Published in final edited form as:

Bioconjug Chem. 2012 March 21; 23(3): 538–547. doi:10.1021/bc200608e.

A New Lyophilized Kit for Rapid Radiofluorination of Peptides

William J. McBride^{*,†}, Christopher A. D'Souza[†], Habibe Karacay[‡], Robert M. Sharkey[‡], and David M. Goldenberg[‡]

[†]Immunomedics, Inc., Morris Plains, New Jersey

[‡]Center for Molecular Medicine and Immunology and the Garden State Cancer Center, Morris Plains, New Jersey

Abstract

Radiolabeling compounds with positron-emitting radionuclides often involves a time-consuming, customized process. Herein, we report a simple lyophilized kit formulation for labeling peptides with ¹⁸F, based on the aluminum-fluoride procedure. The prototype kit contains IMP485, a NODA (1,4,7-triazacyclononane-1,4-diacetate)-MPAA (methyl phenylacetic acid)-di-HSG (histamine-succinyl-glycine) hapten-peptide, [NODA-MPAA-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂], used for pretargeting, but we also examined a similar kit formulation for a somatostatin-binding peptide [IMP466, NOTA-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr] bearing a NOTA ligand to determine if the benefits of using a kit can be extended to other AIF-binding peptides. The NODA-MPAA ligand forms a single stable complex with (AIF)²⁺ in high yields. In order to establish suitable conditions for a facile kit, the formulation was optimized for pH, peptide to Al³⁺ ratio, bulking agent, radioprotectant, and the buffer. For optimal labeling, the kit was reconstituted with an aqueous solution of ¹⁸F⁻ and ethanol (1:1), heated at 100–110 °C for 15 min, and then simply and rapidly purified using one of two equally effective solid-phase extraction (SPE) methods. Al¹⁸F-IMP485 was isolated as a single isomer complex, in high yield (45–97%) and high specific activity (up to 223 GBq/μmol), within 20 min. The labeled product was stable in human serum at 37 °C for 4 h and *in vivo*, urine samples showed the intact product was eliminated. Tumor targeting of the Al¹⁸F-IMP485 in nude mice bearing human colon cancer xenografts pretargeted with an anti-CEACAM5 bispecific antibody showed very low uptake (0.06% ± 0.02 ID/g) in bone, further illustrating its stability. At 1 h, pretargeted animals had high Al¹⁸F-IMP485 tumor uptake (28.1% ± 4.5 ID/g), with ratios of 9 ± 4, 123 ± 38, 110 ± 43 and 120 ± 108 for kidney, liver, blood and bone, respectively. Tumor uptake remained high at 3 h post-injection, with increased tumor/nontumor ratios. The NOTA-somatostatin-binding peptide also was fluorinated with good yield and high specific activity in the same kit formulation. However, yields were somewhat lower than those achieved with IMP485 containing the NODA-MPAA ligand, likely reflecting this ligand's superior binding properties over the simple NOTA. These studies indicate that ¹⁸F-labeled peptides can be reproducibly prepared as stable Al-F complexes with good radiochemical yield and high specific activity using a simple, one-step, lyophilized kit followed by a rapid purification by SPE that provides the ¹⁸F-peptide, ready for patient injection within 30 min.

^{*}Corresponding author: William J. McBride, Immunomedics, Inc., 300 The American Road, Morris Plains, NJ 07950; Telephone 973-605-8200 ext. 233; Fax: 973-605-1340; bmcbride@immunomedics.com. David M. Goldenberg, Center for Molecular Medicine and Immunology, 300 The American Road, Morris Plains, NJ 07950; dmg.gscancer@att.net..

Disclaimer: WJM, CAD, and DMG are employed or have financial interest in Immunomedics, Inc., or IBC Pharmaceuticals, Inc. RMS and HK have no financial interests to declare.

Supporting Information. Description of HPLC methods and HPLC chromatograms are available free of charge via the internet at <http://pubs.acs.org>.

