PET of $\alpha_v\beta_3$-Integrin and $\alpha_v\beta_5$-Integrin Expression with ^{18}F-Fluciclatide for Assessment of Response to Targeted Therapy: Ready for Prime Time?

Angiogenesis, the formation of new blood vessels, is one of the hallmarks of cancer and a key process in the growth of solid tumors (*1*). Therefore, this process could be targeted for the molecular imaging of malignancies and for the treatment of tumors through the inhibition of key processes in the formation of new blood vessels. The imaging of angiogenesis has become increasingly important with the increasing use of targeted antiangiogenic agents, such as bevacizumab (Avastin; Genentech). The latter was shown to have therapeutic efficiency when used in combination with chemotherapy for

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several tumor entities, including colorectal, breast, and lung cancers (*2*). In addition, several tyrosine kinase inhibitors, such as sunitinib, were proven to be effective for a variety of cancer types (*3*). However, because not all patients respond to these targeted therapies, the noninvasive assessment of the response to angiogenic activity is of interest.

The imaging of integrin expression is a potential strategy for monitoring angiogenic activity, as $\alpha_v\beta_3$-integrin and $\alpha_V\beta_5$-integrin play important roles in angiogenesis and metastasis (*4*). On pages 424–430 of this issue of *The Journal of Nuclear Medicine*, Battle et al. (*5*) address this highly relevant strategy; they used the PET tracer ^{18}F-fluciclatide (formerly known as ^{18}F-AH111585), which is selective for $\alpha_v\beta_3$-integrin and $\alpha_v\beta_5$-integrin, to monitor the tumor response to sunitinib in a U87-MG xenograft tumor model in mice. Indeed, a reduction in ^{18}F-fluciclatide uptake in the tumors was observed as early as 2 d after the start of therapy in the treated group and was detectable significantly earlier than volume changes. Moreover, a significant reduction in microvessel density was observed by immunohistochemistry at the end of therapy in the control versus the treated group. Thus, PET of $\alpha_v\beta_3$-integrin and $\alpha_V\beta_5$-integrin expression with ^{18}F-fluciclatide is promising for assessment of the early response to targeted antiangiogenic agents, such as sunitinib.

The article by Battle et al. (*5*) is unique and interesting because there are surprisingly few reports in the literature on response assessment with radiotracers for $\alpha_v\beta_3$-integrin imaging. In one study, PET with ^{18}F-AH111585 visualized a reduction in microvessel density during low-dose paclitaxel therapy in an animal model of Lewis lung cell cancer (LLC), but the uptake of ^{14}C-FDG did not decrease (*6*). The latter report already hinted that ^{18}F-AH111585 might have value for the imaging of angiogenesis and might be superior to nonspecific tracers, such as ^{18}F-FDG. However, no targeted agent other than paclitaxel, a nonspecific antimicrotubule agent, was used. In another study, the SPECT tracer ^{99m}Tc-glucosamino-RGD was used to monitor low-dose paclitaxel therapy in an LLC model at 2 wk after the start of therapy (relatively late) (*7*). Nonetheless, a significant reduction in tumor uptake was demonstrated at that time. Finally, in the study of Dumont et al., ^{64}Cu-DOTA-RGD was used for PET of the activation status of $\alpha_v\beta_3$-integrin during therapy with the Src family kinase inhibitor dasatinib (*8*). Interestingly, dasatinib significantly reduced ^{64}Cu-DOTA-RGD uptake in the treated tumors, whereas the absolute protein concentration of $\alpha_v\beta_3$-integrin remained unchanged. Thus, ^{64}Cu-DOTA-RGD was able to monitor the activation status of $\alpha_v\beta_3$ integrin. However, unlike Battle et al. (*5*), Dumont et al. (*8*) focused on $\alpha_v\beta_3$-integrin expression on tumor cells, not on endothelial cells; therefore, no conclusions concerning the monitoring of antiangiogenic therapy could be drawn. Thus, the study of Battle et al. (*5*) is still the first to deal with PET of $\alpha_v\beta_3$-integrin expression for assessment of the early response to targeted therapy.

This article also has potential clinical relevance because ^{18}F-fluciclatide has already been used in clinical trials. Thus, verification of the promising preclinical results in the clinical arena in the near future seems to be realistic. At present, most clinical data on PET of $\alpha_v\beta_3$-integrin expression pertain to^{18}F-galacto-RGD, which was the first PET tracer applied to $\alpha_v\beta_3$-integrin imaging in humans (*9*). However, despite its favorable imaging characteristics, high metabolic stability, and high radiochemical yield, its rather complex labeling procedure limits more widespread use. This limitation might be overcome by tracers such as ^{18}F-fluciclatide, which was developed industrially and is easily produced (*10*). Initial clinical data are promising. In one study, ^{18}F-fluciclatide was

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administered to 7 breast cancer patients and had no adverse effects (*11*). All lesions visualized by CT were detected. ^{18}F-fluciclatide displayed rapid washout in normal tissues, whereas tumor lesions accumulated the tracer and reached an activity plateau at 40–60 min after injection. Metabolic analysis revealed adequate stability, with 74% intact tracer in the blood at 60 min after injection. Dosimetry was performed in 8 patients; the mean effective dose for a 3.5-h urinary bladder voiding interval was reported to be 0.026 mSv/MBq (*12*). Thus, ^{18}F-fluciclatide is safe and has imaging characteristics favorable for clinical use.

