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FLT: Measuring Tumor Cell Proliferation In Vivo With Positron Emission Tomography and 3′-Deoxy-3′-[^{18}F]Fluorothymidine

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Positron emission tomography (PET) using the radiotracer 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT) can image cellular proliferation in human cancers in vivo. FLT uptake has been shown to correlate with pathology-based proliferation measurements, including the Ki-67 score, in a variety of human cancers. Unlike pathology-based measurements, imaging-based methods, including FLT-PET, are noninvasive, easily repeatable, and less prone to sampling errors. FLT-PET may therefore be a useful tool for assessing tumor aggressiveness, predicting outcome, planning therapy, or monitoring response to treatment. Three recent clinical studies have reported that FLT-PET can accurately predict response very early after the initiation of chemotherapy. Especially with the advent of cytostatic chemotherapy agents, methods of biologically assessing a tumor's response will take on increasing importance, since changes in tumor size will not always be expected. To date, most studies of FLT-PET have focused on validating it as a means of quantifying cellular proliferation and testing its ability to accurately stage cancer. In some settings, FLT-PET has shown greater specificity for cancer than ^{18}F-fluorodeoxyglucose (FDG)-PET, which can show false-positive uptake in areas of infection or inflammation. However, because of FLT's lower overall uptake and higher background activity in liver and bone marrow, FLT-PET should not be considered a potential replacement for staging by FLT-PET. Instead, FLT-PET should be considered a powerful addition to FDG-PET, providing additional diagnostic specificity and important biological information that could be useful in predicting prognosis, planning treatment, and monitoring response.
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The radiotracer 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT; Fig. 1) has potential utility for noninvasively measuring cellular proliferation in vivo in malignant tumors and organ tissues using positron emission tomography (PET). This technology could have exciting implications in oncology, including the possibility of assessing tumor grade and aggressiveness without the use of biopsy. FLT-PET could also be used to predict and monitor response to antitumor therapies, especially early in the course of treatment. With the advent of cytostatic chemotherapy agents, new methods of assessing a tumor's biological response will become especially relevant, because changes in tumor size will not always be expected and standard anatomic imaging will not suffice to assess response. Hence, there is an unmet need for methods of assessing therapeutic effectiveness by criteria other than serial lesion size measurements.

At present, ^{18}F-fluorodeoxyglucose (FDG) is the only PET radiotracer used for the routine clinical evaluation of malignant tumors in a range of body tissues. FDG-PET has high sensitivity for detecting primary tumors and areas of metastasis, providing significant clinical benefit in many cancers. However, FDG-PET also frequently shows false-positive uptake in areas of infection and/or inflammation.[1,2] In addition, it remains unclear whether imaging a tumor's glucose consumption provides a useful indication of aggressiveness or a satisfactory measurement of response to therapy. Hence, molecular imaging techniques that target the pathways of DNA synthesis in proliferating tissues have been developed, including FLT-PET. Increased DNA synthesis is potentially more tumor-specific than high glucose utilization and may correspond more directly with tumor aggressiveness and response to therapy.

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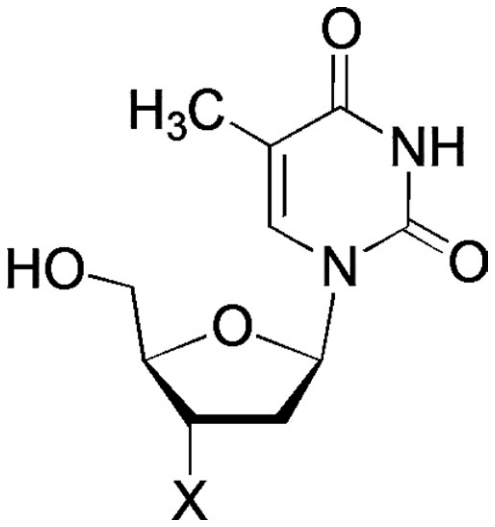
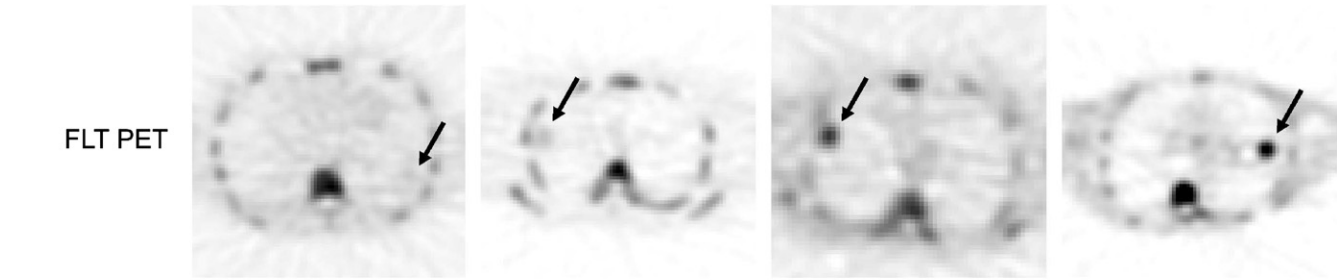


Figure 1 Structure of TdR and FLT. Thymidine (TdR): X = OH, 3′-deoxy-3′-fluorothymidine (FLT): X = F.

Current methods of assessing tumor proliferation are based on pathology specimens and include Ki-67 or PCNA immunohistochemical staining and determination of the S-phase fraction by flow cytometry. These methods have been shown to provide useful clinical information in a variety of human cancers[3–5] and are significantly linked to survival.[5–10] However, these methods require a tissue sample and are therefore invasive and limited by potential morbidity and sampling errors. In contrast, imaging-based proliferation assessments permit the evaluation of an entire tumor and provide information regarding regional heterogeneity in a given tumor, as well as between different individual lesions, all in a single scan. Imaging is noninvasive and can therefore be safely performed for any lesion and repeated multiple times over the course of therapy.