Keywords

bispecific antibody; fluorine-18; pretargeting; peptides; antibodies; molecular imaging; PET; somatostatin

INTRODUCTION

^{18}F is the most commonly used isotope for positron-emission tomography (PET) due to its nearly ideal imaging properties (β^+ 0.635 MeV 97%, $T_{1/2}$ 110 min). The increasing demand for ^{18}F , primarily used to prepare 2- ^{18}F fluoro-deoxyglucose (FDG), has led to greater availability and lower costs. Its ~2-h half-life also is matched well for compounds like peptides that clear rapidly from the blood, and thus there are considerable efforts underway to develop other ^{18}F -labeled peptide-imaging agents. Ordinarily, ^{18}F is attached to peptides by binding it to a carbon atom^{1–4}, but attachments to silicon^{5–8} and boron⁹ also have been reported. Binding to carbon usually involves multistep syntheses, sometimes taking several hours to complete, which can be problematic for an isotope with a 110-min half-life. However, recent advances have reduced synthesis to a single step^{10, 11}, but this method does not work well with peptides containing a tyrosine group and the peptides must be purified by HPLC to increase their specific activity.

Peptides are labeled routinely with radiometals, usually in 15 min and in quantitative yields.^{12,13} For PET imaging, copper-64 and, gallium-68 have been bound to peptides via a chelate with good yields, specific activity, stability, and imaging properties.¹⁴ Since fluoride binds to most metals,¹⁵ we sought to determine if an ^{18}F -metal complex could be bound to a chelate on a targeting molecule. We focused on the binding of an $(\text{Al}^{18}\text{F})^{2+}$ complex, since the aluminum-fluoride bond is one of the strongest fluoride-metal bonds and the $(\text{AlF})^{2+}$ complex is known to bind to ligands.¹⁶ Initial feasibility studies using an ^{18}F -labeled peptide for *in vivo* targeting of cancer with a bispecific antibody (bsMAb) pretargeting system were reported.¹⁷ The pretargeting procedure was shown to be highly sensitive and specific for localizing cancer, even more than ^{18}F -FDG.^{18–23}

In the initial study, we found an $(\text{Al}^{18}\text{F})^{2+}$ complex could bind stably to a 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) ligand in aqueous solution, but the yields were low and the labeled peptide had to be purified by HPLC to obtain the specific activity needed for imaging. We then compared labeling of four different NOTA ligands with $(\text{Al}^{18}\text{F})^{2+}$, and found that while all these ligands formed stable complexes, the isolated yields varied from 5.8% to 87%, depending on the ligand used.²⁴ The peptide with the highest yield, IMP467 (Figure 1), contained the *C-NETA* ligand, which has enhanced binding kinetics for some metals.²⁵ An important additional finding was that IMP467 could be labeled with $^{18}\text{F}^-$ in saline, which is a commercially available source of purified $^{18}\text{F}^-$ typically used for bone imaging.

The investigations with the NOTA compounds provided us with important leads in determining ways to optimize a chelate for binding AIF. We subsequently developed a new ligand that contains 1,4,7-triazacyclononane-1,4-diacetate (NODA) attached to a methyl phenylacetic acid (MPAA) group for IMP485.²⁶ This ligand is synthesized more easily than *C-NETA* and has the added advantage of forming a single stable complex with $(\text{AlF})^{2+}$. Since our original report of NOTA-based chelating agents, the AIF-radiolabeling method has been investigated by several other groups. For example, together with a group of our collaborators, a NOTA-octreotide peptide, IMP466, was fluorinated in good yields with excellent stability and targeting *in vivo*.^{27–29} Others have confirmed the AIF fluorination potential using RGD peptides and some simple derivatives of NODA.^{30–32}

In our earlier reports, we showed that the labeling procedure could be performed simply in solution using a one-pot method.^{24, 26} This success suggested that a lyophilized kit could be prepared similar in concept to those used with ^{99m}Tc.^{33,34} A validated lyophilized kit that enables rapid and reproducible labeling of a peptide by the simple addition of USP ¹⁸F⁻ in saline, a brief heating step, followed by a rapid and simple purification process, as described here, should make the ¹⁸F-labeling of molecules more compatible with the already established good manufacturing practices (GMP) applied to the labeling of ^{99m}Tc-agents. Such kits also could expand the use of ¹⁸F-labeled agents for monitoring the biodistribution and localization of various compounds. While we suspect different agents will have different requirements for kit formulation and optimal labeling, the process we describe here to develop the kits for IMP485 and the somatostatin-binding peptide, IMP466, should provide important insights for preparing agents for use with the AIF-labeling procedure.

