

# $^{18}\text{F}$ Labeling for Immuno-PET: Where Speed and Contrast Meet

In the report by Cai et al. (1) on pages 304–310 of this issue of *The Journal of Nuclear Medicine*, an important milestone was achieved in the race to develop optimal immuno-PET agents, namely, the demonstration that small antibody fragments, in this case, diabodies, can be labeled with  $^{18}\text{F}$  and yield high-contrast PET images of tumors with tumor-to-normal tissue ratios as high as 6.2 in 4 h and with sufficient contrast for imaging as early as 1 h. Although these results were achieved with an optimized tumor xenograft animal model, they are the first step in moving the methodology to the clinic. The interest comes from the promise of using the most

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commonly used PET radionuclide,  $^{18}\text{F}$ , with engineered antibodies, such as diabodies, which are increasingly easier to produce and which correspond to derivatives of the newest therapeutic agents in the cancer field—humanized antibodies. In this example, the tumor antigen was carcinoembryonic antigen, an excellent target for the imaging of many solid tumors, including colon, breast, and medullary thyroid carcinomas, but for which no cold antibody therapy has yet been demonstrated. Thus, the opportunities to use PET for carcinoembryonic antigen-

positive malignancies may be limited to preoperative imaging and radio-immunotherapy, for which therapeutic radionuclides, such as  $^{90}\text{Y}$ , have shown promise (2). However, the methodology can be easily extended to cold therapeutic antibodies, such as anti-Her2 antibodies, for which humanized diabody fragments have already been developed (3,4). Further extension to anti-vascular endothelial growth factor and anti-epidermal growth factor receptor diabodies cannot be far off.

Several points are worth discussion. The first point is: why diabodies? Diabodies (55 kDa) are the smallest engineered fragments that are bivalent, retaining the chief advantage of whole antibodies, namely, avidity (5). Monovalent antibody fragments, such as single-chain variable fragments (scFvs), have never fared well in the clinic, despite their small size (25 kDa), because of their poor tumor retention. scFvs pay a high price for fast blood clearance, which would otherwise make them attractive as imaging agents because of their high tumor-to-blood ratios. However, high tumor-to-blood ratios alone do not make an optimal imaging agent. There are 2 reasons why scFvs fail the test; the first is that they are cleared too rapidly to accumulate in tumors, and the second is their low avidity. They are rapidly washed out from tumors even when they have a high affinity for the target antigen, demonstrating that a bivalent nature or avidity is crucial for high tumor retention (5). On the other hand, larger bivalent antibody fragments, such as  $\text{F}(\text{ab}')_2$  fragments (100 kDa), have slower blood clearance than diabodies, resulting in optimal tumor-to-normal tissue ratios only at prolonged times (18–24 h) relative to those of diabodies (3–5 h). Thus, in the development of ideal immuno-PET agents,

there is a need to compromise between tumor uptake and blood clearance (6).

The second point is the well-known need to match the half-life of the radionuclide to the half-life (blood clearance) of the antibody fragment. Although many potential useful PET radionuclides have been considered, depending on the antibody or antibody fragment,  $^{18}\text{F}$  is the radionuclide of choice because of its wide availability and almost ideal imaging properties (7). Despite these properties, its short half-life (110 min) has discouraged many researchers from considering its use with antibody fragments. What has changed this scenario? The seminal studies of Williams et al. (6) demonstrated not only that diabodies had blood clearance and tumor uptake kinetics that theoretically matched the half-life of  $^{18}\text{F}$  but also that longer-lived PET radionuclides, such as  $^{124}\text{I}$ , led to less ideal results in terms of both tumor-to-normal tissue ratios and time to optimal imaging in a tumor xenograft model system. The study of Cai et al. (1) completes the practical demonstration of the results predicted by the theory. Readers interested in the theory behind the optimization of antibody fragment pharmacokinetics and radionuclide half-lives for imaging (and therapy) should examine the work of Williams et al. (6).