Initial reports on FLT-PET have shown that FLT uptake correlates with other proliferation measurements, including the Ki-67 score, in a variety of human cancers (Fig. 2).[11–21] FLT-PET also has been reported to have high specificity for diagnosing malignancy in primary tumors (Fig. 3)[13,22] and can indicate a response to chemotherapy (Fig. 4)[16,23–25] and subsequent survival.[16] Three studies have reported that early changes in FLT uptake, measured soon after treatment begins, may be able to predict a tumor's eventual response to chemotherapy.[23–25] In addition, in the pretreatment setting, cellular proliferation has been reported to predict subsequent treatment response,[3–5] including in one study using FLT-PET.[26] Thus, FLT-PET may prove useful in selecting treatment before, or even soon after, the beginning of chemotherapy. It remains to be shown whether pretherapy FLT uptake, like pathology-based assessments of cellular proliferation, correlates with overall survival.

However, FLT-PET should not be regarded as an overall staging tool for cancer, as is FDG-PET. Because of lower overall uptake in tumors and higher background activity in the liver and bone marrow, FLT is not expected to have the same outstanding sensitivity as FDG-PET for tumor detection across all organs. Rather, FLT-PET should instead be considered a potentially powerful addition to staging by FDG-PET. In this role, FLT-PET could provide additional diagnostic specificity for proliferating tissues and important biological information that could have implications in treatment selection or monitoring.



Histology	Benign inflammatory lesion	Squamous cell carcinoma	Non-small cell lung cancer NOS	Undifferentiated large cell carcinoma
Avg. diameter	3.9 cm	2.1 cm	1.6 cm	1.6 cm
Ki-67 score	< 5%	20%	70%	90%
FLT maxSUV	0.9	2.6	3.4	4.0
FLT flux	0.8	2.0	2.6	5.4

Figure 2 Example FLT-PET images and corresponding Ki-67 scores in lung lesions. FLT uptake has been found to correlate with proliferation assessed by Ki-67 immunohistochemical scoring in many cancers. These examples show FLT uptake in 3 nonsmall cell lung cancers and 1 benign inflammatory lesion, along with their corresponding Ki-67 scores. NOS, not otherwise specified.

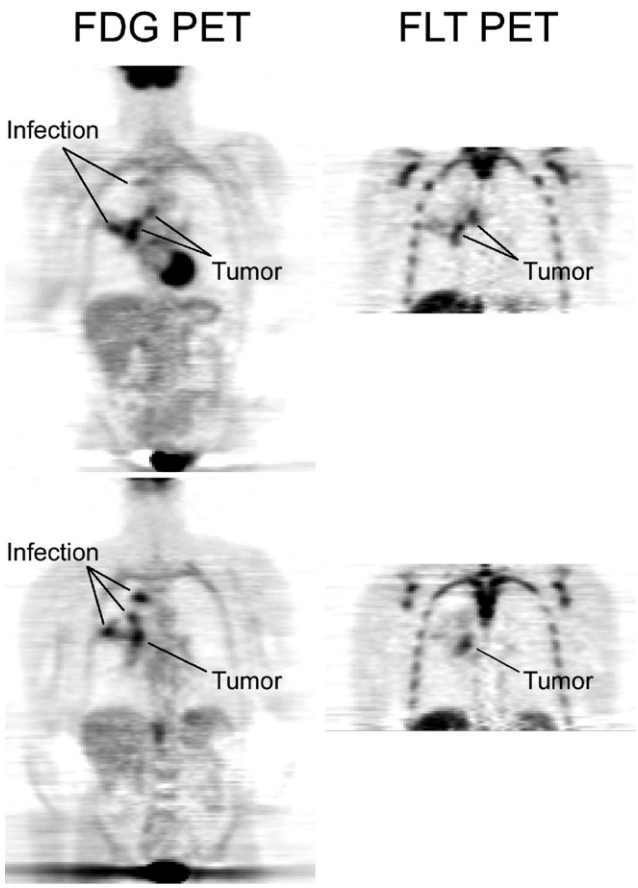


Figure 3 Example of infection and malignancy on FDG-PET and FLT-PET. FLT-PET may have a greater specificity for cancer compared with FDG-PET. In this example, the patient has a right hilar nonsmall cell lung cancer, with significant postobstructive pneumonitis/pneumonia, and malignant adenopathy at a station 4R mediastinal lymph node. Patient is before any therapy. FDG-PET shows uptake in the 2 tumor foci but also suffers from marked FDG uptake in the RUL postobstructive inflammation/infection, which is lateral to and indistinguishable from the primary hilar tumor. FLT-PET, in contrast, shows only minimal uptake in this area, which is easily distinguished from the primary tumor. An additional focus of pneumonitis is present in the medial aspect of the right lung apex, which is highly avid on FDG-PET, but is not seen on FLT-PET.

Early Development of FLT as a Proliferation Tracer

Imaging cell proliferation with PET was first explored with ^{11}C-thymidine (TdR; Fig. 1).[27] The appeal of this approach is the specificity of thymidine for DNA synthesis as opposed to RNA and its ability to incorporate stably into DNA as a means of trapping radiotracer in proportion to cell proliferation. TdR imaging has been validated in cell and human studies as a tracer of proliferation, providing useful images.[28–30] However, the short half-life of ^{11}C (20.4 minutes) and the biologic instability of TdR make it impractical for routine clinical use. TdR is rapidly degraded into labeled metabolites in vivo. As a result, even with careful kinetic modeling, it is difficult to robustly quantify the numerous biological processes that impact observed radiotracer uptake, including the subset of those processes related to cell proliferation.[31–37] For the same reason, static TdR-PET images have limited usefulness. These drawbacks led to the search for a method of tracing the same central biosynthesis pathway, using a molecule that is stable to degradation and incorporates the longer-lived radionuclide ^{18}F ($t_{1/2}$ = 109.8 minutes).