MATERIALS AND METHODS

All commercial chemicals were analytical-grade and used without further purification. L-(+)-ascorbic acid, AlCl₃·6H₂O, 4-morpholineethanesulfonic acid (MES) and sodium hydroxide, 99.99%, were purchased from Sigma-Aldrich (Milwaukee, WI). The N-2 hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) was obtained from Calbiochem (La Jolla, CA). Sodium acetate, α,α-trehalose, potassium biphthalate (KHP), and acetic acid were from J. T. Baker (Phillipsburg, NJ). The analytical and preparative reverse-phase HPLC (RP-HPLC) columns were purchased from Phenomenex (Torrance, CA) and Waters Corp. (Milford, MA). No-carrier-added [¹⁸F]-fluoride was purchased from IBA Molecular (Somerset, NJ), and used for initial serum stability and biodistribution studies. ¹⁸F⁻ in saline (for human use) was obtained from PETNET Solutions (Hackensack, NJ) and used for the kit optimization experiments. Solid-phase extraction (SPE) cartridges (Sep-Pak light QMA, Sep-Pak light Alumina N, Sep-Pak Accell plus CM, and Oasis HLB) were acquired from Waters (Milford, MA). Female nude mice (NCr nu-m), 23.1 ± 2.3 g, were procured from Taconic Farms (Germantown, NY).

The recombinant, humanized, tri-Fab bispecific monoclonal antibody (bsMAb), TF2, was provided by IBC Pharmaceuticals, Inc. (Morris Plains, NJ). TF2 binds divalently to carcinoembryonic antigen (CEACAM5 or CD66e) and monovalently to the synthetic haptan, HSG (histamine-succinyl-glycine).³⁵

IMP485, IMP486, and IMP466

IMP485 [NODA-MPAA-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂] and IMP486 [AlOH-NODA-MPAA-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂; i.e., IMP485 pre-loaded with aluminum] were synthesized as described previously.²⁶ IMP466, which contains the NOTA ligand, was synthesized as described elsewhere.²⁷

IMP485 initial kit formulation

Stock solutions of IMP485 and AlCl₃·6H₂O were dissolved in 2 mM sodium acetate buffer and adjusted to ~pH 4. Ascorbic acid and α,α-trehalose solutions were prepared in DI water.

Prior experience with IMP485 radiolabeling performed in solution-phase provided important insights regarding the basic starting conditions that might be compatible with a lyophilized kit formulation.²⁶ Many of these initial studies were performed using 3-mL vials containing 40 nmoles of IMP485, with the kits formulated in a variety of ways to examine how these changes might affect the radiolabeling yields. Additional information concerning the specific formulation/conditions under evaluation is provided in the *Results*. Briefly, kits were made with varying volumes of the α,α-trehalose stock solution, such that the α,α-

trehalose concentration by weight would be ~2.5, 5, 10, 20 and 50%, respectively, when reconstituted in 0.2 mL of saline. In addition to testing trehalose, other bulking agents (sorbitol, glycine, mannitol and sucrose) were examined. Additional 40-nmol IMP485 kits were formulated with varying amounts of AlCl_3 (40, 36, 32, 28, 24, and 20 nmol) included to examine different peptide to AlCl_3 ratios (1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, and 1:0.5, respectively). Kits containing IMP486 were prepared without the addition of the AlCl_3 solution, because the ligand was prefilled 100% with Al^{3+} according to previously published methods.²⁶

In order to evaluate the effect of pH on radiolabeling yields, 40-nmol kits of IMP485 and IMP486 were adjusted to the pH ranges of 3.3 to 5.1 and 3.4 to 4.98, respectively. A second set of 20-nmol kits of IMP486 was made and adjusted to the pH range 3.61 to 4.4.

Kits were also formulated in various buffers, such as, HEPES/HOAc, MES/HOAc, citrate, ascorbate, and KHP, with and without 0.1 mg ascorbate.

Typically, the filled vials were frozen and lyophilized. However, in one set of studies, the vials containing 40-nmol IMP485, 24 nmol of Al^{3+} , 10 mg of trehalose, and 0.1 mg ascorbic acid, all adjusted to ~1 mL were stored under nitrogen at 2–8°C until lyophilization (no delay, or a 1-, 2-, or 3-day delay).

Final formulation and lyophilization of 20-nmol IMP485 kits

The final formulation consisted of dissolving the peptide (IMP485) in DI water to obtain a 2 mM solution. The $\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$ was dissolved in 2 mM KHP and adjusted to pH 4. Ascorbic acid was dissolved in DI H_2O at 5 mg/mL. The KHP was dissolved in DI H_2O to obtain a 0.06 M solution at pH 3.99 and α, α -trehalose dihydrate was dissolved in DI H_2O to obtain a 5% solution.

