extension reactions were performed on Exo-SAP-it treated (USB) PCR products using the following conditions: 96°C (30 sec), followed by 35 cycles of 96°C (10 sec), 50°C (5 sec), and 60°C (30 sec). Extension products were applied to capillary

electrophoresis in an ABI 3730 analyzer and the data were interpreted using ABI GeneMapper software (version 4.0). Human male genomic DNA (Promega) was used as a wild type control. Spiking primers were mixed to create a pan-positive control mix for the assay.

Statistical methods

Statistical significance of data were evaluated using the non-parametric Wilcoxon Rank Sum (Mann-Whitney U) tests in GraphPad Prism 4. Data were considered significant if $p < 0.05$.

Results

[¹⁸F]-FLT PET/CT Imaging

Five patients with a confirmed wild-type *KRAS* rectal cancer were enrolled in the imaging study. Pre-treatment [¹⁸F]-FLT PET scans were acquired for each patient. The typical biodistribution of [¹⁸F]-FLT was observed in all patients, with elevated activity in bone marrow, urinary excretion, and accumulation in the liver. Elevated [¹⁸F]-FLT uptake was observed in all rectal tumors, with SUV_{max} ranging from 5.03 to 9.62 (**Table 1, Fig. 2**) prior to treatment. Given this elevated uptake compared to surrounding normal rectum, all rectal cancers in this study were easily visualized with [¹⁸F]-FLT PET. [¹⁸F]-FLT uptake in rectal tumors was approximately 7.5 times higher than normal rectum measured in patients without rectal cancer (7.47 vs. 0.98 SUV_{max} , $p = 0.04$). One patient completed baseline imaging and subsequently was withdrawn from the study following an anaphylactic reaction to cetuximab [25, 26]. [¹⁸F]-FLT biodistributions were similar to pre-treatment levels throughout normal tissue after cetuximab treatment alone. Following cetuximab monotherapy, [¹⁸F]-FLT uptake in rectal tumors was reduced in 3/4 patients by 20%, 55%, and 25% in patients, but was elevated by 45% in one patient relative to the pre-treatment scan (**Fig. 3**). After combined cetuximab and chemoradiotherapy, [¹⁸F]-FLT uptake in rectal tumors was reduced by over 70% in all patients compared to the pre-treatment scans. Additionally, reduced [¹⁸F]-FLT uptake was observed in the bone marrow in the final post-treatment scan consistent with radiotherapy [27]. Due to the nature of the rectal tumors, the longest dimension of the tumors could not be reliably measured by CT in these patients.

Immunohistochemistry

Representative high-power fields of Ki67, p27, and TK1 IHC for tumor specimens evaluated in this study are shown in **Fig. 4** and **Fig. 5**. At surgery, Patient 1 demonstrated almost complete reduction in tumor volume, however, as a very small number of tumor cells were histologically detected, the patient was classified as a pathological partial response (PR) partial response to therapy. As patients 2-5 showed pathological complete response (CR) to therapy, no tumor tissue was available to analyze following combined cetuximab and chemoradiotherapy (**Table 1**). In all patients, prominent Ki67 IHC staining was observed in pre-treatment biopsies. Reduced Ki67 was observed in the patients that demonstrated reduced [¹⁸F]-FLT tumor uptake following cetuximab. Patient 3 who also did not exhibit reduced [¹⁸F]-FLT PET at this time point also showed no reduction in Ki67 immunoreactivity. Prior to cetuximab treatment, weak p27 immunostaining was observed in all tumor samples. Cetuximab treatment resulted in elevated nuclear p27 in all patients that

exhibited decreased [^{18}F]-FLT PET, implying that [^{18}F]-FLT PET reflected p27-dependent cell cycle inhibition. In patient 3, where [^{18}F]-FLT PET was not decreased following cetuximab, p27 levels were unchanged from baseline. In agreement with imaging, tumors exhibited robust TK1 immunoreactivity prior to treatment (**Fig. 4**). In agreement with [^{18}F]-FLT PET, fewer TK1-positive cells were observed in patients with decreased [^{18}F]-FLT tumor uptake. Similarly, reduced TK1 immunoreactivity was also observed following combined cetuximab and chemoradiotherapy.

Tumor genotyping

No point mutations in *KRAS*, *BRAF*, *AKT*, *PIK3CA*, *SMAD4*, *PTEN*, or *NRAS* were detected in any tumor sample evaluated in this study (**Table S2**).

Discussion

These studies mirror our previous clinical experience using [^{18}F]-FLT PET to predict pharmacodynamic response to cetuximab in Ménétrier's disease, a rare, premalignant, hyperproliferative disorder of the stomach [8]. We found that [^{18}F]-FLT PET was useful to visualize hyperproliferative tissue, as well as to predict early response to cetuximab therapy.

