PROTOCOL # 2006-127
04/22/2009; Revised 03/31/2011; Revised 02/11/2014; Revised 07/01/2015

WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE
Karmanos Cancer Institute

2006-127 USE OF [F-18] FLT FOR IMAGING WITH POSITRON EMISSION TOMOGRAPHY (PET)

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FAST FACTS

TITLE: Study #2006-127 [F-18] FLUOROTHYMIDINE (FLT) IMAGING WITH PET

OBJECTIVES:

This study will evaluate the use of [F-18] FLT and PET to measure the DNA synthetic pathway in patients to determine the efficacy of this approach in detecting lesions and their response to therapy. Images obtained with FLT PET may be compared to those obtained with conventional radiology obtained as part of routine care. We may also compare imaging proliferation using [F-18] FLT to measurements of gene expression of proliferative pathways made on tumor specimens obtained at biopsy.

PATIENT POPULATION:

1. Prior histologic diagnosis of cancer or awaiting biopsy or surgery for cancer evaluation of a mass detected on physical exam or standard imaging.
2. Age $\geq$18, not pregnant or lactating.
3. The patient must be able to lie still in the PET scanner.
4. Signed informed consent.

STUDY DESIGN:

Imaging will be done with [F-18]FLT and PET up to four times.

EVALUATION:

Prior to PET Imaging: Physical exam, CBC and Multiphasic drawn within 28 days of PET. A negative serum pregnancy test must be obtained in women with child bearing potential within 7 days of the first PET scan. On the day of the scan the staff will verify and record that the patient has not had unprotected intercourse and does not think that she is pregnant. A repeat serum pregnancy test will be done if the patient can't verify this.

PRIMARY ENDPOINTS:

Measurement of the uptake and retention of [F-18] FLT in tumors and normal organs.

STATISTICAL PLAN: Up to 80 patients will be studied.

ANTICIPATED TOXICITIES: None.

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1.0 Introduction

This study will examine the utility of measuring tumor metabolism in vivo by imaging tumor biosynthesis using [F-18] FLT (3'-deoxy-3'-fluorothymidine). Measurements will be made to determine whether this agent and PET are useful in providing a simplified way of imaging cellular proliferation and thymidine kinase levels. FLT is a thymidine analog that was originally developed for the treatment of HIV. Its structure is similar to that of AZT, but the azido group in the 3' position is replaced by fluorine.

Many studies have been conducted using PET in patients with cancer, but most of these have been done using [F-18] fluorodeoxyglucose (FDG) to measure tumor energetics. FLT has the potential for measuring a specific enzymatic step in the synthesis of DNA, the phosphorylation of thymidine by thymidine kinase. Levels of thymidine kinase are increased several fold as cells enter the S phase and begin DNA synthesis. In this way the quantitation of FLT uptake, and hence thymidine kinase levels, may provide a relative measure of cell proliferation.

In addition, imaging studies are regularly conducted including comparisons of different technologies, such as CT and MRI. The approaches are available in a number of centers and such studies have strengthened the rationale for use of PET in monitoring response in cancer patients. (1, 2)

The FLT compound was originally synthesized as a possible anti-neoplastic compound (3) and subsequently studied as an agent to treat HIV (4) once the activity of the related compound, 3'-azidothymidine (AZT), was noted. FLT was thought to be a very promising agent based in part on its slower clearance from the blood and it's minimal binding to plasma proteins. (5) A number of laboratory and animal studies led to a phase I trial in HIV patients (6), which noted increased marrow and hepatic toxicity from FLT and further clinical development of this agent was discontinued. While this toxicity precluded use of FLT as a therapeutic compound, at the miniscule doses used for imaging, such toxicity was not considered to be an issue when used for PET. Thus FLT was labeled with F-18 (7) and a number of synthetic routes have since been developed that produce high yields, with good specific activity, using techniques that can be automated. (8-10) This has led to the commercial availability of a number of possible precursors.

We did the first study of FLT imaging in dogs and patients and it demonstrated that FLT produced high contrast images of proliferative tissues, such as the bone marrow, as well as in tumors. (11) While there was no breakdown of FLT in either species, in humans there is significant glucuronidation of FLT that needs to be taken into account when kinetic models are being developed and tested. (12, 13) FLT is retained in tissues by the action of TK1, which traps the tracer intracellularly as FLT-monophosphate. (14, 15) A small amount of FLT is incorporated into DNA (<2%), which primarily acts as a chain terminator as DNA is synthesized. (16) TK1 is cytosolic and increases several-fold as cells enter their S-phase. (17) Also present within mitochondria is thymidine kinase 2 (TK2), and FLT is not a substrate for TK2. (18) Therefore FLT demonstrates no visible uptake within the heart unlike labeled thymidine, which demonstrates increased cardiac retention. (19)

In general one can model the retention of FLT using a three-compartment model analogous to that utilized for FDG. (20, 21) Over a one hour period of imaging dephosphorylation (k4) appears to be negligible; although it can be detected by careful analysis if imaging is conducted for two hours. (12) A comparison of the estimates of

the overall flux of FLT using the three parameter fit obtained for 60 minutes compared to the four parameter fit over 120 minutes correlated well (r = 0.95), yet the actual value was consistently underestimated with the shorter imaging time. (22) One must take into account the activity in the blood that is present in FLT and its glucuronide, which can be done with analysis of a single blood sample done at the end of the imaging period. (13) On average over the period of 60 minutes 25% of the FLT is converted to the glucuronide, and the unaltered FLT ranged from 57% to 87% in a series of 19 patients. (12, 13, 23) One can also measure the dynamic uptake of FLT using the graphical analysis approach developed by Patlak and Gjedde for use with (24, 25) FDG. In patients with untreated breast cancer both compartmental and graphical approaches give comparable results (r^2 = 0.98, p < 0.0001) and the graphical approach is computationally simpler to perform (26). Even simpler is just to measure SUV. In patients with breast cancer there was a significant correlation with FLT flux (r^2= 0.85, p = 0.0002). SUV was also found to correlate well with net influx constant in patients with colon cancer (r^2=0.84). (21) In measuring treatment response, although it would certainly be simpler just to measure SUV, this would not take into account differences in the clearance and delivery of FLT to the tumor, which might change during the course of therapy. This study may help to determine if kinetic measurements are necessary for accurate measurement of FLT retention or if SUV measurements are sufficient. Another issue that needs careful consideration is the reproducibility of the FLT measurements. Only limited studies of reproducibility have been done with FDG and have demonstrated errors of less than about 20% (27, 28), similar to preliminary studies with FLT. (29) A study in mice with tumor xenographs, where two scans were done on the same day 6 hours apart, demonstrated a coefficient of variation of 14% $\pm$ 10% when the data was expressed as %ID/g (30). It should be noted that mice have native thymidine levels about 10 fold higher than humans (about 10 microM in mice). Since thymidine may compete for FLT uptake, high thymidine levels in rodents may decrease FLT uptake. In fact this competition has been demonstrated in tissue culture and mice and may vary with mouse strain. (30, 31)

We have studied FLT retention in patients with non-small cell lung cancer (NSCLC) to measure the reproducibility of this approach. Nine patients with NSCLC who were untreated or had progressed after previous therapy were imaged twice using FLT and PET within 2 - 7 days. Reproducibility (i.e., error) was measured as the percent difference between the 2 patient scans. We found that the SUVmean obtained from 30-60 minutes had a mean error of 3.6% (range 0.6 – 6.9%, SD 2.3%) and the first and second scans were highly correlated (r^2 = 0.99; p<0.0001). Using shorter imaging times from 25-30 minutes or 55-60 minutes post injection (p.i.) also resulted in small error rates; SUVmean mean errors were 8.4% and 5.7%, respectively. Compartmental and graphical kinetic analyses were also fairly reproducible (r^2 = 0.59; p= 0.0152; r^2 = 0.58; p=0.0175 respectively). FLT imaging of patients with non-small cell lung cancer was quite reproducible with a worst case SUVmean error of 21% when using a short imaging time. Another study has examined the change in FLT retention after the treatment of breast cancer and reported the reproducibility in 9 patients before treatment (32) This study by Kenny et al. found that the standard deviation of the mean percent. difference between the two scans was 10.5% and 15.1% for measurements of the SUV and FLT flux measured by graphical analysis, respectively.

