

## PET Imaging of Tumor Growth: Not as Easy as It Looks

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Positron emission tomography can be used to image tumor proliferation when combined with appropriate labeled tracers, such as the thymidine analog [ $^{18}\text{F}$ ]-3'-deoxy-3'-fluorothymidine. Although thymidine kinase 1 is the principal mechanism of cell trapping, other variables, such as the cellular level of native thymidine, may need to be considered. *Clin Cancer Res*; 18(5); 1189–91. ©2012 AACR.

In this issue of *Clinical Cancer Research*, Zhang and colleagues (1) discuss the use of [ $^{18}\text{F}$ ]-3'-deoxy-3'-fluorothymidine (FLT) and positron emission tomography (PET) in xenografts to measure tumor growth. In their study, variations in the level of native thymidine affected the avidity of the tumor for the tracer.

PET, which is now routinely used in clinical oncology, generally employs fluorodeoxyglucose (FDG) to detect tumors and assess their metabolic activity. Because FDG measures only one aspect of the energetic pathway, investigators have developed other tracers to assess different aspects of metabolism in tumors and normal tissues. FLT, a thymidine analog, was developed to measure cell proliferation because it is trapped in the DNA synthetic pathway (2). Previous PET methods used thymidine itself, labeled with  $^{11}\text{C}$ , but the 20-minute half-life of the radionuclide and the rapid cleavage of the glycosidic linkage limited the routine use of this tracer. FLT, like FDG, is now synthesized commercially and is distributed to centers conducting imaging research. It must be kept in mind that PET simply measures the distribution of activity within a human or animal and many parameters can affect tracer uptake and the interpretation of the images produced. The work presented here by Zhang and colleagues (1) highlights one paramount issue: competition for native thymidine. However, to fully exploit the role of FLT, we must also consider other factors, such as the metabolism and clearance of FLT by glucuronidation, transporters of FLT into the cell, activity of thymidine kinase 1 (TK1), and dephosphorylation of metabolically trapped FLT. The uptake of FLT by inflammatory cells, species differences, and the issue of the blood-brain barrier may also require consideration.

In the simplified model of FLT retention, FLT is taken up from the circulation by tumors and transported into

the cell, where it is trapped by phosphorylation by cytosolic TK1 (Fig. 1; ref. 3). TK1 has been shown to have increased activity when cells are going through S phase; thus, FLT retention reflects proliferative activity. Dynamic imaging and measures of FLT flux into the tumors require assessment of the level of the tracer in the circulation, which is obtained from blood samples or imaging of a vessel. This is combined with a time-activity curve obtained over the tumor to calculate the metabolic rate (using measurements of  $k_1$ ,  $k_2$ , and  $k_3$ ). The phosphorylation of FLT by TK1 is represented in  $k_3$ , but dephosphorylation ( $k_4$ ) is also possible. Fortunately, this is generally a slow process, but with prolonged imaging times it can affect the retention of the tracer. To simplify the use of FLT, including assessment of investigational therapeutic agents, most centers solely obtain a static image ~60 minutes after injection of the tracer. The static image provides the standardized uptake value (SUV), which compares tracer activity in the tumor with the injected dose divided by body weight. Clearly, the easy measurement of SUV hides all the other metabolic variables. In most (but not all) tumors, the pure measurement of the SUV has been found to correlate with other measurements of tumor proliferation, such as Ki-67, obtained from biopsies (4). Furthermore, changes in the SUV were found to be useful in studies that assessed treatment response in both mice and humans (1, 5). The limitations of simple measurements such as the SUV clearly need to be considered, because unexpected variations in FLT uptake may lead to erroneous conclusions.

Although FLT resists degradation, its metabolism varies a great deal among different mammalian species. In rodents and dogs, it is excreted unchanged in the urine. In humans, it is glucuronidated in the liver, and 1 hour after administration, ~25% (range: ~15–43%) of FLT appears as glucuronide in the blood (6). Whether this will be an issue in repeated studies in individual humans requires further study. Regardless, marked liver retention is seen from this metabolism, limiting the use of FLT when hepatic tumors are present. Another important species difference is that the natural level of thymidine in the blood is greater than 100-fold higher

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**doi:** 10.1158/1078-0432.CCR-11-3198