This study represents the first clinical evaluation of [^{18}F]-FLT PET to predict response to neoadjuvant therapy in rectal cancer that includes EGFR blockade with cetuximab. Historically, there have been concerns that [^{18}F]-FLT PET for tumor detection may be limited by the modest uptake of the tracer in many tissues with respect to surrounding normal tissue [11, 12]. Interestingly, however, this phenomenon was not observed with the rectal cancers evaluated in this study, which exhibited excellent signal to noise ratios. Our findings are in agreement with those of others (28, 29), which confirm that [^{18}F]-FLT PET is well-suited for imaging rectal cancer. All patients who completed the study demonstrated a clinical response to therapy. Additionally, [^{18}F]-FLT PET imaging was of added value to CT imaging as [^{18}F]-FLT PET aided in the visualization of rectal tumors that may have been missed with CT alone. Interestingly, [^{18}F]-FLT PET was reduced in 3 of the 4 patients following cetuximab monotherapy, yet one patient exhibited elevated [^{18}F]-FLT uptake at this time point. Overall, we interpret these findings to suggest that [^{18}F]-FLT PET reflected mechanistic insight into tumor responses to cetuximab given that imaging accurately reflected post-treatment levels of p27, an inhibitor of cell cycle. In fact, [^{18}F]-FLT PET results, supported by Ki67 and p27 immunoreactivity, suggest that patient 3 did not experience an anti-proliferative benefit from cetuximab as monotherapy. However, this patient did ultimately benefit from the combined neoadjuvant treatment regimen. A notable limitation of the present study is the small sample size. Building upon the results presented here, we envision that in future studies employing similar regimens, post-cetuximab [^{18}F]-FLT PET could potentially be incorporated as a non-invasive assay to predict those patients most likely to benefit from this drug prior to changes in tumor size ascertainable by anatomical imaging measures, such as CT.

At the conclusion of cetuximab, 5-FU, and radiation therapy, [^{18}F]-FLT uptake was reduced by at least 65% from baseline in all four patients. Given that these patients had received cetuximab as well as chemoradiotherapy, it is not possible to be sure what specific role

cetuximab played in modulating [^{18}F]-FLT PET at this third time point where an enhanced decrease in [^{18}F]-FLT uptake was observed relative to cetuximab alone. However, previously studies have shown that [^{18}F]-FLT PET was reduced following chemoradiotherapy in rectal tumors regardless of clinical outcome [28]. Notably, in this study, reduced [^{18}F]-FLT PET following chemoradiotherapy correlated with complete response, but the limited number of patients evaluated here does not warrant a definitive conclusion.

Conclusion

This pilot study represents the first clinical evaluation of [^{18}F]-FLT PET to predict response to neoadjuvant therapy that included EGFR blockade with cetuximab in patients with rectal cancer. We found that [^{18}F]-FLT PET accurately reflected proliferation in this setting and correlated with increased tumor levels of p27. Our results suggest that [^{18}F]-FLT PET is a promising imaging biomarker of treatment response in this setting and may provide insights into mechanism of response the targeted therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge funding from the National Institutes of Health (R25 CA136440, R25 CA092043, K25 CA127349, P50 CA128323, P50 CA095103, R01 CA140628, RC1 CA145138, P30 DK058404, P50 CA127003, UL1 TR000445), the Kleberg Foundation, and Bristol Meyers Squib. The authors acknowledge Frank Revetta for assistance with immunohistochemistry.

References

1. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009; 45(2):228–47. [PubMed: 19097774]
2. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, et al. Imaging proliferation in vivo with [^{18}F]-FLT and positron emission tomography. *Nat Med*. 1998; 4(11):1334–6. Epub 1998/11/11. [PubMed: 9809561]
3. Shields AF. PET imaging with ^{18}F -FLT and thymidine analogs: promise and pitfalls. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2003; 44(9):1432–4.
4. Manning HC, Merchant NB, Foutch AC, Virostko JM, Wyatt SK, Shah C, et al. Molecular imaging of therapeutic response to epidermal growth factor receptor blockade in colorectal cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2008; 14(22):7413–22. [PubMed: 19010858]
5. Shah C, Miller TW, Wyatt SK, McKinley ET, Olivares MG, Sanchez V, et al. Imaging biomarkers predict response to anti-HER2 (ErbB2) therapy in preclinical models of breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2009; 15(14):4712–21. [PubMed: 19584166]
6. Kim SJ, Lee JS, Im KC, Kim SY, Park SA, Lee SJ, et al. Kinetic modeling of 3'-deoxy-3'- ^{18}F -fluorothymidine for quantitative cell proliferation imaging in subcutaneous tumor models in mice. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2008; 49(12):2057–66.
7. Chen W, Delaloye S, Silverman DH, Geist C, Czernin J, Sayre J, et al. Predicting treatment response of malignant gliomas to bevacizumab and irinotecan by imaging proliferation with [^{18}F]