Before consideration as a PET imaging agent, FLT without a radioactive label was explored as an antiretroviral therapeutic agent for HIV and AIDS.[38–40] At therapeutic doses and with prolonged exposure, some patients experienced toxic effects from FLT, including 2 deaths at greater doses, and the agent was abandoned as a retroviral therapy.[40] However, no adverse effects have been reported from radiotracer doses of FLT, which are several thousand times lower than the lowest and least toxic therapeutic clinical trial dose of FLT. At a minimum specific activity of 3.7 GBq/μmol (0.1 Ci/μmol), a 185 MBq (5 mCi) injection of [^{18}F]FLT represents $\leq$12.2 μg of labeled and unlabeled FLT.[41] Additionally, no significant biological effects were observed in 20 patients in a recent toxicology study conducted by our group.[41]

Radiolabeling FLT for use as a PET proliferation tracer was first proposed and investigated by Grierson and Shields.[42,43] Subsequent cell uptake studies and murine xenograft studies demonstrated that FLT accumulates in cancer cells and tumors in proportion to their rate of cellular proliferation.[44–47] Also, treatment with chemotherapy and radiation has been shown to reduce FLT uptake in these cancer models.[48–57]

Mechanism of FLT Uptake and Retention

FLT imaging takes advantage of the pyrimidine salvage pathway (Fig. 5), which transports circulating thymidine and closely related analogs into cells and phosphorylates them, providing proliferating cells with a pool of nucleotides for use in DNA synthesis. This process supplements the pool of thymidine monophosphate provided by de novo synthesis.[46,58]

After intravenous administration, FLT enters tumor cells both via a nucleoside transporter and partly via passive diffusion.[39] Inside proliferating cells, FLT is accepted as a substrate by thymidine kinase 1 (TK-1), which phosphorylates it, thereby trapping it in cells.[44,58,59] FLT-monophosphate is further phosphorylated to di- and triphosphate forms.[58] However, FLT-triphosphate is not significantly incorporated into DNA,[39,45,47,60] unlike TdR and some other thymidine analogs.[60,61] Thus, the majority of FLT persists as mono- and triphosphates in the cytosol.[39,58] Over time, some FLT is effluxed from cells, due to dephosphorylation of FLT-monophosphate by a putative deoxynucleotidase, but this occurs at a slower rate, creating a significant period of relatively stable tracer retention for imaging.[58]

One major advantage of FLT over TdR is that FLT is not a substrate for degradation by thymidine phosphorylase (TP).[42,43] TP degrades thymidine into a sugar-1-phosphate and labeled thymine, which undergoes further metabolism.[42,43] In contrast, FLT is inert to TP. This property of FLT simplifies PET image analysis and maintains circulating

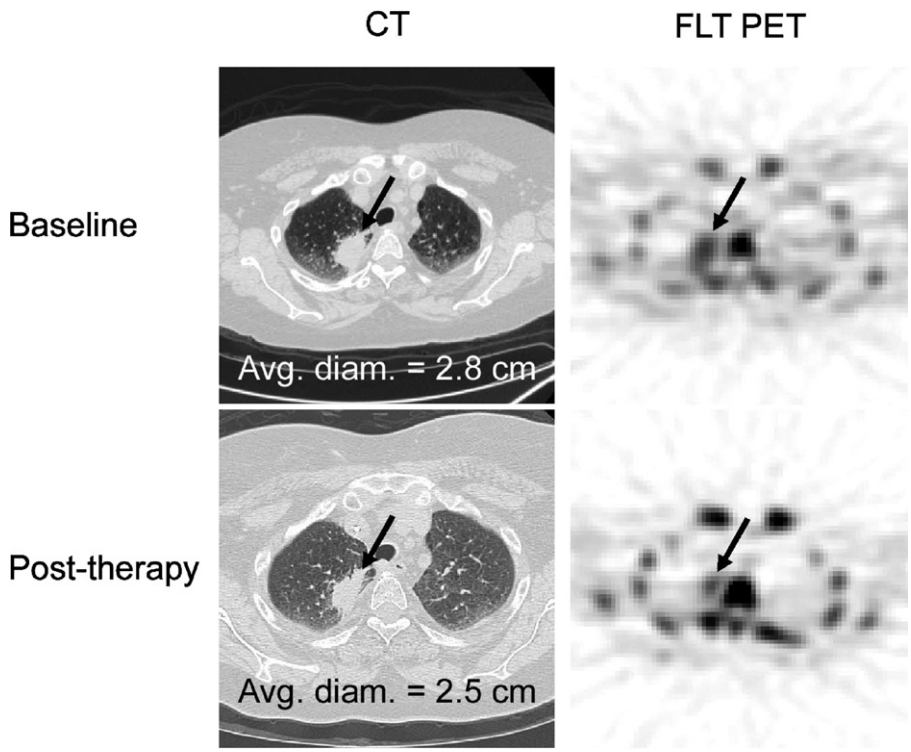


Figure 4 Example of CT and FLT-PET in evaluating response to chemotherapy in nonsmall cell lung cancer. FLT-PET could be used to assess response to chemotherapy. In this subject with nonsmall cell lung cancer, the lesion's size on CT did not show a significant change after chemotherapy. However, the visually assessed level and distribution of FLT uptake was noticeably different.

blood concentrations of FLT at higher levels after injection than with TdR. Also, unlike TdR, FLT is not a substrate for thymidine kinase 2, which is used for mitochondrial DNA replication and repair.[45,59] This specificity means that FLT uptake is exclusively linked to the thymidine salvage pathway in relation to nuclear DNA synthesis.