The bulk solution used to fill vials was prepared by mixing the 2 mM IMP485 solution with the 2 mM Al^{3+} /KHP solution, the ascorbic acid solution, the 0.06 M KHP buffer, and the α, α -trehalose, and then DI H_2O was added to obtain the bulk solution. The solution was adjusted to pH 4.0 with a few microliters of 1 M KOH, and then dispensed in 1-mL aliquots into the vials for lyophilization. The filled vials were frozen and then transferred to the shelf of the Virtis Advantage lyophilizer (Gardiner, NY) cooled to –16 °C. When the vacuum went below 100 mTorr the shelf temperature was increased to 0 °C. After 16 h, the shelf temperature was increased to 20 °C for 4 h. The vials were then sealed under vacuum and removed from the lyophilizer.

Kits were made with 10, 20, 40, 100, and 200 nmol of peptide (using increasing amounts of the stock 2 mM IMP485 solution). For those kits the amount of Al^{3+} added was adjusted to keep the ratio of nmol of peptide to nmol of Al^{3+} constant at 1 nmol of peptide to 0.6 nmol of Al^{3+} . All of the other kit ingredients were unchanged from the kit formulation described above, regardless of the amount of peptide used.

Formulation of IMP466 kits

IMP466 kits were formulated in the same way as IMP485 kits, except the final pH of the kit solution was adjusted to pH 4.1.^{27,28}

Radiolabeling and purification

The lyophilized peptides were radiolabeled by adding $^{18}\text{F}^-$ in 100 to 200 μL saline to the crimp-sealed vial, and then heating to 90–110 °C for 15 min. In some cases, an equal volume of ethanol was added with $^{18}\text{F}^-$. The peptides were purified by one of two methods.

HLB purification was performed by first adding DI water to the vial in fractions (5 mL total volume), which were transferred to a dilution vial and the entire contents of the dilution vial were applied to a Waters HLB column Oasis HLB 1-mL (30 mg) flangeless cartridge. The HLB column was placed on a crimp-sealed vial, drawing the waste liquid containing the unbound ^{18}F into the vial under vacuum. The reaction vial then was washed with aliquots of DI water. The column was eluted directly into a vial containing buffered, lyophilized ascorbic acid (~ pH 5.5, 15 mg), using three, 200 μL portions of 1:1 EtOH/DI water.

The percent isolated yield in the product vial was determined by measuring and adding the activity found in the HLB cartridge, the reaction vial, the water wash, and the product vial to account for the total activity. When the isolated yield calculation was compared to the decay-corrected method, the yields were found to be essentially identical, indicating that all of the ^{18}F -activity was accounted for.

The second method utilized an Alumina N cartridge as described by others.³² At the end of the reaction time, saline was added to the reaction vial, which was drawn into a syringe and passed through the Alumina N cartridge. The vial was washed with more saline, which was also pushed through the Alumina N cartridge into the formulation vial.

Serum stability

The purified radiolabeled IMP485 in 50 μL 1:1 EtOH/ H_2O was mixed with 150 μL of human serum and placed in the HPLC auto-sampler heated to 37 °C and analyzed by RP-HPLC, using an in-line radiation detector.

In vivo studies

All animal studies were approved by CMMI's institutional animal safety committee.

Nude mice bearing subcutaneous LS174T human colon cancer xenografts were injected with 106 μg (~1 nmol) of TF2 anti-CEACAM5 $\times$ anti-HSG bsMAb followed 16 h later with Al^{18}F -IMP485 (1.04 MBq, 5.2×10^{-11} mol, 100 μL , iv) that was prepared using a 40-nmol IMP486 kit to an effective specific activity of 20.4 GBq/ μmol after HLB purification. The animals were necropsied at 1 and 3 h post injection. Other animals were given the Al^{18}F -IMP485 alone and necropsied at the same times.