FLT is retained in the DNA incorporation pathway, but not in DNA itself. To demonstrate that FLT does correlate with tumor growth in patients a number of studies have compared FLT retention to proliferative activity as measured on biopsy specimens to assess Ki-67. (14, 23, 33-36) In general FLT SUV has correlated nicely with Ki-67 in lung, colorectal, and breast cancers, sarcomas, and lymphoma. For example, in the

study of Buck et al. FLT and Ki-67 significantly correlated ($r = 0.92$, $p < 0.0001$) in patients with untreated lung cancer, while the correlation was not as robust with FDG ($r = 0.59$, $p < 0.001$). In one study of breast cancer, FLT retention did not correlate with Ki-67 (37) and in a study of esophageal cancer there was actually a negative correlation. (38) Overall, all of these studies had relatively small numbers of patients.

FLT imaging can also be employed to detect, stage, and assess prognosis in patients with cancer. In patients with lung lesions FLT was more specific than FDG in determining whether a lesion was malignant, in that 4 of 8 benign lesions demonstrated increased activity with FDG, while FLT was negative in all eight. (33) On the other hand, FDG was more sensitive, only missing 1/18 malignant lesions versus 3/18 with FLT. The mean SUV was also higher with FDG than FLT, 4.1 and 1.8 respectively. FLT can also readily detect esophageal cancer, which is aided, in part, by the low retention of FLT in the normal organs of the thorax. (39) In colon cancer, FLT and FDG did almost equally well in detecting extrahepatic disease despite the higher SUVs seen with FDG. (40) Within the liver, however, FDG had a much higher sensitivity than FLT, 97% and 34% respectively. This is partly attributable to the high background uptake of FLT in the normal liver due to metabolism. One area in the body with very low background uptake of FLT, and other pyrimidines, is the brain. The blood-brain barrier allows one to obtain high contrast images of rapidly proliferating tumors, both with FLT and agents such as FMAU. (41-43)

FLT Use in Assessing Treatment

A number of recent studies in both tissue culture and rodents have used FLT to assess response to chemotherapy, endocrine treatment or radiation. In mice implanted with an androgen-dependent prostate cancer, FLT could be readily used to image the tumors. (44) Furthermore, there was a rapid decrease in FLT retention in mice treated by castration or with diethylstilbestrol.

In a study of mice with an implanted fibrosarcoma treated with cisplatin, declines in FLT retention were noted at 24 and 48 hours of 25% and 50% respectively. (45) The changes in FLT retention reflected the declines in TK1 levels. In mice implanted with a murine squamous cell cancer treated with a single dose of 20 Gy of radiation FLT retention decreased by 39% at 6 hours, while a significant decline in FDG retention was not seen until three days. (46) These investigators also examined the use of photodynamic therapy in nude mice implanted with HELA cells. By 24 hours, there was a 64% decline in FLT retention, but not a significant decrease in FDG uptake.

In another study, mice with a human epidermoid carcinoma were treated with a tyrosine kinase inhibitor directed at ErbB. (47) FLT retention declined by over 50% at 48 hours and by almost 80% at 7 days. In this particular study FDG uptake also declined almost as much and the retention of both tracers correlated with tumor measurements of proliferation by staining for proliferating cell nuclear antigen. Thus one may need to determine whether FLT or FDG is needed depending on the tumor being imaged and treatment employed.

One must be careful when interpreting data using FLT to monitor response after the use of antimetabolites. Since these drugs inhibit the synthesis of endogenous thymidine, the cell may compensate by increasing activity within the salvage pathway. In cell lines this resulted in a several-fold increase in FLT retention with 5FU and methotrexate within 24 hours, while FLT retention declined after treatment with cisplatin. (48) In contrast, mice bearing an implanted firbrosarcoma and treated with 5FU resulted in decreased

retention of FLT at 24 and 48 hours. (49) This decline correlated with a decrease in tumor proliferating cell nuclear antigen staining. The difference between these results may reflect differences in the tumor type studied, sensitivity to 5FU, and cell culture versus in vivo. Nevertheless, it serves to caution investigators about the interpretation of FLT imaging soon after treatment with antimetabolites. On the other hand, rapid changes in FLT retention may detect metabolic changes that are reflective of response, particularly if FLT retention increases.

A number of in-human studies have emerged in recent years using both standard of care treatments and novel agents, which support the use of FLT PET to monitor with treatment response in a variety of cancers. In one study, 31 lung cancer patients treated with gefitinib were imaged with FLT PET before and one week after the start of therapy. (50) Using a decrease of SUVmax of greater than 10.9% to indicate response, they found predictive values of about 93% with PET responders having a median time to progression of 7.9 months compared to 1.32 months in non-responders.

Pio, et al., scanned 14 patients with *breast cancer* before treatment, following the first cycle and again after completion of chemotherapy. (51) Change (decline) in tumor SUV after the first course of treatment correlated with subsequent change in tumor size ($r = 0.8$), leading the investigators to conclude that FLT scans beginning 10 min post injection accurately predict tumor response. More recently, Kenny, et al., reported on changes in tumor uptake and trapping of FLT and FDG in 13 breast cancer patients treated with FEC (5-FU, epirubicin and cyclosporine). (32) Follow-up scans were performed 6-12 d after initiation of treatment, and response was defined by CT (RECIST criteria). Tumor SUV at 90 min and Patlak slopes K_i (equivalent to net clearance from blood into the trapping compartment) were significantly reduced on follow-up in all six patients who had complete or partial responses, Average decreases in responding lesions were 41% for SUV and 53% for K_i, whereas non-responding tumors showed average increases of 3.1 and 1.9% for SUV and K_i, respectively. There was a significant difference in FLT parameter changes between patients who responded and those who didn't ($p = 0.02$ for both SUV and K_i).

Two studies in patients with *non-Hodgkin lymphoma* (NHL) have examined the utility of FLT in predicting response to multi-agent immuno-/chemotherapy (rituximab + cyclosporine, adriamycin, vincristine, prednisone; R-CHOP). Hermann, et al., scanned six patients before and again at 1 and 6 wk after R-CHOP. Tumor uptake of FLT (SUV measured at ≥ 45 min post injection) declined by an average of 77% and 85% at those time points. (52) Another group of 16 patients had follow-up scans two days after rituximab and again 2 days after CHOP; there was no significant change after the immunotherapy component, but an average decline of 32% after administration of chemotherapy. Kim and collaborators studied 33 patients with aggressive NHL. (53) FLT follow-up scans were performed before the second cycle of R-CHOP and again after completion of therapy. Response was assessed by conventional imaging, biochemistry and/or FDG. For FLT, average change in tumor SUV was -71% after the first cycle of therapy and -88% after the final cycle. Complete responders had normal FLT scans after both the first and final cycles.

In another study 19 patients with brain tumors receiving bevacizumab and irinotecan were imaged with FLT PET at 1-2 weeks and again at 6 weeks after treatment. 47% responded to treatment and survived three times as long as non responders. Both early and later FLT-PET responses were more significant predictors of overall

survival (1 to 2 weeks, P = .006; 6 weeks, P = .002), compared with the MRI responses (P = .060 for both 6-week and best responses). (1)

In summary, [F-18] FLT appears to be an excellent agent for imaging cell proliferation. It can be readily labeled with F-18, undergoes little catabolism, and is rapidly retained in proliferating tissues. It may provide the advantages of imaging tumor proliferation and ease of use associated with FDG. A number of studies have now demonstrated in culture and in mice that treatment with chemotherapy, radiation, hormonal manipulation, and targeted drugs can result in rapid declines in FLT retention. In many cases, the decline in FLT uptake precedes changes seen in FDG retention. Pilot studies using FLT in assessing treatment response in patients are now just being completed and may need to be expanded if FLT is going to find routine clinical use to assess treatment response. (54)

The essential, rate-limiting step in the retention of FLT is phosphorylation by thymidine kinase (TK). To determine the importance of this enzyme in cellular retention of FLT we may measure the level of TK in tumors and compare this to the results of quantitative imaging. We may also compare the levels of TK with the expression of other genes involved in cell proliferation using DNA microarray, DNA PCR, proteomic, biochemical, and immunocytochemistry technology. A number of assays are available for both TK activity and protein levels. It is routine at our institution to freeze portions of tumors for future analysis as part of research studies. When adequate specimens have been obtained, this may allow for measurement of TK activity levels. Using DNA microarrays we may also be able to measure the relative levels of other markers of cell proliferation for comparison. If the only specimen available is from fixed tissue, then we may do immunostaining which produces a semi-quantitative result. In cases where frozen tissue is available, both staining and biochemical assays may be done for further validation of each assay and comparison. When multiple pathologic specimens are available, for example from the primary and metastatic lesions, we may be able to examine lesion to lesion variability using these in vitro TK assays as well as PET imaging. If biopsies have been done before and after therapy, both specimens may be analyzed using the described techniques to measure changes in response to therapy. We also seek to determine whether FLT is phosphorlylated as predicted. To test this hypothesis some patients who have already had a FLT PET scan, may receive an injection just prior to previously planned surgery. A portion of the tumor may then be analyzed biochemically to measure the FLT in the phosphorylated form.

