INVITED COMMENTARY

PET Imaging with ^{18}F-FLT and Thymidine Analogs: Promise and Pitfalls

Further development and validation of ^{18}F-labeled 3′-deoxy-3′fluorothymidine (FLT) is presented by Buck et al. in this issue of *The Journal of Nuclear Medicine* (*1*). Although the use of PET has exploded within the last few years, this has primarily resulted from the application of ^{18}F-FDG in oncology research and clinical practice. The study of Buck et al. further explores the use of ^{18}F-FLT (Fig. 1) and begins a necessary comparison with ^{18}F-FDG. ^{18}F-FDG has found wide applicability in clinical oncology, since it is simple to use and can be distributed commercially. ^{18}F-FDG is useful in the diagnosis, staging, and restaging of a wide variety of tumor types and in the evaluation of their treatment. Although ^{18}F-FDG is the only PET tracer now routinely used in clinical oncology, many other tracers have been produced and tested by investigators. If PET is going to continue to expand, other tracers that complement the information provided by ^{18}F-FDG will need to be found.

For oncologic use, the measurement of tumor growth and DNA synthesis are attractive targets for imaging. Many investigators have thus been led to test a variety of DNA precursors for tumor imaging. Based on the work originally done in the laboratory using ^{3}H- and ^{14}C-labeled thymidine, synthesis of ^{11}C-thymidine was developed for use with PET (*2,3*). Imaging with ^{11}C-thymidine is of great interest, since it is the native pyrimidine base used in DNA synthesis. Although such an approach is useful in research and validation studies, the practical limitations of this tracer will preclude it from gaining wide acceptance, and it has been evaluated by only a few research centers. Routine clinical use is not practical, given the short half-life of ^{11}C-thymidine and its rapid biodegradation. The result has been a search for analogs of thymidine that might have superior imaging properties. Fortunately, thymidine analogs have been widely studied by both the pharmaceutical industry and academic scientists as possible therapeutic compounds. The study of these analogs was first undertaken by Dr. Charles Heidelberger, who, seeking thymidine analogs that could interfere with DNA synthesis, produced 5-fluorouracil in 1957. Although 5-fluorouracil and its related nucleoside fluorodeoxyuridine have found use as antineoplastic agents, they also undergo rapid catabolism limiting their use for routine imaging. Similar problems have limited the use of other halogenated thymidine analogs (5-iododeoxyuridine and 5-bromodeoxyuridine) substituted in the 5-position on the pyrimidine ring (*4,5*). Imaging with such agents, however, has been useful in understanding their pharmacokinetics. Placing a fluorine in the 2′- or 3′-positions of the sugar, however, has lead to the production of analogs that resist catabolism, since they are stable to thymidine phosphorylase, which cleaves the glycosidic bond.

^{18}F-FLT was originally produced after investigators discovered the anti-HIV properties of azidothymidine. In the initial phase I trial in patients with AIDS, ^{18}F-FLT was found to have higher toxicity at clinically useful doses (100 mg/d given for weeks) (*6*). Such pharmacologic studies of unlabeled ^{18}F-FLT, however, demonstrate that it can safely be given at the tracer doses used by PET. The synthesis of ^{18}F-labeled FLT has been worked out, and it can be made by a couple of different major routes using precursors that are now commercially available. Initial studies of ^{18}F-FLT in animals and subsequent pilot patient studies have demonstrated that the tracer produces images of high contrast of both proliferating tissues and tumors (*7,8*). As predicted, it resists degradation but does undergo glucuronidation. Because the fluorine is placed in the 3′-position in the sugar, ^{18}F-FLT, like its parent drug azidothymidine, works as a terminator of the growing DNA chain. Little ^{18}F-FLT is actually accumulated in DNA; rather, it is retained

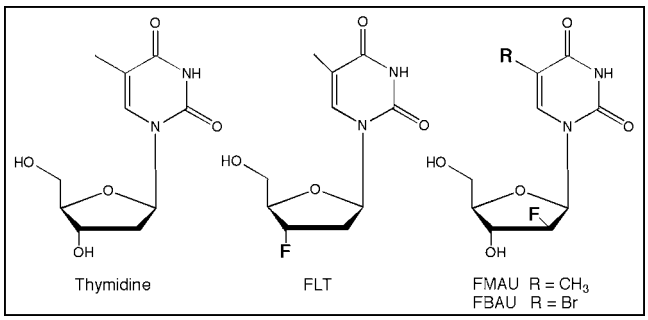

FIGURE 1. Structures of thymidine, ^{18}F-FLT, and related analogs.