The choice of  $^{18}\text{F}$  as the best PET radionuclide for diabodies meets the needs and experience of PET clinicians who are familiar with the equipment used for and the interpretation of conventional  $^{18}\text{F}$ -FDG PET scans. One can predict a rapid learning curve in moving toward routine  $^{18}\text{F}$ -labeled diabody scans on the basis of these considerations and the wide availability of the radionuclide throughout the nuclear medicine community. The

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further use of *N*-succinimidyl-4-<sup>18</sup>F-fluorobenzoate as the labeling agent and automated chemistry stations should allow nuclear medicine laboratories to label diabodies supplied in ready-to-label kits. However, there are still several aspects of the report of Cai et al. (1) that suggest that further improvements are possible. First, the radiochemical yield of the labeled product was only 1.4%, suggesting low incorporation of <sup>18</sup>F-4-fluorobenzoate into the diabody. The conditions of the reaction were also quite harsh, 30 min at 40°C, perhaps accounting for the rather low immunoreactivity of the recovered product, 57%. Problems with the low labeling yield may be attributable to the competing hydrolysis of *N*-succinimidyl-4-<sup>18</sup>F-fluorobenzoate and a suboptimal reagent-to-diabody ratio. These issues may be resolved by either fine-tuning the reaction conditions or using second-generation labeling agents that produce higher yields under milder conditions. Another obvious issue is the need for speed in labeling. Cai et al. (1) compromised with a total labeling time of 30 min, which was sufficient for their study. However, if the reaction time could be reduced to 10 min, then a considerably smaller amount of radionuclide would be required per labeling reaction.

The second major issue regarding the labeling reaction was the low immunoreactivity. In the study of Cai et al. (1), almost half of the product was biologically inactive and was expected to contribute to the normal tissue background activity. If reaction conditions that yield full immunoreactivity can be found, then improvements in both contrast and amount of radionuclide required for an image could be achieved. It is clear from other studies that most antibodies and their fragments can be conjugated to small molecules without a significant loss of immunoreactivity (8), suggesting that this problem can be overcome.

It is ironic that the labeling agent chosen, <sup>18</sup>F-SBF, was shown to label antibody F(ab')<sub>2</sub> fragments as early as 1994 by Page et al. (9) and Vaidyanathan et al. (10), long before small-animal

PET was available. These data suggest that progress in making routine <sup>18</sup>F-labeled immunoconjugates was slow not because of the lack of a labeling reagent but because of the lack of appropriate imaging equipment for the small-animal experiments and the lack of availability of even smaller bivalent antibody fragments. Given the importance of preclinical studies with small-animal models to moving novel agents into the clinic, the large role of small-animal PET should not be surprising (11–13). As more instruments become available, laboratories developing novel antibodies (and their fragments) will have more opportunities to test their immuno-PET capabilities with physiologically relevant animal models.

The role of antibodies in the targeted therapy of cancer has increased the importance of immuno-PET, given the need to judge the suitability of these agents for tumor targeting and for monitoring the tumor response over the course of therapy. The need for appropriate imaging agents is readily apparent. Given the heterogeneity of most cancers, a single type of targeted therapeutic agent does not fit all situations, and preimaging of patients with a fragment of the same antibody makes good sense. Furthermore, the need to monitor therapy with imaging modalities is also crucial given the ability of tumors to develop resistance during the course of therapy, especially for rapidly growing tumors, for which detecting tumor resurgence at the earliest time possible is important. Although many conventional imaging modalities may serve this purpose, immuno-PET has the advantage of tumor specificity, sensitivity, and quantitation. Why use PET and not SPECT? PET allows whole-body imaging and yields quantitative estimates of tumor size and radiation dose for <sup>18</sup>F-based images. These special features of immuno-PET were discussed in detail in a recent review (7).

At this point, diabodies are the clear choice for <sup>18</sup>F labeling because of the close match of their pharmacokinetics with the half-life of <sup>18</sup>F. However, diabodies have a high initial uptake (1–

2 h) in the liver, kidneys, and spleen (1), making it necessary to wait until 4–6 h for tumor-to-organ ratios to be in favor of tumor imaging. Although tumors outside these organs could be imaged as early as 1 h, it is unlikely that clinicians would rely on early scans for diagnosis. Furthermore, the translation of time to imaging from a mouse model to human tumors may require some adjustments.

The final point worth consideration is the need for speed in imaging. It is obvious that imaging agents that require a patient to stay overnight or to return on the following day increase the cost of the procedure and patient inconvenience. Because of the slow clearance of intact antibodies, antibody (non-PET) scans often require a time to imaging (after injection) of up to 3 d. The potential reduction of the time to imaging to as little as 4 h is an enormous improvement that is sure to propel the immuno-PET field forward. Thus, the report of Cai et al. (1) is another important step that may herald the advent of routine immuno-PET in the clinic.

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