Several other radiotracers for the imaging of $\alpha_v\beta_3$-integrin expression have also successfully made the transition from the laboratory to clinical trials; these include ^{68}Ga-1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid [NOTA]-RGD, ^{99m}Tc-NC100692, ^{18}F-RGD-K5, and ^{18}F-^{18}F-FB-E(c[RGDyK])2 (FRGD2) (*13*). However, because few data about these tracers have been published, knowledge about their performance in comparison with that of ^{18}F-fluciclatide or ^{18}F-galacto-RGD is still limited. Nonetheless, the multitude of tracers in initial clinical trials demonstrates the great interest in and hope for PET and SPECT of $\alpha_v\beta_3$-integrin expression as a new imaging biomarker for assessment of the response to targeted antiangiogenic therapy.

However, despite the encouraging results presented by Battle et al. (*5*), some unresolved questions concerning the use of ^{18}F-fluciclatide for response assessment warrant further evaluation in preclinical and clinical studies. First, the reduction in microvessel density observed after the end of therapy in that study does not necessarily allow for the conclusion that the reduction in tracer uptake was directly caused by the reduction in microvessel density alone. The main reason is that the U87-MG cell line highly overexpresses $\alpha_v\beta_3$-integrin on the tumor cells themselves as well as on the neovasculature. Moreover, no histopathologic workup was done at relevant early times after the start of therapy. In addition, the possibility that tumor cell density as a whole was reduced cannot be excluded; therefore, the reduction in tracer uptake might simply have been due to the smaller amounts of viable tumor cells. This factor is a problem in the interpretation of the PET signal for $\alpha_v\beta_3$-integrin expression in the clinical arena as well because it is known that a variety of human tumors overexpress $\alpha_v\beta_3$-integrin not only on tumor cells but also on endothelial cells of the neovasculature (*14*). Thus, the interpretation of the PET signal for $\alpha_v\beta_3$-integrin expression is complex, and the value of imaging of $\alpha_v\beta_3$-integrin expression as a genuine biomarker of angiogenesis depends on the tumor type analyzed. However, as mentioned earlier, a study with the LLC model, which expresses $\alpha_v\beta_3$-integrin only on endothelial cells and not on tumor cells, also revealed the first promising results for response assessment with ^{18}F-fluciclatide (^{18}F-AH111585) (*6*).

Still largely unresolved is whether the imaging of $\alpha_v\beta_3$-integrin expression for response assessment is superior to other imaging techniques more widely used for this purpose, such as PET with ^{18}F-FDG or, more recently, ^{18}F-FLT as a proliferation marker (*15,16*). However, most studies with these tracers have focused more on the assessment of the response to conventional chemotherapy and less on the response to targeted therapy. Again, initial preclinical data comparing ^{18}F-fluciclatide with ^{14}C-FDG in the LLC model during paclitaxel therapy indicated the superiority of ^{18}F-fluciclatide over ^{14}C-FDG; however, more detailed in vivo preclinical imaging studies and clinical data comparing ^{18}F-FDG with ^{18}F-fluciclatide are needed.

Concerning other imaging modalities, the assessment of angiogenesis in the clinical arena is still most commonly performed by measuring functional parameters, such as blood flow, blood volume, and vessel permeability, as surrogates of angiogenic activity. These methods include dynamic contrast-enhanced (DCE) CT, DCE MRI and, less often, ultrasound and PET with perfusion tracers such as ^{15}O-H_2O (*17*). In clinical studies, DCE CT and, especially, DCE MRI are currently the most widely used techniques (*18*). However, results concerning the power of DCE MRI to predict responses in clinical studies are heterogeneous and probably depend markedly on the tumor type, therapy regimen, and methodology used for imaging (*19*). Thus, the strategy of measuring molecular parameters of angiogenesis, such as $\alpha_v\beta_3$-integrin, instead of functional parameters is an interesting alternative or adjunct to DCE MRI or DCE CT. However, a direct comparison of the clinical value of molecular imaging and functional imaging of angiogenesis is still needed.

The concerns about the histopathologic correlate of the signal from ^{18}F-fluciclatide and about a comparison with functional imaging techniques might be addressed by a planned phase II study of patients with brain tumors, lung cancers, squamous cell carcinoma of the head and neck, differentiated thyroid carcinoma, sarcoma, and melanoma. The aim of that study is to correlate dynamic and static ^{18}F-fluciclatide PET with histologic parameters of angiogenesis (including $\alpha_v\beta_3$-integrin expression) and DCE CT (*20*). Such correlative studies of functional imaging and molecular imaging of angiogenesis can be elegantly performed in a one-step approach with modern PET/CT scanners and potentially also with future hybrid MRI/PET machines. It is hoped that such studies will elucidate which technique or even combination of techniques is optimal for use as an imaging biomarker of angiogenesis for assessment of the response to targeted therapy. Therefore, the answer to the question of whether ^{18}F-fluciclatide and similar tracers for $\alpha_v\beta_3$-integrin imaging are ultimately of clinical value seems imminent.

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