2.0 Rationale

The overall goal of the project is to define how in vivo measurements of tumor metabolism can be used to predict and evaluate response to therapy. [F-18] FLT may prove to be a good imaging agent for tumors that can be used to diagnose tumor proliferation and response to therapy. Measurements of metabolism made during or after therapy should be far more sensitive measures of tumor injury than the more conventional approach of observing the tumor for changes in size.

3.0 Objectives

This pilot study will evaluate the use of [F-18] FLT and PET to measure tumor proliferation and the activity of thymidine kinase. This study may also assess the changes that occur after therapy. Finally we may compare the retention of [F-18] FLT in comparison to that of routine clinical measurements, such as CT, MRI or other imaging studies. One goal of this study is to better understand the abnormalities of thymidine

metabolism in tumors in vivo. We may also compare imaging proliferation data using [F-18] FLT to measurements of gene expression of proliferative and biochemical pathways made on tumor specimens obtained at biopsy.

4.0 Patient Eligibility

4.1 Prior histologic diagnosis of cancer or awaiting biopsy or surgery for cancer evaluation of a mass detected on exam or standard imaging. This includes solid tumors as well as hematologic malignancies.

4.2 Patients who have received no prior therapy are eligible, as well as those who have received prior treatment.

4.3 Patients must be able to lie still for the tests. Their girth and weight must be suitable to enter the gantry, which varies per tomograph.

4.4 Patients must be $\geq$18 years old.

4.5 Women of child bearing potential must not be pregnant or breastfeeding. A negative serum pregnancy test must be obtained in women with child bearing potential within 7 days of the first PET scan. Men and women with reproductive potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) on study entry and for the duration of study participation. On the day of the scan the staff will verify and record that the patient has not had unprotected intercourse and does not think that she is pregnant. A repeat serum pregnancy test will be done if the patient can't verify this.

4.6 Patients must sign an informed consent indicating that they are aware of the investigational nature of this study, in keeping with the policies of the cancer center.

4.7 Physical exam, CBC and Multiphasic (including electrolytes, BUN, creatinine, total bilirubin, AST, and ALT) must be done within 28 days of PET imaging, but eligibility is not restricted by these results. The CBC and Multiphasic may be drawn at the time of imaging.

4.8 The medical monitor, Dr. Lois Ayash, at minimum, may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research, shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; shall have the responsibility to promptly report their observations and findings to the IRB or other designate official and the Human Research Protection Office at the Department of Defense. The monitor may also observe recruitment and enrollment procedures and the consent process for individuals, groups or units overseeing study interventions and interactions, reviewing monitoring plans and reports; overseeing data matching, data collection and analysis.

5.0 Recruitment

Patients may be sought from Oncology, Radiation Oncology, Medicine, and Surgery clinics at the Karmanos Cancer Institute, Detroit Medical Center, and affiliated hospitals. Physicians, Nurse Practitioners, or Certified Physician Assistants will offer patients the opportunity to enroll. The Multi-disciplinary teams will assist in identifying patients for study.

6.0 Benefits

While it is anticipated that patients will have no direct benefit from this imaging study, some subjects may gain benefit from the use of PET in helping to define the extent of their tumor. In some subjects PET may help to evaluate their response to treatment. While subjects may not directly benefit from the study, future patients should benefit since this work may well lead to improved methods to diagnose and treat tumors.

7.0 Risks

The potential risks inherent in this study are as follows: Radiation dose carries an associated risk to the patient. The radiation absorbed dose to the whole body for [F-18] FLT will be limited to about 11.5 mSv per scan. The machine also requires an attenuation scan, which is used to determine the density of the body, and involves a dose of 0.2 mSv when done with a germanium-68 rod sources or 3 mSv when done with low dose CT. This depends on whether a regular dedicated PET or PET/CT is used.

The placement of intravenous catheters has the associated risk of making the patient temporarily uncomfortable and a small bruise may form. The lidocaine used for the local anesthetic, when administered in 100 times larger doses than used for this protocol, has been associated with lightheadedness, nervousness, confusion, convulsions, and shock. Extremely rarely, allergic reactions occur with smaller doses. As with any drug, there may be unanticipated adverse effects but no further toxicity is anticipated. A slight bruise may form where the needle has been in a vessel. There is a slight risk of infection at the site, but sterile technique reduces this risk nearly completely.

A possible risk from blood sampling is bleeding. No more than 30 mL (two tablespoons) of blood may be collected for each imaging study. This may result in a maximum of 120 mL being drawn over a maximum of four studies.

The patient may also experience claustrophobia from the imaging ring apparatus.

For the dose of FLT used in this study there are no anticipated toxicities. FLT was originally developed for the treatment of HIV infections (6). In the initial human trial a starting dose of 0.125 mg/kg q12 hr resulted in grade 3 hematologic toxicity in 6 out of 10 subjects. At this dose the mean AUC12 (area under the curve during a 12 hour dosing interval) was 417ng*h/mL. When the dose was adjusted to give a AUC12 of 300ng*h/mL grade 2 or greater anemia was the dose limiting toxicity and developed within 4 weeks of treatment at that level. At 200ng*h/mL 3/14 subjects developed anemia, and 1/15 and 0/15 at 100 and 50 ng*h/mL respectively. Granulocytopenia developed in 5 subjects receiving >200ng*h/mL, but this was not seen at lower doses. Impaired vibration sense was also noted with FLT with a median onset of 40 days of treatment. This occurred in 1, 2, 5, and 2 subjects at an AUC12 of 300, 200, 100 or 50ng*h/mL respectively. This toxicity was not dose limiting in any subjects. Two patients have died of unexpected liver failure after FLT. They had received about 12 weeks of therapy, one at a dose of 200ng*h/mL and the other was on a fixed dose

schedule of 10 mg/day.

Two more recent phase II studies have used FLT at lower doses to treat HIV for four weeks and found this agent to be safe. (55, 56) Katlama et al. conducted a study in 15 refractory HIV patients who were treated with FLT 7.5 mg once daily for four weeks along with their other drugs. The median viral load decreased by -1.13 log10 from 3.93 log10 copies/ml at baseline. FLT "was well tolerated, with no serious adverse events and no treatment withdrawals." Ghosn et al., conducted a randomized phase II trial of using placebo, FLT 0.5, 1, and 2 mg daily for 4 weeks in 72 patients with resistant HIV. They found decreases in viral load of -0.42 log(10) and -0.30 log(10) in the 2 and 1 mg arms, respectively. Again they reported that FLT was well tolerated.

Dosimetry estimates in rats have been validated using estimates previously employed only in humans and large animals. Normal-tissue absorbed dose estimates for the [F-18] labeled chemotherapy agent [F-18] 5-fluorouracil ([F-18] 5-FU) were derived by PET imaging- and by tissue harvesting-based methods in anesthetized rats imaged dynamically for up to 2 h after injection of [F-18] 5-FU (n=16). Combined polynomial and exponential functions were used to model the harvesting-based and imaging-based time-activity data. The measured time-activity data were extrapolated to modeled (i.e., Standard Man) human organs and human absorbed doses calculated. Organ activities derived by imaging-based and by harvesting-based methods were highly correlated (r>0.999) as were the projected human dosimetry estimates across organs (r=0.998) obtained with each method. The tissues calculated to receive highest radiation dose by both methods were related to routes of excretion (bladder wall, liver, and intestines). The harvesting-based and imaging-based methods yielded effective dose (ED) of 2.94E-2 and 2.97E-2 mSv/MBq, respectively. This small-animal PET study provides radiation dose estimates with statistical and logistical advantages over traditional tissue harvesting-based methods. (57)

In our imaging studies we will inject up to 280 Mbq (8 mCi) with a specific activity of at least 3500 Mbq/microM (100 Ci/mMole). This would be a dose of 0.1 microM or dose of about 0.02 mg. Therefore, we will be injecting less than 1% of the dose the daily therapeutic dose used in initial studies. Typically our specific activity will be ten times higher, so the injected dose of drug will be ten fold lower. Furthermore, toxicity took weeks to develop even at the high therapeutic doses used in the phase I study. Based on this work no toxicity is anticipated as part of these imaging studies.

