

Proliferation Imaging to Measure Early Cancer Response to Targeted Therapy

□□ Commentary on Sohn et al., p. 7423

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Abstract Positron emission tomography imaging using thymidine and analogues labeled with positron emitters provides noninvasive and quantitative estimates of regional cellular proliferation. This *CCR Translations* summary reviews the biological basis for proliferation imaging and discusses recent results using ^{18}F -fluorothymidine-positron emission tomography to measure response to targeted therapy in the context of prior studies and potential future applications.

In this issue of *Clinical Cancer Research*, Sohn and colleagues from the Asan Medical Center in Korea show that fluorothymidine (FLT)-positron emission tomography (PET) can also provide an early measure of cancer response to targeted therapy (1). Aberrant cellular proliferation has been recognized as a hallmark of cancer, and increased cellular proliferation is an essential feature of tumor growth (2). Targeted therapies may result in cytostasis instead of cytotoxic effects; however, successful treatment results in a decline in cellular proliferation in either case. Therefore, cellular proliferation assays can provide a particularly timely approach to response evaluation. The potential of cellular proliferation as an indicator of cancer therapeutic response has been recognized for some time, leading to early studies measuring sensitivity to cancer drugs by *in vitro* testing (3). This approach used the incorporation of thymidine labeled with ^{14}C or ^3H into DNA to generate the DNA labeling index as a measure of proliferation. More recently, serial cellular proliferation assays in tumor biopsy material has also been used in drug efficacy evaluations (4). This background, and the limitations of *in vitro* testing as a measure of *in vivo* treatment response, provided the impetus for *in vivo* imaging methods for noninvasive assessments of tumor proliferation.

The rate of incorporation of thymidine into DNA has been recognized as a quantitative measure of cellular proliferation since the pioneering work of Cleaver (5). Although other nucleosides are incorporated in both RNA and DNA, thymidine is incorporated only into DNA. The rate of thymidine incorporation is proportional to the rate of DNA synthesis and is therefore a quantitative marker of cellular proliferation. Thymidine delivered to proliferating cells is incorporated into DNA along the extrinsic or salvage pathway (Fig. 1), where it is phosphorylated to form TTP, a substrate for DNA polymerase. The extrinsic pathway competes with the intrinsic or *de novo*

pathway, which forms thymidine nucleotides from deoxyuridine nucleotides. The rate-limiting step in the extrinsic pathway is thymidine phosphorylation by thymidine kinase, whereas the rate-limiting step in the intrinsic pathway is at thymidylate synthase. Thymidine flux through both pathways is ultimately tied to the rate of TTP incorporation into DNA.

Synthesis of labeled thymidine using the positron label, ^{11}C , was an early development in PET (reviewed in ref. 6). Models for inferring thymidine incorporation into DNA from dynamic ^{11}C -thymidine PET imaging were developed, validated, and applied to patient studies. These studies showed the ability to measure response to chemotherapy within 1 week of treatment (7). Early studies also showed that imaging could measure pharmacodynamic response to targeted therapy. For example, Wells et al. (8) found a transient increase in thymidine incorporation through the extrinsic pathway in response to treatment with a thymidylate synthase inhibitor.

Although ^{11}C -thymidine PET accurately depicts cellular proliferation, the short half-life of ^{11}C (20 min) and the need to account for labeled metabolites limit the practicality of ^{11}C -thymidine PET for routine clinical use. The search for an analogue with a longer-lived isotope label and less catabolism yielded several candidates, the most promising of which has been FLT (9). FLT differs from thymidine in that it is not incorporated into DNA. FLT is trapped in cells by phosphorylation, and its uptake is therefore dependent on the cellular activity of thymidine kinase. Because thymidine kinase activity generally reflects the proliferative state of the cell, the rate of cellular trapping of FLT provides an indirect measure of thymidine incorporation into DNA and therefore an approximate measure of cellular proliferation. Studies *in vitro* and in patients have shown that FLT uptake correlates with other measures of cellular proliferation (reviewed in ref. 6); however, the relationship between thymidine uptake and FLT uptake varies for different cancer cell types. FLT has recently been shown to measure early response to chemotherapy for a number of cancers (for example, breast cancer; ref. 10), with results similar to early response results previously shown for ^{11}C -thymidine.