- fluorothymidine positron emission tomography: a pilot study. *J Clin Oncol.* 2007; 25(30):4714–21. [PubMed: 17947718]
8. McKinley ET, Smith RA, Tanksley JP, Washington MK, Walker R, Coffey RJ, et al. [18F]FLT-PET to predict pharmacodynamic and clinical response to cetuximab therapy in Menetrier's disease. *Ann Nucl Med.* 2012; 26(9):757–63. [PubMed: 22821337]
 9. Muzi M, Spence AM, O'Sullivan F, Mankoff DA, Wells JM, Grierson JR, et al. Kinetic analysis of 3'-deoxy-3'-18F-fluorothymidine in patients with gliomas. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2006; 47(10):1612–21.
 10. Soloviev D, Lewis D, Honess D, Aboagye E. [(18)F]FLT: an imaging biomarker of tumour proliferation for assessment of tumour response to treatment. *Eur J Cancer.* 2012; 48(4):416–24. [PubMed: 22209266]
 11. McKinley ET, Ayers GD, Smith RA, Saleh SA, Zhao P, Washington MK, et al. Limits of [18F]-FLT PET as a biomarker of proliferation in oncology. *PLoS One.* 2013; 8(3):e58938. [PubMed: 23554961]
 12. Zhang CC, Yan Z, Li W, Kuszpit K, Painter CL, Zhang Q, et al. [(18)F]FLT-PET imaging does not always "light up" proliferating tumor cells. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2012; 18(5):1303–12. [PubMed: 22170262]
 13. McKinley ET, Zhao P, Coffey RJ, Washington MK, Manning HC. 3'-Deoxy-3'-[18F]-Fluorothymidine PET imaging reflects PI3K-mTOR-mediated pro-survival response to targeted therapy in colorectal cancer. *PLoS One.* 2014; 9(9):e108193. [PubMed: 25247710]
 14. Aide N, Kinross K, Cullinane C, Roselt P, Waldeck K, Neels O, et al. 18F-FLT PET as a surrogate marker of drug efficacy during mTOR inhibition by everolimus in a preclinical cisplatin-resistant ovarian tumor model. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2010; 51(10):1559–64.
 15. Zhu Z. Targeted cancer therapies based on antibodies directed against epidermal growth factor receptor: status and perspectives. *Acta pharmacologica Sinica.* 2007; 28(9):1476–93. [PubMed: 17723181]
 16. Cappuzzo F, Ciuleanu T, Stelmakh L, Cicenias S, Szczesna A, Juhasz E, et al. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. *The lancet oncology.* 2010; 11(6):521–9. [PubMed: 20493771]
 17. Rothenberg ML, LaFleur B, Levy DE, Washington MK, Morgan-Meadows SL, Ramanathan RK, et al. Randomized phase II trial of the clinical and biological effects of two dose levels of gefitinib in patients with recurrent colorectal adenocarcinoma. *J Clin Oncol.* 2005; 23(36):9265–74. [PubMed: 16361624]
 18. Saltz LB, Meropol NJ, Loehrer PJ Sr. Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol.* 2004; 22(7):1201–8. [PubMed: 14993230]
 19. Lenz HJ, Van Cutsem E, Khambata-Ford S, Mayer RJ, Gold P, Stella P, et al. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. *J Clin Oncol.* 2006; 24(30):4914–21. [PubMed: 17050875]
 20. Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol.* 2007; 25(22):3230–7. [PubMed: 17664471]
 21. Oh SJ, Mosdzianowski C, Chi DY, Kim JY, Kang SH, Ryu JS, et al. Fully automated synthesis system of 3'-deoxy-3'-[18F]fluorothymidine. *Nucl Med Biol.* 2004; 31(6):803–9. [PubMed: 15246372]
 22. Dias-Santagata D, Akhavanfard S, David SS, Vernovsky K, Kuhlmann G, Boisvert SL, et al. Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. *EMBO Mol Med.* 2010; 2(5):146–58. [PubMed: 20432502]
 23. Su Z, Dias-Santagata D, Duke M, Hutchinson K, Lin YL, Borger DR, et al. A platform for rapid detection of multiple oncogenic mutations with relevance to targeted therapy in non-small-cell lung cancer. *The Journal of molecular diagnostics: JMD.* 2011; 13(1):74–84. [PubMed: 21227397]