RESULTS

Kit formulation

Bulking Agents—A lyophilized kit containing such small amounts of product requires a bulking agent. Thus, starting with 40 nmol IMP485 kits (containing 20 nmol Al^{3+} , ascorbate/acetate buffer, pH 4.0), we examined five different bulking agents to assess which would produce an acceptable cake with minimal impact on the radiolabeling reaction. Kits were formulated with 10 mg of each bulking agent with identical amounts of the other formulation reagents, adjusted to approximately the same pH. After lyophilization, the kits were labeled by adding ~74 MBq $^{18}\text{F}^-$ in 200 μL saline (no ethanol added) and heated to ~105 °C for 15 min and then purified by the HLB method. The isolated yields were 83%, 42%, 82%, 66%, and 81% for sorbitol, glycine, mannitol, sucrose, and α,α -trehalose, respectively. The sorbitol formulation collapsed to a gum on lyophilization, while both the mannitol and α,α -trehalose formulations formed acceptable cakes and labeled in high yield. Changing the final concentration of α,α -trehalose in the kit (40 nmol IMP485, 200 μL $^{18}\text{F}^-$ in saline, 105 °C) from 2.5 to 50% (5 mg to 100 mg/kit) by weight had no effect on radiolabeling yields, with an average of $83.3 \pm 0.65\%$ ($n=5$) for all concentrations of α,α -trehalose tested.

IMP485 kits could be stored at 2–8 °C under nitrogen for up to three days before lyophilization without impacting radiolabeling yields.

Effect of pH—We found previously that radiolabeling is pH-sensitive and needs to be adjusted to the ligand and possibly even to the peptide and the ligand.²⁴ Whereas the optimal pH for IMP461, IMP466, and IMP467 was reported to be 4.1, 4.1, and 4.5, respectively,^{24, 27} the optimal pH for both IMP485 and IMP486 kits was 4.0 ± 0.2 . In both cases, the yields fell rapidly outside the ideal pH zone. For example, using 20-nmol kits of IMP486 (containing 10 mg trehalose, 0.1 g ascorbate, 20 nmol Al^{3+} , in acetate buffer) labeled with 200 μL 1.8–2.4 GBq $^{18}\text{F}^-$ in saline (no ethanol added), the yields were 50%, 63%, 66%, 55%, and 50% at pH 3.6, 3.8, 4.0, 4.2, and 4.4, respectively. It is important to note that the lower yields in this study reflected the use of 20-nmol kits rather than 40 nmol-kits used in the previous section.

Effect of buffer—In this series, 40-nmol kits containing 20 nmol Al^{3+} and 10 mg trehalose were formulated using MES, HEPES, KHP, citrate, ascorbate and acetate buffers, all adjusted to pH 4 before lyophilization. We noted that when reconstituted with saline, the pH of some of the kits changed after they were lyophilized. The kits initially formulated with acetate buffers without ascorbic acid had the largest pH change after lyophilization, probably due to loss of acetic acid during freeze-drying. The radiolabeling yields of those kits were lower (45–71%). The KHP-buffered kit had the best yield in the ascorbate-free batch (71% yield). The pH was more consistent between formulation and lyophilization with most of the buffers tested when 0.1 mg of ascorbic acid was present in the vials. The yields derived from kits formulated with HEPES, MES, NaOAc, and KHP in the presence of 0.1 mg of ascorbic acid were similar (83–86 % isolated yield). Thus, ascorbic acid appears to serve as a significant non-volatile buffer that stabilizes the pH similarly before and after lyophilization, allowing better control of pH, which, in turn, ensures optimal labeling efficiency.

To determine the effect of KHP on the labeling efficiency, kits with 0.1, 1.22, 8, 16, and 32 μmol of KHP were tested. Kits having 1.22 μmol of KHP maintained the pH at the desired level (4.0 ± 0.2) and gave a better labeling yield than 0.1 μmol KHP kits. The kits with ≥ 8 μmol of KHP had progressively lower yields. We suspect that KHP and ascorbate might be acting both as buffers and as transfer ligands to increase the labeling yields with those excipients. Citric acid is not a good buffer for AlF labeling, giving low yields even when only 50 μL of 2 mM citrate was used in the presence 0.1 mg ascorbate.

Radioprotectants—Ascorbic or gentisic acid often are added to radiopharmaceuticals during preparation to minimize radiolysis. Ascorbic acid was included routinely in vials used to isolate the HLB-purified product, but we also examined the importance of having ascorbic acid added during the labeling procedure. When IMP485 (20 nmol, containing 10 mg trehalose and 10 nmol Al^{3+}) was formulated with 0.1, 0.5 and 1.0 mg of ascorbic acid/acetate buffers at pH 4.1–4.2 and labeled with $^{18}\text{F}^-$ in 200 μL saline, final yields were 51, 31 and 13%, respectively, suggesting 0.1 mg of ascorbic acid was the maximum amount that could be included in the formulation without reducing yields. Formulations containing gentisic acid/acetate did not label as well as the ascorbic acid kits.