The 8 mCi dose of FLT was chosen since previous experience with FLT when using 10 mCi has demonstrated that this produces acceptable imaging statistics. We have now reduced the dose to allow for four studies over the course of a treatment trial. Given the low dose of radiation, no immediate toxicity is anticipated or could one expect to observe any long term toxicity in cancer patients who receive multiple radiologic procedures. The risk from radiation exposure of this magnitude is too small to be measured directly and is considered to be low in comparison with other everyday risks.

A summary Table 1 of the relevant dosimetry is provided with the total body exposure as well as the two other target organs of most concern. The bladder wall (independent on the voiding scenario) is the target organ with the liver receiving then next most significant radiation exposure in both man and woman. The Total Body Dose and the Bladder and Liver radiation exposure is provided in Table 1. (From NCI Investigators Brochure and H. Vesselle (58)).

Table 1

Organ of Interest	Men mGy/MBq (mrad/mCi)	Woman mGy/MBq (mrad/mCi)
Total Body Dose	Scenario 1 1.23 E-02 (46) Scenario 2 1.26 E-02 (47)	Scenario 1 1.56 E-02 (58) Scenario 2 1.59 E-02 (59)
Bladder	Scenario 1 1.79 E-01 (662) Scenario 2 7.91 E-02 (293)	Scenario 1 1.74 E-01 (646) Scenario 2 7.76 E-02 (287)
Liver	Scenario 1 4.51 E-02 (167) Scenario 2 4.54 E-02 (168)	Scenario 1 6.42 E-02 (238) Scenario 2 6.45 E-02 (239)

Scenario 1: Single bladder voiding at 6 h after [F-18] FLT administration with a 10% post-voiding bladder residual decayed to infinity. This scenario assumed no urine reaccumulation after 6 h. Scenario 2: First bladder voiding at 2 h after [F-18] FLT administration with a 10% post-voiding residual; urine reaccumulation between 2 and 6 h at a rate determined by the bladder curve fit; second bladder voiding at 6 h with a 10% post-voiding residual decayed to infinity. This scenario assumed no urine reaccumulation after 6 h. The first scenario is conservative, whereas the second has a more realistic voiding scheme.

The individual organ and total body doses associated with [F-18] FLT-PET are comparable to those for similar nuclear medicine procedures. The effective dose for an 8 mCi FLT dose in standard man is estimated to be 5.39 mSv and for a standard female is 6.42 mSv. These doses were calculated using the data supplied for FLT residence times (NCI Investigators Brochure and H. Vesselle (58) and using the OLINDA (Organ Level INternal Dose Assessment) program developed and distributed by Vanderbilt University (see dosimetry in Appendix 1). (59)

These figures compare with the effective dose due to the diagnostic CT scan of the chest for measurements of tumor volume and diameter is in the range of 6 mSv. (60) Patients getting up to four PET scans as part of this protocol, generally have multiple CT scans as part of routine care. The additional radiation exposure will add minimally to the exposure from such routine care. The annual background radiation in the United States is approximately 3.6 mSv. It can also be compared to the annual amount of radiation allowed for a radiation worker such as a CT technologist or radiologist, which equals 50 mSv per year.

PET can be done with attenuation obtained using external germanium-68 rod sources or CT, depending on the scanner employed. Studies have shown that the dose absorbed using germanium-based transmission were less than 0.2 mSv. (61) The effective dose of a whole body low dose CT for attenuation correction of the PET data is in the range of 3 mSv. (62)

In the unlikely event that any immediate toxicity is found during the course of imaging, it will be recorded and reported as described below. Given the dose of drug and radiation exposure, no measurable delayed toxicity is expected. This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 for toxicity and Adverse Event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). All appropriate treatment areas should have access to a copy of the CTCAE version 3.0.

Reaction	Reporting Obligation for A, B, and C.
A. All life-threatening events (Grade 4), which may be due to drug Administration [Note 2]	within 24 hours [Note 1]
B. All fatal events (Grade 5) while on study (or within 24 hours of imaging).	A written report to follow within 10 working days. [Note 3]
C. First occurrence of any **previously unknown** clinical event (regardless of grade).	[Notes 4 and 5]

Note 1 Telephone number available 24 hours daily: (301) 230-2330 (Recorder after hours).

Note 2 See DCTD/NCI Common Terminology Criteria for Adverse Events.

Note 3 Report to: Investigational Drug Branch, PO Box 30012, Bethesda, MD, 20824.

Note 4 A list of all known toxicities can be found in the protocol document or consent form.

Note 5 **Reactions judged *definitely* not treatment-related should not be reported.** However, a report should be submitted if there is a reasonable suspicion of drug effect.

8.0 PET Procedure

8.1 Pretreatment Evaluation

Physical exam, CBC, Multiphasic (including electrolytes, BUN, creatinine, total bilirubin, AST, and ALT), within 28 days of PET imaging, but eligibility is not restricted by these results. The CBC and Multiphasic may be drawn at the time of imaging. Women of child bearing potential must not be pregnant or breastfeeding. A negative serum pregnancy test must be obtained in women with child bearing potential within 7 days of the first PET scan. Men and women with reproductive potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) on study entry and for the duration of study participation. On the day of the scan the staff will verify and record that the patient has not had unprotected intercourse and does not think that she is pregnant. A repeat serum pregnancy test will be done if the patient can't verify this.

Tumor Types: Since this is to be integrated with phase 1-3 trials, this protocol is open to all tumor types.

8.2 Imaging Procedure

1) After careful positioning, the patient will be made as comfortable as possible

but may have to lie on the imaging table for up to 2.5 hours depending on the scanner and whether dynamic imaging, whole body imaging, or both are obtained.

2) Placement of intra-venous catheters.
3) Vital signs: temperature, pulse, and blood pressure may be recorded before and after the imaging study.
4) An attenuation scan may be done with by CT (on PET/CT) or a rotating Ge-68 source (on dedicated PET).
5) Intravenous injection of up to 280 MBq [F-18] FLT followed by tomographic imaging and venous blood sampling. Periodic blood samples may be taken during the imaging period for a maximum of 10 samples with a total of 30ml (120 mL maximum blood withdrawal for up to four imaging studies). All blood activity measurements are made using gamma counting and are recorded into a computer for later analysis. Urine samples may be collected during the study if needed for metabolite analysis, but this is not required.
6) After the [F-18] FLT is infused into the subject dynamic imaging up to 60 min may be done over the tumor. This may be followed by static imaging of the rest of the body. Imaging data may be collected for up to 150 min. Dedicated PET or PET/CT scanners will be used for imaging. Depending on scanner availability, the dynamic scan may be performed on a dedicated PET scanner and the whole body scan on the PET/CT or both scans may be done on the same scanner.
7) The patient must empty his/her bladder immediately after imaging.
8) Depending on the results of the first scan, patients may receive up to three additional scans which may be done during or after treatment to determine the effect of therapy upon [F-18] FLT uptake.
9) When available, previously obtained biopsies, done as part of routine clinical care, may be obtained from the pathologist and assayed for enzymes and genes associated with tumor proliferation. These may include, but are not limited to thymidine kinase and thymidylate synthase. These and other markers of tumor proliferation and metabolism may be measured using DNA/ RNA microarray, PCR, proteomic, biochemical, and immunocytochemistry technology. These results may be used to determine the utility and appropriateness of PET scans.
10) We also seek to determine whether FLT is phosphorlylated in vivo as predicted. To test this hypothesis some patients who have already had a FLT PET scan, may receive another injection just prior to previously planned surgery. A portion of the tumor may then be analyzed biochemically to measure the FLT in the phosphorylated form. Such a procedure would count toward the maximum of 4 FLT injections per patient.
11) The “off treatment” date for this study will be the date of the last FLT PET scan performed. Follow-up data on the patient’s clinical course after imaging may be obtained for comparison with the imaging results.
12) The FLT PET scans will be scheduled relative to the initiation of treatment.