In this issue of *Clinical Cancer Research*, Sohn and coworkers show that FLT-PET can be useful as an early measure of cancer response. In a series of lung cancer patients treated with the epidermal growth factor receptor-targeted agent, gefitinib, a decline in FLT uptake after a week of treatment portended a

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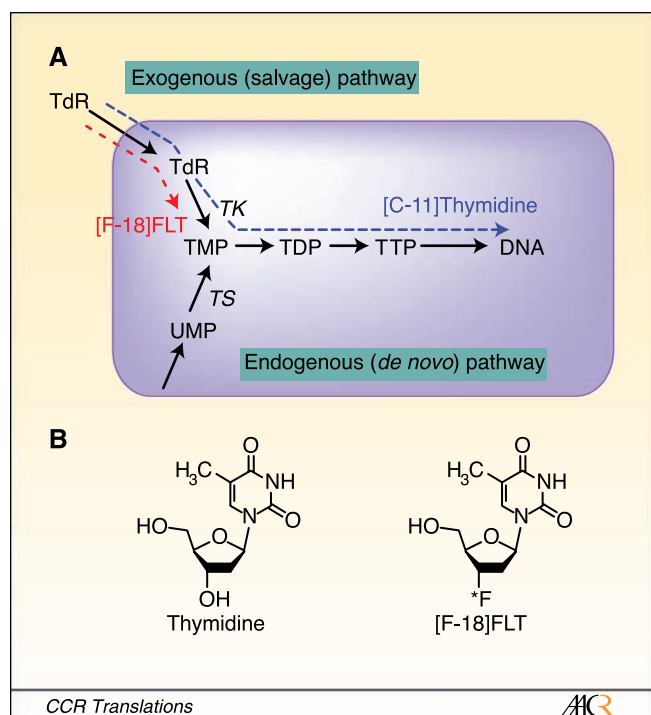


Fig. 1. A, diagram of exogenous (*salvage*, top) pathway for thymidine (*TdR*) incorporation into the DNA vs. the endogenous (*de novo*, bottom) pathway. Flux through both pathways is ultimately controlled by the rate of DNA synthesis. Flux through the exogenous pathway is controlled by thymidine kinase (*TK*), and flux through the endogenous is controlled by thymidylate synthase (*TS*). Dashed line, the pathway traced by ^{11}C -thymidine; dotted line, the path traced by ^{18}F . B, structures of thymidine and fluorothymidine (*FLT*).

response to treatment documented by tumor size changes, whereas patients that ultimately had tumor progression had a slight average increase in FLT uptake at 1 week. Retrospective application of an 11% decline in FLT uptake at 1 week as a response threshold provided <90% sensitivity and specificity for predicting response. The potential utility of an approach that would indicate the early efficacy, or perhaps more importantly the futility, of targeted treatment is immediately obvious. This is particularly true in the case of epidermal growth factor receptor-directed lung cancer therapy, in which response has been shown to be variable and affected by mutations in the epidermal growth factor receptor. Furthermore, because targeted agents such as gefitinib are often combined with cytotoxic chemotherapy, it can be difficult to discern the individual contributions to response (or nonresponse) of each agent. By showing that serial FLT-PET can measure early response to

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gefitinib, the work of Sohn and colleagues expands the application of proliferation imaging and FLT-PET to the rapidly emerging field of targeted, individualized cancer therapy.

The results of the Sohn study also raise some important questions for further study. The investigators used a post hoc threshold for decline in FLT uptake to test the predictive value of serial FLT-PET; however, further prospective studies will be needed to validate these estimates. In addition, the investigators relied on simple static measures of FLT uptake (standardized uptake value) to measure the change in the FLT phosphorylation rate with treatment; however, recent studies of FLT kinetics suggest that the standardized uptake value may be misleading as a measure of FLT phosphorylation (11). Intracellular trapping of phosphorylated FLT seems to be at least partially reversible, resulting in finite washout rates that may affect static measures such as standardized uptake value. Therapy that affected this washout of FLT might therefore lead to underestimated or overestimated response. Early studies of FLT-PET and novel therapy should therefore include more detailed kinetic analysis to evaluate drug effects on FLT kinetics before relying on more approximate uptake measures such as standardized uptake value.

Another important consideration is whether or not cellular proliferation is always the right response measure for targeted therapies. There are therapies that exert their effects primarily through metabolic alterations or by promoting tumor-programmed cell death, rather than directly decreasing cellular growth rates. A previous study showed that early response to gefitinib was identified by changes in glycolysis measured by ^{18}F -fluorodeoxyglucose that preceded a decline in cellular proliferation (12). Approaches for imaging cell death have also been developed and have undergone early human testing. These alternative imaging biomarkers may also be helpful for evaluating response to targeted cancer therapy and may be used in the preclinical stage of drug development to guide imaging in early clinical trials.

The former spectrum of cancer therapy had limited choices for therapeutic agents and only the change in tumor size to evaluate treatment efficacy. The new era of targeted and individualized cancer therapy raises the need for more sophisticated measures of early treatment efficacy both in new therapy evaluation and treatment decision making in individual cancer patients. Cellular proliferation imaging is certain to play a role in the era of targeted cancer therapy. We are just beginning, however, to understand how best to use this new tool to the benefit of cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.