Peptide to aluminum ratio—The optimal IMP485 to Al^{3+} mole ratio was 1:0.6, but good yields were obtained with ratios from 1:0.5 to 1:1.

Recommended kit formulation buffer—The culmination of the above testing led us to conclude that the optimal formulation buffer for the IMP485 kits contained the peptide with

Al³⁺ in a 1:0.6 ratio, along with 0.1 mg of ascorbic acid, 1.22 mol of KHP, and 10 mg of α,α -trehalose dihydrate adjusted to pH 4.0 ± 0.2 .

Optimizing radiolabeling yields

We reported previously that adding ethanol can enhance the ¹⁸F⁻ radiolabeling yields.²⁶ Therefore, various ratios of saline to ethanol were tested with 20-nmol IMP485 kits in 400 μ L total volume and 60 MBq of ¹⁸F⁻ that were heated at 107 °C for 15 min. The vials tested contained 100%, 75%, 50% and 25% saline mixed with ethanol affording Al¹⁸F-IMP485 in isolated yields of 48.8, 67.1, 86.2 and 84.4%, respectively. The data indicate that yields are best when the mixture contains between 50 and 75% ethanol. Comparable radiolabeling yields of 83% and 81% were obtained when 20-nmol kits of IMP485 and IMP486 were labeled with ~1.5 GBq of ¹⁸F⁻ in 100 μ L of saline and 100 μ L of EtOH, respectively.

Using the recommended formulation buffer, we next prepared IMP485 kits with 10, 20, 40, 100, and 200 nmol of peptide and then test-labeled with ¹⁸F⁻ (~37 MBq) in 400 μ L of 1:1 saline/ethanol (peptide concentration during radiolabeling ranged from 25 to 500 μ M) for 15 min at 50, 70, 90, 100, and 110 °C. At this point, we also changed our SPE-purification procedure, favoring an Alumina N cartridge reported by others.³² The Alumina N method reduces the time required to isolate the purified product, since it flows through the cartridge rather than binding to the resin and requiring elution, as in the HLB purification procedure. RP-HPLC analysis of IMP485 and IMP466 purified by HLB and Alumina N showed <1% to undetectable amounts of ¹⁸F. The results clearly indicate that the reaction must be heated to 100 to 110 °C to obtain the maximum yield (Figure 2), and nearly quantitative radiolabeling yields can be obtained with peptide concentrations of 100 μ M (40 nmol of peptide in 400 μ L 1:1 saline/ethanol) or higher.

The optimum reaction time was studied next with 20-nmol IMP485 kits labeled with ~37 MBq ¹⁸F⁻ in 400 μ L of 1:1 ethanol/saline at 108 °C for 5 to 60 min. Maximum yields were obtained after 15 min of heating, with a specific activity of ~1 GBq/ μ mol (Table 1). Indeed extending the incubation period to 30 minutes did not improve the yield appreciably, with any gain made in yield being offset by the ¹⁸F decay occurring over this additional time. These same kits showed optimal labeling yields again within 15 minutes using ~2.5 GBq of ¹⁸F⁻ in 400 μ L 1:1 ethanol saline at 110 °C, and while the isolated yields decreased somewhat with this higher level of activity, the specific activity increased to ~75 GBq/ μ mol. Kits prepared with ¹⁸F⁻ received on 4 separate days, with ~1.68 to 2.57 GBq of ¹⁸F⁻, had yields ranging from 70 to 84% and specific activities from 48 to 85 GBq/ μ mol (Supplemental Table 1).

To assess the labeling yields with even higher amounts of ¹⁸F, we performed one additional procedure using a 20-nmol IMP485 kit and 10.8 GBq of ¹⁸F⁻ in 400 μ L 1:1 ethanol saline at 108 °C for 15 min. After purification through an Alumina N cartridge and in a total reaction and purification time of just ~20 min, 4.46 GBq (45.6% yield) of Al¹⁸F-IMP485 was obtained with a specific activity of 222.8 GBq/ μ mol (assumes no loss of IMP485).