8.3 Comparative PET Imaging

Some of the patients imaged with [F-18] FLT may be imaged again after treatment with chemotherapy. Such patients may be undergoing treatment as part of their routine clinical care or on approved research studies and they may be offered the opportunity to undergo repeated imaging, up to and including three times, during the course of treatment. This will allow us to determine whether

FLT uptake changes in response to therapy. Results may be compared to standard clinical measures of treatment response using anatomic imaging with CT and MRI to measure tumor size.

8.4 PET Scanner

PET studies will be performed using a dedicated PET or PET/CT scanner.

8.5 Image Analysis

Images may be analyzed by two approaches: a) qualitative assessment of the uptake images, noting tumor to normal tissue uptake and b) mathematical modeling of ROI data from the individual uptake images at several times and the blood curve. Several methods of analysis are being tested for the evaluation of [F-18] FLT images and results. One or more analysis approach may be used for each scan.

1) Visual inspection of the images to see if one can detect uptake in tumors and normal tissues.

2) Quantitation by the method of the standardized uptake value (SUV) in a region of interest. SUV is defined as the activity per gram in the tissue divided by the activity per gram in the whole body. If the tracer distributes throughout the body in a completely uniform manner, then the SUV for any tissue would by 1.0. Implicit in the definition is the requirement that the tomograph be properly calibrated so that the activity in tissue can be determined in microCi/ml. This is then divided by the microCi's injected, divided by body weight in grams.

3) We have developed a graphical approach to the modeling of thymidine uptake (63). This is based on the work of Patlak (25) and Gjedde (24), who developed a method for analysis of dynamic FDG data using a relatively simple model that can be evaluated by generating a non-linear plot.

4) We have also formulated a compartmental approach to the modeling of thymidine retention. (64) Compartmental models are formulated as a number of pools of activity connected by rate constants. In the thymidine model we have included compartments for the incorporation of thymidine into DNA, as well as the distribution of the metabolites.

Compartmental models are used for data analysis in conjunction with the method of parameter optimization. This approach requires an input function, usually a blood or plasma time-activity-curve. In our thymidine model this includes the quantitation of thymidine, CO_2, and other metabolites in the blood. The tissue time-activity-curve is determined from dynamic PET images. These curves are then fit using the compartmental modes and a parameter optimization program.

5) Measurement of response by FLT PET. We are now completing a study to test the reproducibility of FLT PET measurements in 9 patients with untreated or progressive non-small cell lung cancer (NSCLC). The patients were imaged with FLT PET two times about 1 week apart. The mean percent *differences* for SUVmax and SUVmean were 7.8% (range 0.8-16.5) and 5.5% (range 0.6 -7.0), respectively. Based on these results we will initially use a decline of 20% in SUVmean as a

definition of "PET response". As part of these studies we may also examine other cutoffs using approaches such as receiver operating curve (ROC) analysis.

8.6 TK Analysis

A number of methods to assay TK levels are available. Each has its strengths and so they will be compared. Enzyme activity levels will be measured with a biochemical technique, protein levels will be measured using Western blotting and an immunostaining approach, and mRNA levels will be measured using DNA chip technology. The DNA chip technology, will also allow us to do simultaneous measurements of other genes associated with tumor proliferation for correlation with the imaging results. Comparisons are needed since TK expression can be regulated by both translational and post-translational mechanisms, which may complicate comparisons of activity levels with protein and mRNA levels (65, 66). TK protein levels and/or enzyme activities in tumor tissue will therefore be measured directly, where tissue is available. Total cell protein will be prepared from frozen tissue samples, and assayed for enzyme activity as described previously (67). TK protein levels may also be measured using the KCI Proteomics Core.

TK protein levels in extracts will be determined by Western blotting as previously described (65). Approximately twenty micrograms of protein from each sample will be electrophoresed on a 15% SDS-polyacrylamide gel, blotted to PVDF membranes, and probed with a rabbit polyclonal antiserum that was raised against recombinant human TK protein (68). The bound antibody will be detected with a HRP-conjugated anti-rabbit antibody and a chemiluminescence detection reagent (Pierce Supersignal Chemiluminescence Reagent). The relative amounts of TK in each sample will be determined by densitometric scanning of several different film exposures. This rabbit antiserum has also been used successfully to detect TK expression in intact cells by immunofluorescence microscopy. (65)

Measurement of TK and other Markers of Proliferation with DNA Arrays:

Microarray technology enables massive gathering of biological data with chips providing hybridization-based expression monitoring. Microarrays containing sequences representative of human genes allow the expression analysis of portions of the entire human genome in a single analysis. These genome chips provide remarkable amounts of information for human health and drug discovery. The microarrays that we will use measure the levels of 1200 cancer related genes including TK, DNA polymerase alpha, thymidylate synthase, cell cycle controls, etc. Microarray technology is the central element of functional genomics.

Microarray analysis may be done to rapidly identify individual genes associated with tumor growth. We will use this ability in particular to evaluate genes that are markers of cell proliferation in addition to TK. This will allow us to determine whether TK differences between tumors, and over time in the same tumor, provide an accurate reflection of the tumors' proliferative state. Other genes that will be of particular interest include those in the DNA synthetic pathways including thymidylate synthase, DNA polymerase alpha etc. When possible,

gene expression associated with tumor proliferation will be confirmed by biochemical or immunological techniques as described above.

8.7 Biochemical Analysis

Patients who have had a prior FLT scan and have a planned surgical resection for clinical reasons may have another FLT injection 1-3 hours prior to resection. A portion of the tumor, to be determined by the surgeon and pathologist, may then be allocated for biochemical analysis. The tumor may be homogenized in acid and the precipitate removed by centrifugation. The activity in the supernatant and precipitate may be determined by gamma spectrometry. The supernatant may then be analyzed by high performance liquid chromatography (HPLC) to measure the activity in native FLT, FLT-phosphate, and other metabolites.

9.0 STATISTICAL CONSIDERATIONS

9.1 Study Design

This protocol serves as an ancillary study to image patients with FLT PET, hence statistical analysis will depend on the patient populations being imaged, the pairing with other conventional and protocol treatments, and use in obtaining pilot data. Examples of the type of studies to be performed under this protocol are given below.

1) Pilot FLT PET imaging to be done in new tumor types. In patients with tumor types that have not been previously imaged with FLT PET we will image 3 – 5 patients of each tumor type to determine whether FLT is readily retained and allows for tumor identification and quantification.

2) Pilot studies using FLT PET to measure metabolic changes with treatment. Patients undergoing treatment, either conventional treatment off-study or as part of an approved therapeutic trial, may undergo FLT PET pilot imaging studies to determine if PET may be useful in expanded phase II trials. Any such approved therapeutic trial would have its own statistical design and analysis plan, and sample size requirement.

3) Exploratory studies of the utility of FLT PET in conjunction with phase I and II trials. This may include a subset of treated patients or all patients on a trial. FLT PET may be used in phase II trials to determine preliminary data about the sensitivity of imaging to measure response. Any such phase I or phase II trial would have its own statistical design and analysis plan, and sample size requirement.

For any of the above types of pilot studies, PET Images will be used to calculate the concentration of [F-18] FLT in tumor and its retention over time, as described above. Lesions seen with PET will be compared to more conventional results obtained from routine clinical care. PET will also be used to monitor response to treatment. In patients being imaged before and after therapy we will compare the quantitative changes measured by PET with therapeutic response as determined by routine clinical studies.

4) Pilot studies may also compare FLT retention with other measurements obtained using genetic, immunologic, and biochemical assays of cell proliferation and DNA synthesis (described in Sections 8.6 and 8.7).

9.2 Statistical Analysis

9.2.1 Primary objective

For any of the above types of pilot investigations, or source protocol studies, the FLT PET data obtained under this ancillary protocol would be summarized with descriptive statistics. The main variables of interest would include: standardized uptake value (SUV), FLT retention, thymidine kinase (TK), thymidylate synthase (TS), as well as changes in SUV, TK, or TS pre/post therapy.

9.2.2 Secondary objective

One statistical objective of this study is to estimate the "PET response" rate in cancer patients treated with experimental therapies, as defined in other studies. PET response is defined in Section 8.5.5 as a decrease in mean SUV of ≥ 20%.

9.2.3 Precision of statistical estimates

The sample size of any of the 3 types of studies described in Section 9.1 cannot be predicted in advance. However, it is still useful to determine the precision in the statistical estimate of a mean or a proportion, for a spectrum of likely sample sizes, as shown in Table 2.