Phosphorylation by TK-1 is the rate limiting step in FLT accumulation in proliferating cells, causing FLT to accumulate in proportion to TK-1 activity.[43–46,49,62–65] Because its retention does not result from direct incorporation into DNA, it has been suggested that FLT is not a true tracer of proliferation.[66,67] Nevertheless, the enzymatic activity of TK-1 is tightly regulated to correspond with cellular proliferation. TK-1 expression is typically low in the G_0/G_1 phase of the cell cycle, and is selectively upregulated just before and during S-phase.[68–72] TK-1 is then rapidly degraded after G2 or M-phase.[68–72] In practice, TK-1 activity is representative of S-phase fraction[44–46] and FLT uptake as a measurement of TK-1 activity correlates strongly with other markers of cellular proliferation, including PCNA and Ki-67 score.[49,52,56,62,73,74] Indeed, the metabolic trapping of FLT is analogous in concept to that of FDG, which depends on transport by Glut-1 and phosphorylation by hexokinase as a marker for glucose utilization, without further progression down the glycolytic pathway.[42,63–65]

In more aggressive tumors, there may be a more complex control of TK-1 activity where high TK-1 levels may no longer be tightly coupled with cell cycle stage, but may be associated with other facets of tumor aggressiveness, such as high mutation rate in tumor cells.[44,71,75] Thus, TK-1 dependent FLT uptake may reflect tumor aggressiveness, in addition to cell proliferation.

FLT Radiosynthesis

Since the initial practical radiosynthesis of FLT at the University of Washington and Wayne State University,[42,43] several innovations have been adopted, with the aim of simplifying the synthesis and improving yield.[76,77] Currently, the favored FLT production method is the one described by Machulla and coworkers,[76] which is a one-pot process that can be conveniently automated. A GMP-grade labeling precursor for this process is commercially available (ABX Biochemicals, Germany). The U.S. National Cancer Institute (NCI) has chosen this method, following United States Pharmacopeia [797, 823] guidance, for an investigational new drug application (IND), approved by the U.S. Food and Drug Administration (FDA). NCI encourages investigators to cross-file on this IND, to investigate FLT-PET in cancer.

Quantifying FLT Uptake

There are 2 approaches to image analysis with FLT-PET. One simple method, which is practical in the clinic uses the standardized uptake value (SUV) derived from static images. The second method uses dynamic image sequences and kinetic

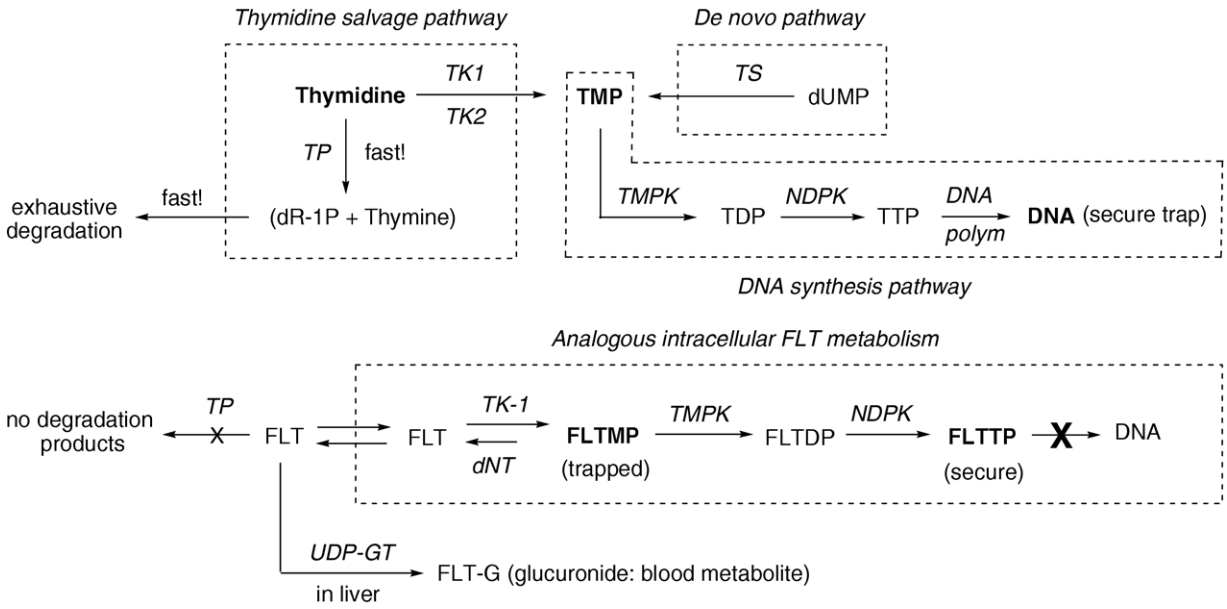


Figure 5 Thymidine salvage pathway and DNA (thymidine) synthesis pathway. Thymidine (TdR) enters cells via the thymidine salvage pathway and is trapped due to DNA incorporation. However, TdR is also rapidly broken down by thymidine phosphorylase (TP). FLT is not a substrate for TP and is trapped in cells after phosphorylation by TK-1, even though it is not incorporated into DNA. Abbreviations: (a) *enzymes*: TP (thymidine phosphorylase); TK (thymidine kinase: TK-1 (cytosolic), TK-2 (mitochondrial)); dNT (5′-deoxynucleotidase); TS (thymidylate synthase); TMPK (thymidylate kinase); NDPK (nucleotide diphosphate kinase); DNA polym (DNA polymerase); (b) *substrates*: TdR (thymidine); FLT (3′-deoxy-3′-fluorothymidine); dR-1P (2-deoxy-D-ribose-1a-phosphate); TMP, TDP, TTP (nucleotides); FLTMP, FLTDP, FLTTP (FLT nucleotides); dUMP (2′-deoxyuridine monophosphate).

