Al^{18}F Labeling of Affibody Molecules

TO THE EDITOR: Glaser et al. recently described the labeling of ^{18}F-$Z_{HER2:2891}$-Cys-NOTA-$(COOH)_2$-AlF (^{18}F-12) (*1*) and compared it in vivo to the biodistribution of that Affibody (Affibody AB) with ^{18}F attached to carbon and silicon, as well as an ^{111}In-DOTA-Affibody. They reported that the Al^{18}F-labeled Affibody had a similar biodistribution to the ^{111}In-Affibody, as previously noted by Heskamp et al. (*2*), and also observed that the Al^{18}F-labeled Affibody had high uptake and retention in the kidney (~80 percentage injected dose [%ID], like the ^{111}In-Affibody). This is presumably because the small-sized Affibody is eliminated through the kidneys, where it is rapidly catabolized, with the resulting Al^{18}F complex residualized in the renal tubules in the same manner as the ^{111}In-DOTA complex (*3*). In contrast, when the carbon- and silicon-labeled Affibody molecules are metabolized in the kidney, the ^{18}F-labeled metabolites are eliminated from the kidney cells, greatly reducing renal uptake. Although this clearly serves as an advantage for this product, much like differences between radioiodinated and radiometal-labeled antibody fragments, it is important to emphasize that renal uptake of the Al^{18}F-Affibody product is a property of the Affibody targeting agent and not the Al^{18}F complex. Previous studies with our pretargeting peptide (*4*) and the Al^{18}F-NOTA-pegylated arginine-glycine-aspartic acid dimer (PRGD2) peptide (*5*) both showed excellent renal clearance in the mouse models, and the Al^{18}F-NOTA-PRGD2 peptide also had good renal clearance in humans (*6*). It should also be noted that the ^{18}F-Affibody labeled through a carbon atom had high hepatobiliary clearance (40–50 %ID in the intestines), whereas the Al^{18}F-labeled Affibody had low uptake in the intestines. The high hepatobiliary accretion might be considered at least as undesirable as the high renal retention, depending on the use of the agent.

Glaser et al. also reported a 2-fold lower labeling yield for their Al^{18}F-Affibody than the Al^{18}F-labeling yield of a similar Affibody bearing the same NOTA ligand (11% vs. 21%), and this despite the fact that Heskamp et al. used a lower amount of the Affibody (*2*). Although we cannot discount the possibility that slight differences in the Affibody structure could have influenced the yields, we strongly suspect the yield differences are attributable to the lack of a co-solvent in the labeling procedure used by Glaser et al. Indeed, we have shown that the use of a co-solvent generally improves yields 2-fold (*7*).

Thus, we believe it is important when comparing labeling technologies to attempt to optimize or normalize each procedure, or if not empirically assessed, to state the conditions that might have affected yields when this information has been published previously. Second, whereas the nonresidualizing ^{18}F-linkage used by Glaser et al. provided lower renal uptake, there likely are other situations, such as in target cells with a more rapid metabolism, in which a residualizing form of ^{18}F afforded by the AlF method would be preferred (*8*).

DISCLOSURE

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REPLY: We welcome the opportunity to respond to McBride and colleagues' comments on our article (*1*) in this journal and would like to reflect on the raised points from our perspective.

First, one would not be wrong to assume that the Al^{18}F-chelator protocol has now been recognized by the radiopharmacy community as an innovative and powerful protocol to stably radiolabel biomacromolecules using a simple and direct approach.

Although we certainly have to accept the reported data as they stand, their interpretation seems to have regrettably caused some disagreement with readers. If McBride et al. state that the "renal uptake of the Al^{18}F-Affibody product is a property of the Affibody targeting agent and not the Al^{18}F complex," we would like to stress that the biodistribution profile as such is of course always a combination of properties of the peptide plus labeling group.

In this context, we also would like to point out that the principal aim of our study was to compare the different bioconjugation protocols and the corresponding pharmacokinetics for a single Affibody motif only. Evidently, our data have revealed differences in the biodistribution and excretion of the ^{18}F-labeled Affibody molecule that can be attributed to the radiolabeling moiety. Our biodistribution results demonstrated that the ^{18}F-AlF–labeled HER2-Affibody molecule was not cleared from the kidneys after 3 h.

Further, whereas we fully accept the conclusion by McBride et al. that "high hepatobiliary accretion might be considered at least as undesirable as the high renal retention," we also think this always will depend on the intended application. The decisive criterion for the suitability of the tracer will be uptake in the actual organ and secondary tumors to be targeted. In addition, species-related differences in the metabolism also cannot be ruled out. In any case, the ultimate information on tracer suitability should be provided by a dosimetry study.

As for differences in radiolabeling yield compared with Heskamp et al. (*2*), this is to be explained by the method of measuring. Although we reported in Table 1 the isolated non–decay-corrected radiochemical yield, the referenced study used instant thin-layer chromatography and high-performance liquid chromatography. In our study, analytic high-performance liquid chromatography of a reaction mixture containing ^{18}F-$Z_{HER2:2891}$-Cys-NOTA-$(COOH)_2$-AlF indeed showed labeling efficiencies of up to 49% (see also Supplemental Fig. 5). Thus, significant loss of product occurred during the purification step using a gel filtration cartridge. Unfortunately, authors do not always explicitly mention the method of measuring radiochemical yields, and this can easily lead to confusion in the interpretation of such data.

Finally, we think the suggested use of an organic co-solvent such as acetonitrile is an intriguing aspect. We agree that our protocol has room for improvements both in the labeling step and in the purification efficiency as mentioned above. Clearly, this matter will have to be addressed in future work. However, the published protocol achieved its purpose of delivering tracer for a biodistribution study.

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