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intracellularly after phosphorylation by thymidine kinase 1 (*9*). Very much analogous is the imaging of the glucose use pathway with ^{18}F-FDG after trapping by hexokinase (*10*). Both compounds therefore reflect accumulation by transport and subsequent activation by the first step in the utilization pathways. ^{18}F-FLT does not reflect the whole of DNA synthesis, just as ^{18}F-FDG does not reflect the whole of glucose use. This limitation may prove to be important in some situations, but so far, it has not been a problem. In cell lines, ^{18}F-FLT retention correlated with ^{3}H-thymidine uptake ($r = 0.88$, despite the fact that DNA incorporation was 0.2% and more than 90%, respectively, for the 2 tracers (*11*)). In a few studies, thymidine kinase 1 levels have been found to correlate with cell proliferation. The study of Buck et al. and a recent study of Vesselle et al. clearly add to this literature in demonstrating the ^{18}F-FLT retention in lung cancer correlates with tumor proliferation (*1,8*). The average standardized uptake value (SUV) for ^{18}F-FLT was found to correlate nicely with Ki-67 immunohistochemistry, with $r = 0.92$ and 0.84, in the studies of Buck et al. and Vesselle et al., respectively. Dynamic imaging was also performed in the study of Vesselle and improved the correlation slightly. S-phase fraction, measured by flow cytometry, also correlated with ^{18}F-FLT retention. This correlation appears to be better than that between Ki-67 staining and the kinetic measurement of ^{11}C-thymidine retention ($r = 0.58$), although the latter was done on a mixed group of abdominal tumors (*12*). Although the data with ^{18}F-FLT look promising, one still must prove that ^{18}F-FLT will track proliferation in at least most situations. This issue is particularly important after therapy, since one could envision situations in which DNA synthesis might be impaired, while thymidine kinase 1 activity persisted for a time.

As the PET community continues to validate the use of ^{18}F-FLT, the determination of how it compares with ^{18}F-FDG will be critical. Are there situations in which ^{18}F-FLT might be more accurate in diagnosis, staging, and the assessment of treatment response? The study of Buck et al. (*1*) begins to address this issue. Their study demonstrated that ^{18}F-FDG produces higher-contrast images (mean SUV, 4.1) than does ^{18}F-FLT (mean SUV, 1.8). Although ^{18}F-FLT can miss slowly proliferative tumors, its specificity was 100% in their study. On the other hand, ^{18}F-FLT has not always been 100% specific. In our dog studies we found that ^{18}F-FLT is taken up in some normal lymph nodes, and in a patient with sarcoidosis we have seen increased ^{18}F-FLT retention in involved lymph nodes. It is important to realize that the inflammatory reaction can involve proliferation of some cellular elements. At this point, it will be important to determine which clinical situations merit the use of ^{18}F-FLT, rather than ^{18}F-FDG. This determination will require careful study, taking into account the strengths and limitations of each tracer. ^{18}F-FLT has lower background activity in the brain and thorax. On the other hand, ^{18}F-FLT is limited in its ability to image in the bone marrow because of normal cellular proliferation and in the liver because of glucuronidation. The most demanding use of PET in oncology may be the early assessment of treatment response. If one can document that PET reliably demonstrates treatment failure, then a switch to second-line therapy may be indicated. ^{18}F-FDG has been studied for this use in a few trials. Further trials with both ^{18}F-FDG and ^{18}F-FLT are clearly needed before oncologic physicians will be comfortable with this application.

Finally, ^{18}F-FLT is not the only pyrimidine analog that can be used to measure proliferation. FMAU (1-(2′-deoxy-2′-fluoro-β-D-arabinofurano-syl)thymine) and FBAU (1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-bromouracil) were initially labeled with ^{11}C and ^{76}Br, respectively, and more recently, both have been labeled with ^{18}F (*13–15*). These compounds are incorporated into DNA, which offers theoretic advantages (*16*). Further studies will be needed to determine whether such tracers offer practical improvements over ^{18}F-FLT. Differences in metabolism may be more important in determining which tracer may best be used for different tumors or areas of the body. For example, the high retention of FMAU in the liver may impair imaging in the upper abdomen compared with ^{18}F-FLT, while FMAU is less readily cleared into the bladder than ^{18}F-FLT, leading to improved FMAU imaging of the pelvis.

In summary, the development of PET will require new tracers that track different cellular pathways for use in oncology. ^{18}F-FLT offers a promising approach to imaging cellular growth and has now been used at several centers around the world. The recent studies are an important step in validating and determining the best way to use this tracer.

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