modeling of tracer uptake.[63–65] This approach is uniquely able to track FLT uptake and retention over time and to estimate the rates of the underlying biological processes that contribute to observed radiotracer levels. As a result, kinetic model-based results have shown stronger correlations with tumor proliferation rates than SUVs[11,19,64] and have provided additional insights into the biology of malignant tumors.[64,65] With either method, partial volume correction can be employed to compensate for the limited resolution of current PET scanners, which leads to the underestimation of radiotracer uptake in small tumors.[11,78]

For FLT, Muzi and coworkers have described a kinetic model suitable for analyzing dynamic FLT-PET images of both somatic and brain tumors.[63–65] Kinetic analysis using a model with 2 tissue compartments yields estimates of rate constants characterizing FLT transport and retention within cells as well as the overall flux of FLT into cells. The FLT flux also can be estimated using the simplified graphical method developed by Patlak and coworkers.[79,80] A key prerequisite for kinetic analysis is the availability of an input function in the form of a blood or plasma time activity curve. This can be derived either from counting the activity in repeated arterial samples drawn during imaging or from fitting a historical FLT arterial curve to individual patient data derived from venous sampling.

Over time, after FLT injection, there is a low-level production of FLT-glucuronide in the liver. The presence of FLT-glucuronide does not affect radiotracer uptake in proliferating tissues but does affect the measured blood concentration of FLT at later time points because not all observed radioactivity in blood or plasma represents FLT available to enter proliferating cells. Kinetic analysis takes into account the fraction of this metabolite in blood, using published methods for quickly and routinely analyzing its contribution to blood activity.[81,82] On FLT-PET images, high tracer levels in the liver are due to the production of this glucuronide, not to elevated proliferation.

Although more technically demanding and often limited to a single field of view, dynamic imaging with kinetic modeling should be the initial approach to evaluate FLT in any new tumor type or clinical application. The additional precision and biological information provided by this method can then be utilized to develop simplified dynamic imaging protocols and to determine whether static imaging could suffice in a given clinical setting. This approach may be especially appropriate when FLT is used to assess tumor grade and prognosis, or to predict and monitor tumor response to therapy, rather than as a staging method. Because of its simplicity of implementation and the ability to scan over multiple fields of view, static FLT-PET imaging may ultimately be the method which transitions successfully to the clinic. This method has already shown high reproducibility.[24] However, the ongoing work of validating FLT-PET as an accurate tracer of proliferation across the spectrum of human tumors and clinical settings (including after specific therapies) should be conducted using the biological insights provided by kinetic modeling and dynamic imaging.

Safety

The safety of radiotracer doses of ^{18}F-FLT has been examined from the standpoints of both radiation exposure and drug toxicity, by our group at the University of Washington.[41,83] In terms of radiation exposure, ^{18}F-FLT administered at a dose of 2.59 MBq/kg (0.07 mCi/kg) with a maximum dose of 185 MBq (5 mCi) produced similar whole-body and bladder wall radiation doses[83] as have been reported for FDG, per unit of activity injected.[84] With FLT administration, the brain and heart received lower radiation doses than with a comparable dose of FDG, whereas the liver and bone marrow received somewhat higher doses because of glucuronidation of FLT in the liver and high proliferation in the marrow. Whole-body and individual organ radiation doses after a 185 MBq (5 mCi) administration of ^{18}F-FLT are well below the FDA's maximum suggested individual study and annual doses for radioactive research drugs.[85] Nevertheless, care should be exercised to observe these limits if administering higher doses of ^{18}F-FLT or conducting repeat scans in research patients.

In terms of any potential for drug toxicity, a single, 185-MBq (5 mCi) intravenous dose of ^{18}F-FLT, at a minimum specific activity of 3.7 GBq/μmol (0.1 Ci/μmol; a conservative estimate), corresponds with a total labeled and unlabeled FLT mass of 12.2 μg.[41] Comparing the integrated exposure to FLT in the systemic circulation, this dose is nearly 3000 times lower than the lowest cumulative dose observed to produce any toxicity (peripheral neuropathy) in clinical trials of FLT as antiretroviral therapy.[40,41] In addition, no neurological or other adverse effects were observed in any of 20 subjects receiving a single ^{18}F-FLT radiotracer dose of 2.59 MBq/kg (0.07 mCi/kg) with a maximum dose of 185 MBq (5 mCi).[41]

No dietary restrictions are necessary before FLT-PET, unlike with FDG-PET. In the current literature on FLT-PET scanning in humans, some studies required prescan fasting whereas others did not. Both types of studies produced quality images and reached similar conclusions regarding their significance and interpretation. This convenience of FLT-PET imaging could be especially significant to diabetic patients and will not be affected by fasting compliance issues. FLT uptake is also unlikely to be affected by patient movement during the prescan uptake period, unlike FDG uptake, which is influenced by skeletal muscle activity.[86]

Clinical Investigations of FLT-PET

To date, the majority of clinical studies of FLT-PET in humans have focused on validating it as an accurate measurement of proliferation in a broad spectrum of cancers (Fig. 2), and assessing its usefulness for diagnosing and staging malignancies (Fig. 3). Future studies should test FLT-PET's usefulness for predicting and monitoring response to therapy (Fig. 4), and for predicting overall tumor aggressiveness and patient outcome. Already, a few studies have begun to do so in lymphoma,[25] glioma,[16] and breast cancer,[23,24] with positive findings.