Table 2. Precision of statistical estimates

	Statistic estimated	
N	Mean[a]	Proportion[b]
5	0.74s	0.224
10	0.52s	0.158
15	0.42s	0.129
20	0.37s	0.112

[a]The sample mean will be within this many standard deviation (s) units of the true mean, with 90% confidence.

[b]Table entries are the maximum standard error (SE) of the estimated proportion.

Examples of continuous endpoints for which an estimated mean would be calculated would include SUV, TK, and others listed in Section 9.1. Examples of binary endpoints for which an estimated proportion would be calculated would include PET response rate, as mentioned in Section 9.2.

9.3 Study Accrual Rate and Study Duration

We anticipate an accrual rate of 20 patients/year, hence four years will be needed to accrue 80 patients. This sample size (N=80) will allow us to study several histologic tumor types and their response to therapy. Accrual of 80 patients should permit pilot studies of FLT PET as described in Section 9.1. The exact number of such pilot studies that may eventually be conducted under this ancillary protocol cannot be predicted in advance.

10.0 PATIENT REGISTRATION and DATA RETENTION

All patients shall be registered with the Comprehensive Cancer Center Clinical Trials Office (CTO) located in the Mid-Med Professional Building. The CTO assigns the data manager and alternate for this protocol. The CTO phone number is 313-576-8981 or 313-576-9790.

Data is retained by the Clinical Trials Office for at least 5 years after study completion. Kinetic analysis of the imaging results is to be retained at Shields' Hudson Webber research laboratory for at least 5 years. Imaging records are retained by the PET Center and periodically archived and stored for at least 5 years. The Principal Investigator oversees record management.

11.0 DATA SAFETY and MONITORING

11.1 Scheduled meetings will be held monthly or more frequently depending on the activity of the protocol. These meetings will include the protocol investigators and data managers involved with the conduct of the protocol.

11.2 During these meetings the investigators will discuss matters related to:

- Safety of protocol participants (Adverse Event reporting)
- Validity and integrity of the data
- Enrollment rate relative to expectation of target accrual, characteristics of participants
- Retention of participants, adherence to the protocol (potential or real protocol violations)
- Data completeness on case report forms and complete source documentation

11.3 Completed Data and Safety Monitoring Reports of these regular investigator meetings will be kept on file in the office of the Clinical Trials Core. The Clinical Study Coordinator assigned to the clinical trial will be responsible for completing the report form. The completed reports will be reviewed and signed by the Principle Investigator (PI) or designee. The signed forms will then be forwarded to the Director, Clinical Trials Core for review of completeness and processing with the Data and Safety Monitoring Committee.

11.4 The Barbara Ann Karmanos Cancer Institute Data and Safety Monitoring Committee will meet on a monthly basis to review the prior months Serious Adverse Event forms and Data and Safety Monitoring study specific reports that have been filed.

12.0 REFERENCES

1. 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 [18F] fluorothymidine positron emission tomography: a pilot study. J Clin Oncol. 2007 Oct 20;25(30):4714-21.
2. Perini R, Choe R, Yodh AG, Sehgal C, Divgi CR, Rosen MA. Non-invasive assessment of tumor neovasculature: techniques and clinical applications. Cancer Metastasis Rev. 2008 Dec;27(4):615-30.
3. Langen P, Etzold G, Hintsche R, G. K. 3'-Deoxy-3'-fluorothymidine, a new selective inhibitor of DNA-synthesis. Acta Biol Med Ger. 1969;23(6):759-66.
4. Matthes E, Lehmann C, Scholz D, Rosenthal HA, Langen P. Phosphorylation, anti-HIV activity and cytotoxicity of 3'-fluorothymidine. Biochem Biophys Res Commun. 1988 Jun 16;153(2):825-31.
5. Lundgren B, Bottiger D, Ljungdahl-Stahle E, Norrby E, Stahle L, Wahren B, et al. Antiviral effects of 3'-fluorothymidine and 3'-azidothymidine in cynomolgus monkeys infected with simian immunodeficiency virus. J Acquir Immune Defic Syndr. 1991;4(5):489-98.
6. Flexner C, van der Horst C, Jacobson MA, Powderly W, Duncanson F, Ganes D, et al. Relationship between plasma concentrations of 3'-deoxy-3'- fluorothymidine (alovudine) and antiretroviral activity in two concentration-controlled trials. J Infect Dis. 1994;170(6):1394-403.
7. Grierson J, Shields A. A strategy for the labeling of [F-18]-3'-deoxy-3'-fluorothymidine: [F-18]FLT. J Labelled Comp and Radiopharm. 1995;37:606-7.
8. Grierson J, Shields A. Optimizing labeling substrate structure for 3'-deoxy-3'-[F-18]fluorothymidine: [F-18]FLT. J Nucl Med. 1999;40:83P.
9. Grierson JR, Shields AF. Radiosynthesis of 3'-deoxy-3'-[(18)F]fluorothymidine: [(18)F]FLT for imaging of cellular proliferation in vivo. Nucl Med Biol. 2000 Feb;27(2):143-56.
10. Machulla H-J, Blocher A, Kuntzsch M, Wei R, Grierson J. Simplified labeling approach for synthesizing 3'-deoxy-3'-[18F]fluorothymidine ([18F]FLT). J Radioanalytical Nuclear Chemistry. 2000;243:843-6.
11. Shields A, Grierson J, Dohmen B, Machulla H-J, Stayanoff J, Lawhorn-Crews J, et al. Imaging Proliferation In Vivo with [F-18]FLT and Positron Emission Tomography. Nature Medicine. 1998;4:1334-6.
12. Muzi M, Vesselle H, Grierson JR, Mankoff DA, Schmidt RA, Peterson L, et al. Kinetic analysis of 3'-deoxy-3'-fluorothymidine PET studies: validation studies in patients with lung cancer. J Nucl Med. 2005 Feb;46(2):274-82.
13. Shields A, Briston D, Chandupatla S, Douglas K, Lawhorn-Crews J, Collins J, et al. A Simplified Analysis of [F-18] 3'-Fluorothymidine Metabolism and Retention. Eur J Nucl Med Mol Imaging. 2005;32:1269-75.
14. Wagner M, Seitz U, Buck A, Neumaier B, Schultheiss S, Bangerter M, et al. 3'-[18F]fluoro-3'-deoxythymidine ([18F]-FLT) as positron emission tomography tracer for imaging proliferation in a murine B-Cell lymphoma model and in the human disease. Cancer Res. 2003 May 15;63(10):2681-7.
15. Grierson JR, Schwartz JL, Muzi M, Jordan R, Krohn KA. Metabolism of 3'-deoxy-3'-[F-18]fluorothymidine in proliferating A549 cells: validations for positron emission tomography. Nucl Med Biol. 2004 Oct;31(7):829-37.
16. Seitz U, Wagner M, Neumaier B, Wawra E, Glatting G, Leder G, et al. Evaluation of pyrimidine metabolising enzymes and in vitro uptake of 3'-[(18)F]fluoro-3'-deoxythymidine ([(18)F]FLT) in pancreatic cancer cell lines. Eur J Nucl Med Mol Imaging. 2002 Sep;29(9):1174-81.
17. Sherley JL, Kelly TJ. Regulation of human thymidine kinase during the cell cycle. J Biol Chem. 1988;263(17):8350-8.