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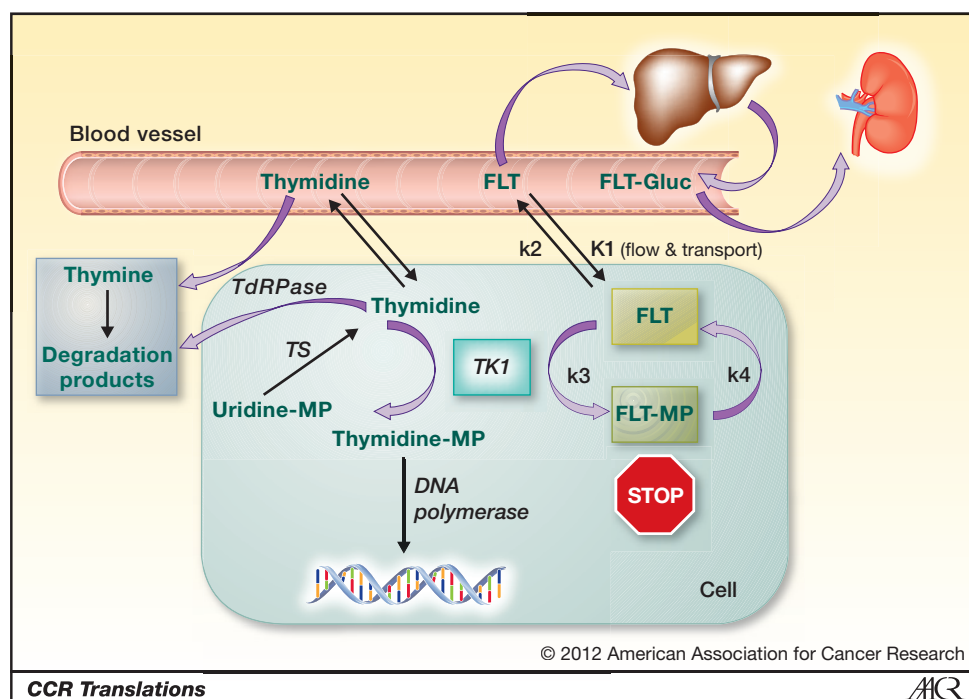


Figure 1. Diagram of FLT and thymidine uptake and metabolism, including phosphorylation by TK1, synthesis of thymidine by TS, and degradation by TdRPase. Thymidine, but not FLT, is incorporated by DNA polymerase. FLT is metabolized to its glucuronide in the liver and excreted by the kidney. The rate constants used in modeling of FLT uptake ( $K_1$ ,  $k_2$ ,  $k_3$ , and  $k_4$ ) are indicated. TdRPase, thymidine phosphorylase.

in mice and rats than in dogs and humans (7). In some preclinical studies, the high level of thymidine in rodents was shown to decrease tracer uptake. The reliance by Zhang and colleagues on murine xenografts in their study may alter our ability to fully translate their results to patients.

From the bloodstream, FLT and thymidine are transported across the cell membrane by both equilibrative (ENT1 and ENT2) and concentrative (CNT1 and CNT3) transporters, which have different affinities for thymidine and FLT (8). The added complexity of the conformation of the transporters and how these differences may affect FLT retention require further study. Once in the cell, the exogenous thymidine and FLT obtained from the blood mix with the endogenously synthesized pool of thymidine. In the implanted cell lines studied by Zhang and colleagues, this pool was shown to vary significantly and compete for the trapping of FLT. It remains to be determined how common a problem this is in tumors clinically. Most of the thymidine that is used by cells in DNA synthesis comes from endogenous synthesis. Once in the cell, the FLT can be metabolically trapped by phosphorylation by TK1. As noted above, TK1 and cell proliferation generally increase concomitantly; however, the control of TK1 is complex and includes posttranslational mechanisms. Drugs that interfere with the endogenous synthesis of thymidine can produce rapid increases in TK1 activity and lead to marked retention of labeled thymidine and FLT (9).

This "flare" phenomenon can be used in pharmacodynamic measurements of the effect of agents that block thymidylate synthase (TS), such as 5-fluorouracil and nolatrexed.

We also need to consider the normal distribution of FLT in various organs *in vivo*. Little FLT is seen in the brain because of the low proliferative rate and the inability of thymidine and FLT to cross the intact blood-brain barrier. When the blood-brain barrier is broken down in tumors, high contrast is seen compared with normal brain parenchyma (10). FLT retention may be seen in the brain when breakdown occurs because of tissue necrosis or when inflammation is present. Inflammatory cells anywhere in the body, which often are proliferating, can retain FLT and incorrectly suggest that a tumor has spread. Some investigators have shown that FLT can be used to image the effects of immunotherapy (11).

This brief summary highlights some of the variables that can alter the tumor retention of FLT. Fortunately, many of these variables become less important when one compares a baseline and post-treatment scan in a single patient. Zhang and colleagues (1) found that FLT retention readily measured the effect of treatment when used in tumors that showed increased FLT uptake at baseline. FLT appears to be a promising method for assessing response to treatment; however, investigators need to understand its mechanism of retention. Such information is important for a proper interpretation of the results obtained with any tracer in

oncology, because PET imaging of tumor growth is not as easy as it looks.

#### Disclosure of Potential Conflicts of Interest

A.F. Shields has an ownership interest in Radiology Corporation of America, and is a consultant to Siemens, Bristol-Myers Squibb, and GE.

#### Grant Support

National Cancer Institute grants CA39566 and CA22453.

Received December 28, 2011; accepted January 11, 2012; published OnlineFirst January 24, 2012.

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