Lung Cancer

Four studies have demonstrated that FLT uptake at PET correlates with the proliferation rate of human lung tumors.[11–14] Compared with Ki-67 immunohistochemical scoring performed on biopsy or resection specimens, reported correlations ranged from $r = 0.60$ to $r = 0.92$. Vesselle and coworkers found a greater correlation with Ki-67 scores when FLT uptake was quantified using kinetic modeling parameters,[11,64] rather than a simple SUV (SUV_{max}, $r = 0.61$; partial volume corrected SUV_{max}, $r = 0.67$; SUV_{mean}, $r = 0.72$; and modeled FLT flux, $r = 0.89$). The importance of these findings stems from the prognostic value of tumor proliferation in nonsmall cell lung cancer.[9,10] FLT-PET should therefore be investigated as an independent predictor of prognosis for early stage nonsmall cell lung cancer, where it could permit the selection of patients at risk for recurrence who would most benefit from adjuvant chemotherapy following surgical resection.

FLT-PET also has been evaluated for primary lung cancer detection and was found to have high accuracy, despite the fact that FLT has lower uptake in lung cancer than does FDG.[13,14,22,87] Four studies that compared the diagnostic sensitivity of FLT-PET and FDG-PET in detecting primary lung cancer reported sensitivities of 72% to 100% and 89% to 100%, respectively.[13,14,22,87] Two of these studies which also included benign lesions reported specificities of 86% to 100% and 57% to 73% for FLT-PET and FDG-PET, respectively.[13,22] Although all studies assessed these diagnostic parameters in relatively small patient populations (17-35 subjects), they tell a consistent story: FDG is the more sensitive tracer, whereas FLT may be the more specific for malignancy (Fig. 3). In a fifth study assessing diagnostic and staging accuracy, FLT-PET results were compared directly against FDG-PET results without biopsy confirmation, so it remains unknown whether dissimilarities between the 2 modalities were attributable to false negatives on FLT-PET, false positives on FDG-PET, or a combination.[88]

In assessing lymph node metastases from primary lung cancer, 2 studies reported FLT-PET sensitivities of 33% to 53% for lymph node status and specificities of 98% to 100%, whereas FDG had sensitivities of 44% to 77% and specificities of 95% to 100%.[13,22] Therefore, in lung cancer, FLT-PET combined with FDG-PET could potentially improve the specificity of staging and provide information regarding tumor growth rate and aggressiveness which could impact prognosis and/or treatment selection.

The effect of chemotherapy and radiotherapy on FLT uptake in lung cancer still needs to be examined. Specifically, it should be explored whether decreases in FLT uptake after therapy correlate with clinical response by size criteria, whether they predict progression-free survival and overall survival, and how soon after the initiation of therapy a successful response can be predicted by FLT-PET. With the advent of cytostatic chemotherapy agents such as erlotinib (Tarceva), which can produce a response in lung cancer without associated tumor shrinkage, it will be necessary to have

alternative imaging tools to assess the biological effectiveness of such treatment. FLT-PET may play such a role.

Brain Malignancies

FLT, unlike FDG, is only slightly able to cross the intact blood–brain barrier.[89,90] For this reason and because of low proliferation in normal brain tissues, FLT has very low background uptake in the brain. However, FLT is taken up significantly by high-grade gliomas and other brain tumors associated with significant blood-brain barrier breakdown.[15–17,91] As a result, FLT-PET can create images of high-grade brain malignancies with greater tumor/background contrast than possible with FDG-PET.[15,16] FDG, in comparison, has high background uptake in the normal brain, in particular in gray matter. This physiologic uptake makes image interpretation difficult and can obscure malignant tumors or sites of tumor recurrence with FDG uptake equal to or less than background.[92–94]

Because of this difference, FLT-PET can have distinct advantages over FDG-PET in diagnosing high-grade brain tumors. To demonstrate this point, Choi and coworkers[15] performed FLT-PET on a series of 22 patients with suspicious brain lesions with FDG uptake equal to or less than background (ie, those which would be extremely difficult to detect by FDG-PET). In this sample, FLT-PET successfully detected 11 of 14 cancerous tumors and accurately showed no uptake in 5 of 8 benign lesions. Three false positive findings were found in 1 subacute infarction, 1 multiple sclerosis lesion, and 1 focus of radiation necrosis.

However, FLT's limited penetration of the intact blood–brain barrier also means that low-grade tumors without significant breakdown of the blood-brain barrier have very low uptake of FLT and can frequently go undetected.[15,16,65,91] As a result of this dichotomy, overall sensitivities of 79% to 82% have been reported for the detection of brain malignancies with FLT-PET.[15,16,91]

Overall, Choi and coworkers have reported that FLT uptake correlates very well with tumor grade at pathology ($r = 0.87$), and have proposed that FLT uptake may therefore be able to function as a noninvasive means of grading brain tumors.[15] FLT uptake has been found to correlate strongly with tumor proliferation assessed by Ki-67 immunohistochemistry in gliomas of all grades ($r = 0.84$ and $r = 0.82$, respectively).[15,16]

In gliomas, cellular proliferation assessed by Ki-67 immunohistochemistry is a well-characterized marker of clinical outcome,[6,7] suggesting that FLT-PET may also be able to noninvasively predict outcome in gliomas. Chen and coworkers recently tested this hypothesis in a series of 25 patients with newly diagnosed or recurrent gliomas.[16] In this series, FLT-PET visualized all 18 high-grade (III/IV) gliomas, but none of 4 grade II gliomas or 3 gliomas with long-term stability used as negative controls. FLT-PET was also an outstanding indicator of prognosis. All 14 patients who had disease recurrences and died during the follow up period also had visually positive FLT-PET results, including 5 patients with negative FDG-PET findings. The mean FLT SUV of this group was twice that of survivors. On the other hand, none of the 7 patients with visually negative FLT-PET findings (4 with low-grade gliomas and 3 with long-term stable disease) had a relapse or died during the follow-up period. These findings demonstrate the potential of FLT-PET for predicting survival after treatment.