24. Lovly CM, Dahlman KB, Fohn LE, Su Z, Dias-Santagata D, Hicks DJ, et al. Routine multiplex mutational profiling of melanomas enables enrollment in genotype-driven therapeutic trials. *PLoS One*. 2012; 7(4):e35309. [PubMed: 22536370]
25. O'Neil BH, Allen R, Spigel DR, Stinchcombe TE, Moore DT, Berlin JD, et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. *J Clin Oncol*. 2007; 25(24):3644–8. [PubMed: 17704414]
26. Patel DD, Goldberg RM. Cetuximab-associated infusion reactions: pathology and management. *Oncology (Williston Park)*. 2006; 20(11):1373–82. discussion 82, 92-4, 97. [PubMed: 17112000]
27. Agool A, Slart RH, Thorp KK, Glaudemans AW, Cobben DC, Been LB, et al. Effect of radiotherapy and chemotherapy on bone marrow activity: a 18F-FLT-PET study. *Nucl Med Commun*. 2011; 32(1):17–22. [PubMed: 21166089]
28. Wieder HA, Geinitz H, Rosenberg R, Lordick F, Becker K, Stahl A, et al. PET imaging with [18F]3'-deoxy-3'-fluorothymidine for prediction of response to neoadjuvant treatment in patients with rectal cancer. *Eur J Nucl Med Mol Imaging*. 2007; 34(6):878–83. [PubMed: 17195074]
29. Dehdashti F, Grigsby PW, Myerson RJ, Nalbantoglu I, Ma C, Siegel BA. Positron emission tomography with [(18)F]-3'-deoxy-3'-fluorothymidine (FLT) as a predictor of outcome in patients with locally advanced resectable rectal cancer: a pilot study. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging*. 2013; 15(1):106–13. [PubMed: 22684813]

Fig. 1. Study schema

Baseline [^{18}F]-FLT PET/CT scan and tissue biopsy were collected prior to treatment. A second [^{18}F]-FLT PET/CT scan were collected following three weekly infusions of cetuximab. Subsequently, patients received continuous infusion of 5-FU and radiotherapy in conjunction with weekly cetuximab. Prior to surgery, a final [^{18}F]-FLT PET/CT scan was obtained and tissue was collected from the surgical resection.

Fig. 2. $[^{18}\text{F}]$ -FLT PET/CT imaging

Transverse, coregistered $[^{18}\text{F}]$ -FLT PET/CT images for each patient completing the study are shown at pre-treatment, post-cetuximab therapy, and after combined cetuximab-chemoradiotherapy. Tumors are denoted by arrow. Serial intra-patient images windowed relative to baseline.

Fig. 3. Quantification of $[^{18}\text{F}]\text{-FLT}$ PET imaging
 $[^{18}\text{F}]\text{-FLT}$ uptake in rectal cancer (SUV_{max}) at each imaging time point.

Fig. 4. Ki67, p27, and TK1 immunohistochemistry in patient 1

Representative 40x fields are shown. Ki67 IHC staining was reduced from pre-treatment levels following both single and combined therapy. Increased p27 immunoreactivity was observed after cetuximab monotherapy compared to pre-treatment, and p27 was further increased after completion of cetuximab and chemoradiotherapy. TK1 staining was greatly reduced at both time points.

Fig. 5. Ki67 and p27 immunohistochemistry in patients 3-5

Representative 40x fields are shown. Prior to treatment Ki67 staining was observed in almost all tumor cells for all patients. Almost no p27 immunoreactivity was detected in any patient prior to treatment. After cetuximab, Ki67 staining was not changed in patient 3 or 4, however, was greatly reduced in patient 5. p27 staining was inversely related to [^{18}F]-FLT PET. In patient 3 when [^{18}F]-FLT PET was not reduced following cetuximab treatment, no p27 induction was observed. However, patients 4 and 5, where ^{18}F -FLT PET was reduced after treatment, increased p27 staining was seen compared to pre-treatment tissue. No tissue was available from these patients at surgery following cetuximab and chemoradiotherapy treatment.

Table 1

Patient characteristics, [^{18}F]-FLT PET imaging, and clinical results.

Patient ID	Sex	Age	Baseline ^{18}F -FLT PET (SUV _{max})	Post cetuximab ^{18}F -FLT PET (SUV _{max})	Change from baseline ^{18}F -FLT PET	Post cetuximab and chemoradiotherapy ^{18}F -FLT PET (SUV _{max})	Change from baseline ^{18}F -FLT PET	Clinical Outcome
1	M	39	6.66	5.45	-18.24%	2.21	-66.82%	PR
2	F	45	5.03	N/A				
3	F	48	7.29	10.58	45.06%	1.84	-74.79%	CR
4	M	58	9.62	4.29	-55.4%	2.51	-73.94%	CR
5	M	62	8.77	6.52	-25.59%	1.8	-79.5%	CR