When Al¹⁸F-IMP485 was mixed with at least a 20-fold molar excess of TF2 and then analyzed by SE-HPLC, the peptide shifted from a retention time of ~17 min to 9 min, the retention time of TF2, indicating the labeled peptide retained HSG-binding, antibody-binding (Supplemental Figure 1, traces A and B).

In order to determine if the same kit formulation could be applied to another peptide, IMP466, a somatostatin-binding peptide described previously was examined.^{27,28} Kits were made with the same ingredients as IMP485, but the pH was adjusted to 4.1 prior to lyophilization. Initial labeling was performed in concert with IMP485 using ~2.5 GBq

of $^{18}\text{F}^-$ (refer to Table 1), examining reaction times between 15 and 30 minutes. As shown in Table 2, maximum yields were obtained within 15 min at 110 °C. The lower yields for IMP466 compared to IMP485 suggested that the NODA-MPAA ligand affords higher yields as compared to the simple NOTA ligand. Another study compared $^{18}\text{F}^-$ labeling of IMP466 to IMP485 with increasing amounts of each peptide (10, 20, 40, 100, and 200 nmol) using ~ 14 to 20 MBq of $^{18}\text{F}^-$ in 400 μL 1:1 ethanol saline at 110 °C for 15 min (Supplemental Table 2). The data demonstrate that each peptide could be radiolabeled to near quantitative levels, but the simple NOTA required 5-times more moles of peptide than the NODA-MPAA containing IMP485. To illustrate this point further, we prepared an octreotide analog containing the NODA-MPAA (IMP490; Supplemental data) and formulated in acetate buffered kits in the same manner as IMP485. The ^{18}F -radiolabeling yield in the acetate-buffered kits for IMP490 and IMP485 was similar, supporting our position that the NODA-MPAA ligand improves yields as compared to the simple NOTA.

Stability and biodistribution of Al^{18}F -IMP485

RP-HPLC of Al^{18}F -IMP485 (1.9 MBq/ μmol) incubated in fresh human serum at 37 °C for 4 h showed no evidence of destabilization (Supplemental Figure 2), being identical to the original labeled peptide HPLC trace (data not shown). The labeled product was also injected into tumor-bearing mice. Al^{18}F -IMP485 had low uptake in all normal tissues (Figure 3C), e.g., $0.18 \pm 0.03\%$ and $0.17 \pm 0.06\%$ injected dose/gram (ID/g) at 1 h for liver and blood, respectively, with higher uptake in the kidneys ($3.33 \pm 0.56\%$ ID/g and $2.27 \pm 0.29\%$ ID/g at 1 and 3 h, respectively), which is expected because the peptide is eliminated in the urine. Bone uptake was also low, averaging $0.11 \pm 0.02\%$ ID/g at 1 h, decreasing to $0.08 \pm 0.03\%$ ID/g at 3 h, indicating that the Al^{18}F complex was stably bound to the peptide. Tumor uptake of Al^{18}F -IMP485 alone was $0.31 \pm 0.11\%$ and $0.09 \pm 0.02\%$ at 1 and 3 h, respectively. However, in animals pretargeted with TF2, tumor uptake was $28.1 \pm 4.5\%$ ID/g and $26.5 \pm 6.0\%$ ID/g at 1 h and 3 h, respectively, illustrating that the peptide retained HSG binding antibody (Figure 3A). High tumor uptake in the TF2-pretargeted animals resulted in high T/NT ratios, e.g., 123 ± 38 , 9 ± 4 , and 110 ± 43 at 1 h, and 189 ± 43 , 12 ± 3 , and 1240 ± 490 at 3 h post injection of the peptide in liver, kidney, and blood, respectively (Figure 3B).

RP-HPLC traces of urine taken from a mouse 50 min after Al^{18}F -IMP485 injection were identical to the injected product, again indicating the stability of the Al^{18}F binding to the ligand (Figure 4). Samples of urine from the mice given the peptide alone examined by SE-HPLC also showed that the radiolabeled peptide shifted to a shorter retention time after TF2 addition, indicating that all of the activity was attached to the peptide and that the peptide affinity for TF2 was unchanged, even after injection and excretion in urine (Supplemental Figure 1, traces C and D).