18. Munch-Petersen B, Cloos L, Tyrsted G, Eriksson S. Diverging substrate specificity of pure human thymidine kinases 1 and 2 against antiviral dideoxynucleosides. J Biol Chem. 1991;266(14):9032-8.
19. Jansson O, Bohman C, Munch-Petersen B, Eriksson S. Mammalian thymidine kinase 2: Direct photoaffinity labeling with [^{32} P]dTTP of the enzyme from spleen, liver, heart and brain. Eur J Biochem. 1992;206:485-90.
20. Shields A, Grierson JR, Muzik O, Stayanoff JC, Lawhorn-Crews J, Obradovich JE, et al. Kinetics of 3'-deoxy-3'-[F-18]fluorothymidine uptake and retention in dogs. Molecular Imaging BIology. 2002:83-9.
21. Visvikis D, Francis D, Mulligan R, Costa DC, Croasdale I, Luthra SK, et al. Comparison of methodologies for the in vivo assessment of 18FLT utilisation in colorectal cancer. Eur J Nucl Med Mol Imaging. 2004 Feb;31(2):169-78.
22. 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 Feb;46(2):371-80.
23. Kenny LM, Vigushin DM, Al-Nahhas A, Osman S, Luthra SK, Shousha S, et al. Quantification of cellular proliferation in tumor and normal tissues of patients with breast cancer by [18F]fluorothymidine-positron emission tomography imaging: evaluation of analytical methods. Cancer Res. 2005 Nov 1;65(21):10104-12.
24. Gjedde A. Calculation of cerebral glucose phosphorylation from brain uptake of glucose analogs in vivo: A re-examination. Brain Res Rev. 1982;4:237-74.
25. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. J Cereb Blood Flow Metab. 1983;3:1-7.
26. Shields AF, Briston DA, Chandupatla S, Douglas KA, Lawhorn-Crews J, Collins JM, et al. A simplified analysis of [18F]3'-deoxy-3'-fluorothymidine metabolism and retention. Eur J Nucl Med Mol Imaging. 2005 Nov;32(11):1269-75.
27. Minn H, Zasadny KR, Quint LE, Wahl RL. Lung cancer: reproducibility of quantitative measurements for evaluating 2-[F-18]-fluoro-2-deoxy-D-glucose uptake at PET. Radiology. 1995;196(1):167-73.
28. Weber WA, Ziegler SI, Thodtmann R, Hanauske AR, Schwaiger M. Reproducibility of metabolic measurements in malignant tumors using FDG PET. J Nucl Med. 1999 Nov;40(11):1771-7.
29. Shields AF, Lawhorn-Crews JM, Briston DA, Zalzala S, Gadgeel S, Douglas KA, et al. Analysis and reproducibility of 3'-Deoxy-3'-[18F]fluorothymidine positron emission tomography imaging in patients with non-small cell lung cancer. Clin Cancer Res. 2008 Jul 15;14(14):4463-8.
30. Tseng JR, Dandekar M, Subbarayan M, Cheng Z, Park JM, Louie S, et al. Reproducibility of 3'-deoxy-3'-(18)F-fluorothymidine microPET studies in tumor xenografts in mice. J Nucl Med. 2005 Nov;46(11):1851-7.
31. Mankoff DA, Shields AF, Krohn KA. PET imaging of cellular proliferation. Radiol Clin North Am. 2005 Jan;43(1):153-67.
32. Kenny L, Coombes RC, Vigushin DM, Al-Nahhas A, Shousha S, Aboagye EO. Imaging early changes in proliferation at 1 week post chemotherapy: a pilot study in breast cancer patients with 3'-deoxy-3'-[(18)F]fluorothymidine positron emission tomography. Eur J Nucl Med Mol Imaging. 2007 Mar 2.
33. Buck AK, Halter G, Schirrmeister H, Kotzerke J, Wurziger I, Glatting G, et al. Imaging proliferation in lung tumors with PET: 18F-FLT versus 18F-FDG. J Nucl Med. 2003 Sep;44(9):1426-31.
34. Cobben DC, Elsinga PH, Suurmeijer AJ, Vaalburg W, Maas B, Jager PL, et al. Detection and grading of soft tissue sarcomas of the extremities with (18)F-3'-fluoro-3'-deoxy-L-thymidine. Clin Cancer Res. 2004 Mar 1;10(5):1685-90.

35. Francis DL, Freeman A, Visvikis D, Costa DC, Luthra SK, Novelli M, et al. In vivo imaging of cellular proliferation in colorectal cancer using positron emission tomography. Gut. 2003 Nov;52(11):1602-6.
36. Vesselle H, Grierson J, Muzi M, Pugsley JM, Schmidt RA, Rabinowitz P, et al. In Vivo Validation of 3'deoxy-3'-[(18)F]fluorothymidine ([(18)F]FLT) as a Proliferation Imaging Tracer in Humans: Correlation of [(18)F]FLT Uptake by Positron Emission Tomography with Ki-67 Immunohistochemistry and Flow Cytometry in Human Lung Tumors. Clin Cancer Res. 2002 Nov;8(11):3315-23.
37. Smyczek-Gargya B, Fersis N, Dittmann H, Vogel U, Reischl G, Machulla HJ, et al. PET with [18F]fluorothymidine for imaging of primary breast cancer: a pilot study. Eur J Nucl Med Mol Imaging. 2004 May;31(5):720-4.
38. van Westreenen HL, Cobben DC, Jager PL, van Dullemen HM, Wesseling J, Elsinga PH, et al. Comparison of 18F-FLT PET and 18F-FDG PET in esophageal cancer. J Nucl Med. 2005 Mar;46(3):400-4.
39. Dittmann H, Dohmen BM, Paulsen F, Eichhorn K, Eschmann SM, Horger M, et al. [18F]FLT PET for diagnosis and staging of thoracic tumours. Eur J Nucl Med Mol Imaging. 2003 Oct;30(10):1407-12.
40. Francis DL, Visvikis D, Costa DC, Arulampalam TH, Townsend C, Luthra SK, et al. Potential impact of [18F]3'-deoxy-3'-fluorothymidine versus [18F]fluoro-2-deoxy-D-glucose in positron emission tomography for colorectal cancer. Eur J Nucl Med Mol Imaging. 2003 Jul;30(7):988-94.
41. Choi SJ, Kim JS, Kim JH, Oh SJ, Lee JG, Kim CJ, et al. [18F]3'-deoxy-3'-fluorothymidine PET for the diagnosis and grading of brain tumors. Eur J Nucl Med Mol Imaging. 2005 Jun;32(6):653-9.
42. Sun H, Sloan A, Mangner TJ, Vaishampayan U, Muzik O, Collins JM, et al. Imaging DNA synthesis with [18F]FMAU and positron emission tomography in patients with cancer. Eur J Nucl Med Mol Imaging. 2005 Jan;32(1):15-22.
43. Chen W, Cloughesy T, Kamdar N, Satyamurthy N, Bergsneider M, Liau L, et al. Imaging proliferation in brain tumors with 18F-FLT PET: comparison with 18F-FDG. J Nucl Med. 2005 Jun;46(6):945-52.
44. Oyama N, Ponde DE, Dence C, Kim J, Tai YC, Welch MJ. Monitoring of therapy in androgen-dependent prostate tumor model by measuring tumor proliferation. J Nucl Med. 2004 Mar;45(3):519-25.
45. Leyton J, Latigo JR, Perumal M, Dhaliwal H, He Q, Aboagye EO. Early detection of tumor response to chemotherapy by 3'-deoxy-3'-[18F]fluorothymidine positron emission tomography: the effect of cisplatin on a fibrosarcoma tumor model in vivo. Cancer Res. 2005 May 15;65(10):4202-10.
46. Sugiyama M, Sakahara H, Sato K, Harada N, Fukumoto D, Kakiuchi T, et al. Evaluation of 3'-deoxy-3'-18F-fluorothymidine for monitoring tumor response to radiotherapy and photodynamic therapy in mice. J Nucl Med. 2004 Oct;45(10):1754-8.
47. Waldherr C, Mellinghoff IK, Tran C, Halpern BS, Rozengurt N, Safaei A, et al. Monitoring antiproliferative responses to kinase inhibitor therapy in mice with 3'-deoxy-3'-18F-fluorothymidine PET. J Nucl Med. 2005 Jan;46(1):114-20.
48. Dittmann H, Dohmen BM, Kehlbach R, Bartusek G, Pritzkow M, Sarbia M, et al. Early changes in [(18)F]FLT uptake after chemotherapy: an experimental study. Eur J Nucl Med Mol Imaging. 2002 Nov;29(11):1462-9.
49. Barthel H, Cleij MC, Collingridge DR, Hutchinson OC, Osman S, He Q, et al. 3'-deoxy-3'-[18F]fluorothymidine as a new marker for monitoring tumor response to antiproliferative therapy in vivo with positron emission tomography. Cancer Res. 2003 Jul 1;63(13):3791-8.
50. Sohn H-J, Yang Y-J, J-S Ryu, Oh S, Im K, Moon D, et al. [18F]Fluorothymidine Positron Emission Tomography before and 7 Days after Gefitinib Treatment Predicts Response in Patients with Advanced Adenocarcinoma of the Lung. Clin Cancer Res. 2008;14:7423-9.