Lymphoma

The majority of clinical work on FLT-PET in lymphoma has been conducted by Buck and coworkers. This group has shown in a series of 25 patients that FLT SUV_{max} was strongly correlated with cellular proliferation assessed by Ki-67 immunohistochemistry ($r = 0.84$).[18] In addition, there was a clear dichotomy between 14 aggressive and 11 indolent lymphomas in this series, with the former group having mean FLT SUV_{max} and Ki-67 scores of 8.8 and 83%, respectively, and the latter group having mean FLT SUV_{max} and Ki-67 scores of 3.7 and 14%, respectively. These findings are in accord with a much smaller series by Buchmann and coworkers.[95]

On the basis of these initial results, lymphoma appears to display a much greater uptake of FLT than any other malignancy, possibly due in part to a high fraction of proliferating cells in aggressive lymphomas. However, even indolent lymphomas with a mean Ki-67 score of 14% had a mean FLT SUV_{max} of 3.7. This level of FLT uptake is considerably higher than the mean for any other malignancy examined to date. In fact, overall FLT uptake was comparable in magnitude to FDG uptake.[18,25] In detection of lesions, Buck and coworkers found that in 34 patients, FLT detected more lymphoma lesions than were detected by routine clinical staging (490 versus 420). However, given the high uptake of FLT in normal bone marrow and the propensity for marrow involvement in lymphoma, the clinical usefulness of FLT-PET in staging lymphoma is still likely to be limited. Future research should focus on FLT's role in predicting and assessing response to therapy, with FDG-PET remaining the main lymphoma staging tool.

Recently, the same group examined the ability of FLT-PET to predict response to chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in non-Hodgkin's lymphoma (NHL).[25] In 22 patients, there was a mean 68% decrease in FLT uptake just 2 days after receiving the first dose of CHOP, a 77% decrease at 7 days, and an 85% decrease at 40 days compared with baseline, after a second cycle of CHOP. Furthermore, uptake measurements within 1 week after the first CHOP administration corresponded significantly with eventual response, determined by computed tomography (CT) and magnetic resonance imaging (MRI) criteria over 3 months later, after 6 cycles of CHOP. Mean FLT SUV during the first week was 1.5 in complete responders, versus 2.6 in partial responders. Only one patient had progressive disease, and 2 days after CHOP, this patient had an FLT SUV of 9.0, the highest uptake value observed in any patient, at any posttherapy time point. These results demonstrate the potential of FLT for early assessment of treatment response in lymphoma, affording the possibility

of switching therapy regimens quickly in cases of nonresponse. Because relapse only occurred in 2 patients, correlation between residual FLT uptake or decrease in FLT uptake and overall outcome was not attempted. However, future studies should examine this question.

Breast Cancer

Two recent studies have demonstrated the potential of FLT for early assessment of response to chemotherapy in breast cancer.[23,24] In 14 patients with metastatic breast cancer, Pio and coworkers[23] performed FLT-PET before and 2 weeks after initiation of chemotherapy, which was cytotoxic for four patients and hormonal only for 5. In these patients, the percent decrease in FLT uptake observed over the first two weeks of therapy correlated strongly with late CT size changes ($r = 0.74$) and CA27.29 tumor marker levels ($r = 0.79$) measured 3.3 and 5.8 months later, respectively. In contrast, changes in FDG uptake over the first two weeks were not significantly correlated with these response measurements. Also, in three patients in whom the directionality of FLT and FDG uptake changes differed, FLT was the more accurate clinical indicator.

Similarly, Kenny and coworkers measured FLT uptake in 13 patients with 17 breast cancer lesions, immediately before and 1 week after initiation of treatment with combined 5-FU, epirubicin, and cyclophosphamide, and compared these values with patients' eventual response by CT RECIST criteria after 60 days.[24] The 6 patients who were found to have a partial or complete response at 60 days also had a greater percent reduction in tumor FLT uptake during the first week, compared with the 6 patients ultimately diagnosed with stable disease.

In breast cancer, FLT uptake has been shown to correlate with cellular proliferation assessed by Ki-67 immunohistochemistry.[19] In 12 patients, a greater correlation coefficient was observed when quantifying FLT uptake using kinetic modeling parameter estimates ($r = 0.92$) than when using either the 21- or 60-minute postinjection SUV_{max} ($r = 0.71$ and $r = 0.79$, respectively). This advantage of kinetic modeling is analogous to observations in lung cancer.[11,64] The fraction of tracer uptake at 5 minutes retained at 60 minutes (FRT) produced an equally excellent correlation ($r = 0.92$). However, another study by Smyczek-Gargya and coworkers found no significant correlation ($r = 0.1$) between FLT uptake and proliferation by Ki-67 scores derived from core biopsies of 12 breast cancers.[96] Although it would be easy to attribute these negative results to a small sample size or to biopsy sampling limitations, future studies in breast cancer should examine this issue for the purpose of clarification.

In terms of staging, Smyczek-Gargya and coworkers reported that in 12 patients, although FLT uptake was lower than FDG uptake in primary breast cancers, tumor/background contrast was equivalent because of low background FLT uptake in the normal breast.[96] FLT-PET successfully detected 13 of 14 primary breast cancers and 7 of 8 axillary lymph node metastases. Been and coworkers also reported good tumor/background uptake ratios with FLT-PET in breast cancer.[21] However, in this study, FLT only detected 8 of 10 primary breast cancers and 2 of 7 patients' lymph node metastases. Because of the limited numbers of patients in these studies, larger trials will have to be conducted to determine whether FLT-PET should play any role in axillary lymph node staging. However, even in the best case, it is unlikely that FLT-PET would replace sentinel lymph node mapping and excision for accurately evaluating the axilla. Instead, FLT-PET is most likely to play a role in assessing response to therapy, especially in locally advanced breast cancers treated with neoadjuvant chemotherapy.

Colorectal Cancer

Francis and coworkers reported imaging 17 patients with primary or metastatic colorectal cancer (CRC) using FLT-PET.[97] Using FLT-PET, they successfully detected all 6 primary CRCs, all 6 peritoneal metastases, and 5 of 6 lung metastases. Disappointingly, however, they detected only 11 of 32 liver metastases because of high background activity in the liver, caused by hepatic glucuronidation of FLT. Because the liver is a key site of possible metastasis for CRCs, FLT is unlikely to provide comprehensive staging on its own. Its role will more likely be in combination with FDG-PET, in the evaluation of treatment response in rectal cancer patients undergoing induction chemoradiotherapy.

Esophageal Cancer

Only van Westreenen and coworkers have specifically examined FLT uptake in esophageal cancer, finding that, in their series of 10 patients, they successfully detected 8 of 10 primary esophageal cancers with FLT-PET, compared with 10 of 10, with 2 false-positive findings using FDG-PET.[98] Both tracers successfully detected only 2 of 8 primary lymph node metastases, and only one of these metastases was detected by both tracers.

Surprisingly, in this study, there was not a significant positive correlation between FLT uptake and cellular proliferation assessed by Ki-67 scoring ($r = -0.76$).[98] Negative results also were obtained in a similar comparison by Dittmann and coworkers, in 9 patients with thoracic tumors, including 5 esophageal carcinomas ($r = 0.24$).[87] Because both of these studies were extremely small, and because the van Westreenen study may have been hampered by using extremely short emission scans of only 2 minutes per bed position, it remains to be seen whether these findings reveal an unusual property of esophageal carcinoma, or are merely an anomaly.

Head and Neck Cancer

Two groups of authors have examined the diagnostic and staging accuracy of FLT-PET in head and neck cancer. In a series of 17 primary and recurrent laryngeal cancers, Cobben and coworkers reported that although FLT had lower uptake in primary tumors than FDG, both tracers successfully detected 15 of 17 primary tumors, with 1 false-positive finding with FDG-PET and 2 with FLT-PET.[99] In addition, Troost and coworkers reported that in a series of 10 stage II-IV squamous cell head and neck cancers, FLT successfully de-

tected 3 of 3 metastatic cervical lymph node metastases but also showed increased uptake in a large number of benign lymph nodes.[20] This effect was found to be caused by high proliferation of B-lymphocytes in germinal centers in lymph nodes, with Ki-67 scores that were actually greater than in squamous cell carcinoma nodal metastases. The number of germinal centers was also greatly increased in false-positive lymph nodes. This study therefore revealed a significant and hitherto unexplored source of false-positive FLT lymph node findings which warrants further characterization, especially to determine in what clinical situations this type of finding is likely to occur. Overall, FLT uptake was found to correlate significantly with proliferative activity in lymph nodes quantified by either Ki-67 or deoxyuridine staining of pathology specimens ($r = 0.69$ and $r = 0.74$, respectively).[20]

Melanoma

Cobben and coworkers have investigated FLT-PET in a series of 10 patients with stage III melanoma.[100] FLT-PET correctly detected the extent of locoregional lymph node metastasis in all patients and correctly upstaged 2 patients. Of a total of 30 pooled histologic samples, FLT correctly detected 22 of 25 malignant foci and correctly showed no uptake in 3 of 5 benign foci. In an additional 11 foci detected by FLT for which histologic confirmation was not available, clinical correlation revealed that FLT was true-positive in 5, false-positive in 4, and 2 could not be definitively evaluated but were likely true-positive. Thus, FLT may show some ability to contribute to the noninvasive locoregional staging of melanoma. However, as in the setting of breast cancer, FLT-PET should not be expected to be able to improve on the sensitivity of lymphoscintigraphy and sentinel lymph node excision. The overall noninvasive staging of melanoma will likely remain the domain of FDG-PET/CT. The role of FLT-PET in initial prognostic assessment or in assessing response to therapy in melanoma still needs to be explored.

Soft Tissue Sarcoma of the Extremities

Cobben and coworkers also examined FLT-PET in 19 patients with 20 soft-tissue sarcomas of the extremities.[101] They found that FLT-PET detected all sarcomas and also identified additional malignant foci in 3 of the patients, with significant clinical implications. Benign, false-positive lesions were also detected by FLT-PET in 3 of the patients. Either the FLT SUV or the tumor/nontumor uptake ratio was able to distinguish between low- and high-grade sarcomas. These measurements also correlated with proliferation by immunohistochemical scoring and the French and Japanese grading systems for sarcoma (all correlations between $r = 0.55$ and $r = 0.75$). In addition, Been and coworkers have examined FLT-PET before and after treatment with hyperthermic isolated limb perfusion (HILP) in 10 patients, concluding that pretreatment FLT uptake correlates with mitotic index ($r = 0.82$) and predicts clinical response to treatment.[26]

The Future of FLT-PET

^{18}F-FLT is a promising tracer for which the basic mechanism of cellular uptake and retention is known. Further cell uptake studies should be conducted to understand how cellular uptake of FLT is affected by various therapeutic interventions, across many cell lines. With well established dosimetry, no known toxicity for radiotracer doses, and an IND that can be cross-filed, FLT is ready for a thorough validation in larger trials addressing clinically relevant questions. FLT's role will likely be (1) to establish patient prognosis through measurement of primary tumor proliferation rate and (2) to predict and evaluate tumor response to therapy. In most clinical settings, it is expected to be applied as strong adjunct to staging by FDG-PET.

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