51. Pio BS, Park CK, Pietras R, Hsueh WA, Satyamurthy N, Pegram MD, et al. Usefulness of 3'-[F-18]fluoro-3'-deoxythymidine with positron emission tomography in predicting breast cancer response to therapy. Mol Imaging Biol. 2006 Jan-Feb;8(1):36-42.
52. Herrmann K, Wieder HA, Buck AK, Schoffel M, Krause BJ, Fend F, et al. Early response assessment using 3'-deoxy-3'-[18F]fluorothymidine-positron emission tomography in high-grade non-Hodgkin's lymphoma. Clin Cancer Res. 2007 Jun 15;13(12):3552-8.
53. Kim S-K, Kim S, Kang JY, Jang SJ, Kang SH, Lee H-Y, et al. Characterization and response monitoring for aggressive non-Hodgkin's lymphoma using 18F-fluoro-deoxythymidine J Nuc Med. 2007;48:350P.
54. Bading JR, Shields AF. Imaging of cell proliferation: status and prospects. J Nucl Med. 2008 Jun;49 Suppl 2:64S-80S.
55. Ghosn J, Quinson AM, Sabo N, Cotte L, Piketty C, Dorleacq N, et al. Antiviral activity of low-dose alovudine in antiretroviral-experienced patients: results from a 4-week randomized, double-blind, placebo-controlled dose-ranging trial. HIV Med. 2007 Apr;8(3):142-7.
56. Katlama C, Ghosn J, Tubiana R, Wirden M, Valantin MA, Harmenberg J, et al. MIV-310 reduces HIV viral load in patients failing multiple antiretroviral therapy: results from a 4-week phase II study. Aids. 2004 Jun 18;18(9):1299-304.
57. Kesner AL, Hsueh WA, Czernin J, Padgett H, Phelps ME, Silverman DH. Radiation dose estimates for [18F]5-fluorouracil derived from PET-based and tissue-based methods in rats. Mol Imaging Biol. 2008 Nov-Dec;10(6):341-8.
58. Vesselle H, Grierson J, Peterson LM, Muzi M, Mankoff DA, Krohn KA. 18F-Fluorothymidine radiation dosimetry in human PET imaging studies. J Nucl Med. 2003 Sep;44(9):1482-8.
59. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med. 2005 Jun;46(6):1023-7.
60. McNitt-Gray MF. AAPM/RSNA Physics Tutorial for Residents: Topics in CT. Radiation dose in CT. Radiographics. 2002 Nov-Dec;22(6):1541-53.
61. Almeida P, Bendriem B, de Dreuille O, Peltier A, Perrot C, Brulon V. Dosimetry of transmission measurements in nuclear medicine: a study using anthropomorphic phantoms and thermoluminescent dosimeters. Eur J Nucl Med. 1998 Oct;25(10):1435-41.
62. Brix G, Lechel U, Glatting G, Ziegler SI, Munzing W, Muller SP, et al. Radiation exposure of patients undergoing whole-body dual-modality 18F-FDG PET/CT examinations. J Nucl Med. 2005 Apr;46(4):608-13.
63. Mankoff DA, Shields AF, Graham MM, Link JM, Krohn KA. A graphical analysis method for estimating blood-to-tissue transfer constants for tracers with labeled metabolites. J Nucl Med. 1996;37(12):2049-57.
64. Mankoff DA, Shields AF, Graham MM, Link JM, Eary JF, Krohn KA. Kinetic analysis of 2-[carbon-11]thymidine PET imaging studies: compartmental model and mathematical analysis. J Nucl Med. 1998;39(6):1043-55.
65. Carozza MA, Conrad SE. Regulation of thymidine kinase protein stability in serum-stimulated cells. Cell Growth Differ. 1994;5(8):901-8.
66. Kauffman MG, Kelly TJ. Cell cycle regulation of thymidine kinase: residues near the carboxyl terminus are essential for the specific degradation of the enzyme at mitosis. Mol Cell Biol. 1991;11(5):2538-46.
67. Stuart P, Ito M, Stewart C, Conrad SE. Induction of cellular thymidine kinase occurs at the mRNA level. Mol Cell Biol. 1985;5(6):1490-7.
68. Dagher SF, Conrad SE, Werner EA, Patterson RJ. Phenotypic conversion of TK-deficient cells following electroporation of functional TK enzyme. Exp Cell Res. 1992;198(1):36-42.

Appendix 1

TABLE 1 Cumulated Activity and Residence Time of ^{18}F-FLT for 37-MBq Injection and Standard Man

Source organ	n	$\tilde{C}$ (kBq-h/g)	SD $\tilde{C}$ (kBq-h/g)	Organ wt (g)	$\tilde{A} = \tilde{C} \cdot$ organ wt (MBq-h)	SD for $\tilde{A}$ (MBq-h)	τ (h)	SD τ (h)
Adrenals	1	1.929	0.339*	16.3	0.031	0.006	0.001	0.0002
Brain	1	0.229	0.040*	1420.0	0.325	0.057	0.009	0.0015
LLI	4	1.060	1.053	143.0	0.152	0.151	0.004	0.0041
Stomach	3	2.178	0.654	260.0	0.566	0.170	0.015	0.0046
Blood	11	1.136	0.198	454.0	0.516	0.090	0.014	0.0024
Heart wall	10	1.195	0.348	316.0	0.378	0.110	0.010	0.0030
Kidney	3	5.192	1.819	299.0	1.552	0.544	0.042	0.0147
Liver	6	6.165	1.641	1910.0	11.774	3.134	0.318	0.0847
Lung	11	0.572	0.157	1000.0	0.572	0.157	0.015	0.0042
Pancreas	2	2.368	1.586	94.3	0.223	0.150	0.006	0.0040
Marrow	11	7.696	2.272	1120.0	8.620	2.545	0.233	0.0688
Spleen	5	1.768	0.839	183.0	0.324	0.153	0.009	0.0041
Bladder†								
Voiding scenario 1	5	61.622	45.700‡	211.0	13.002	9.641	0.351	0.2606
Voiding scenario 2		25.589			5.399		0.146	
Testes	3	1.360	0.753	39.1	0.053	0.029	0.001	0.0008
Remainder§		0.872	0.160	66234.3	57.755	10.473	1.561	0.2831

* SD for organs with only 1 curve were calculated in proportion to SD of remainder.

† Bladder average and SD of 5 patients.

‡ SD for bladder was calculated from 5 patients with bladder curves.

§ Remainder of body for 73.7-kg man. Both voiding scenarios yield same remainder values.

wt = weight; LLI = lower large intestine.

TABLE 2 Cumulated Activity and Residence Time of ^{18}F-FLT for 37-MBq Injection and Standard Woman

Source organ	n	$\tilde{C}$ (kBq-h/g)	SD $\tilde{C}$ (kBq-h/g)	Organ wt (g)	$\tilde{A} = \tilde{C} \cdot$ organ wt (MBq-h)	SD for $\tilde{A}$ (MBq-h)	τ (h)	SD τ (h)
Brain	1	0.406	0.064*	1410	0.572	0.090	0.015	0.0024
Breast	5	0.495	0.128	361	0.179	0.046	0.005	0.0012
LLI	1	0.913	0.150*	109	0.100	0.016	0.003	0.0004
Stomach	1	2.171	0.347*	195	0.423	0.068	0.011	0.0018
Blood	6	1.598	0.381	347	0.555	0.132	0.015	0.0036
Heart wall	6	1.712	0.341	241	0.413	0.082	0.011	0.0022
Kidney	1	6.070	1.550†	248	1.505	0.389	0.041	0.0105
Liver	3	9.163	1.320	1400	12.828	1.848	0.347	0.0499
Lung	6	0.705	0.613	651	0.459	0.399	0.012	0.0108
Marrow	6	10.704	3.553	1050	11.239	3.730	0.304	0.1008
Spleen	3	3.864	0.633	123	0.475	0.078	0.013	0.0021
Bladder‡								
Voiding scenario 1	5	61.622	45.700§	160	9.860	7.310	0.266	0.1976
Voiding scenario 2		25.589			4.094		0.111	
Remainder‖		1.142	0.170	50505	57.689	8.430	1.559	0.2278

* SD for organs with only 1 curve were calculated in proportion to SD of remainder.

† SD for kidney was calculated by pooling male and female kidney curves.

‡ Bladder average and SD of 5 patients.

§ SD for bladder was calculated from 5 patients with bladder curves.

‖ Remainder of body for 56.8-kg woman. Both voiding scenarios yield same remainder values.

wt = weight; LLI = lower large intestine.

Vesselle H, Grierson J, Peterson LM, Muzi M, Mankoff DA, Krohn KA. ^{18}F-Fluorothymidine Radiation Dosimetry in Human PET Imaging Studies J Nucl Med. 44:1482-1488.