DISCUSSION

We reported previously a simple one-pot method for preparing ^{18}F -labeled peptides using the AIF method in solution.²⁶ Our primary goal here was to simplify the method even further by examining whether peptides coupled with AIF-binding ligands could be formulated and lyophilized so that upon reconstitution with $^{18}\text{F}^-$ in saline, the final product could be isolated quickly and easily.

Most ^{18}F -peptide radiolabeling processes are complicated, take several reaction steps, and require specialized and costly equipment, as well as highly trained personnel, but recently there has been some progress in simplifying labeling procedures. For example, one method involves binding ^{18}F to silicon using isotopic exchange to displace ^{19}F with ^{18}F either as a two-step prosthetic labeling method⁵ or as a direct labeling of peptides and proteins.⁶⁻⁸ The

exchange is performed at room temperature in a DMSO solution over 5 min, producing the directly ^{18}F -labeled peptide in 38% yield and with a specific activity of up to 56 GBq/ μmol .⁶ One drawback of this method is that the silicon-binding substrate is lipophilic, which can lead to high liver uptake for ^{18}F -labeled peptides. However, hydrophilic groups can be added to the peptide to compensate for the lipophilicity of the silicon acceptor.⁶

A one-step carbon-fluoride ^{18}F -labeling method for peptides also has been explored.^{10, 11} In that procedure, the peptide contains a trimethylammonium-leaving group attached to an aromatic ring containing an electron-withdrawing group. The $^{18}\text{F}^-$ is dried down (10 min) with K222/ K_2CO_3 or Cs_2CO_3 , and heated (90 °C) with the peptide for 10 min to substitute the ^{18}F for the trimethylammonium group. The reaction mixture is then purified by HPLC to produce the high specific activity ^{18}F -labeled peptide (170 GBq/ μmol) in 15% decay-corrected isolated yield. The radiolabeled peptide showed good tumor targeting with minimal hepatobiliary clearance, probably due to the addition of two hydrophilic cysteic acid groups on the peptide.

Taking the lead from simple methods to label chelate-conjugated antibodies and peptides quantitatively with radiometals, we examined a similar approach for ^{18}F . Knowing that ^{18}F binds avidly to aluminum, we showed the feasibility of binding $(\text{AlF})^{2+}$ to a NOTA chelate attached to peptide used in our bsMAb pretargeting system;¹⁷ however, because of low and variable yields, other related NOTA-based ligands were explored that gave more consistent and higher yields and specific activity.²⁴ More recently, a new NODA-MPAA ligand was evaluated for peptide labeling that forms a single, stable, octahedral complex with $(\text{AlF})^{2+}$ with the fluorine in an axial position,²⁶ whereas the earlier ligandpeptides (IMP449, IMP461, and IMP467) formed two complexes. Having a single complex would be important for some receptor-targeting agents if there were differences in affinity when multiple complexes occur. Another notable difference with the new ligand is that the same labeling yields are obtained with the preformed aluminum complex (IMP486) and the peptide formulated with Al^{3+} in the kit. Labeling yields with the other Al-NOTA-peptide complexes were consistently lower than those obtained by mixing the NOTA-peptide with Al^{3+} and then heating with $^{18}\text{F}^-$ in saline. All of these advances led to the current effort to develop a simple one-step kit that would provide a useful platform for commercial development. Indeed, the AIF technology perfectly lends itself to a simple kit.

In addition to exploring suitable Al^{18}F -binding ligands, another one of the early hurdles in this work was the source of $^{18}\text{F}^-$. Unfortunately, there is no single consistent source and specification for $^{18}\text{F}^-$, and in our early studies, we found $^{18}\text{F}^-$ contained variable amounts of metals and even other trace radionuclides, making it necessary to purify $^{18}\text{F}^-$ before use.³⁶ However, $^{18}\text{F}^-$ in saline used for bone imaging gave more consistent labeling results. Starting with an already approved radiopharmaceutical is an important first step toward the development of a commercial kit.

Based in our prior experience in developing a simple lyophilized kit for $^{99\text{m}}\text{Tc}$ -labeling of an antibody Fab' fragment,³⁴ we knew the most important aspect of developing a kit was the formulation buffer. Working with our prototype peptide, IMP485, that is being used, for pretargeted imaging studies, we examined a number of issues related to consistent labeling and proper lyophilization. Important to the lyophilization process was the use of a suitable bulking agent. While a few candidates appeared to be suitable, we elected to use α,α -trehalose, since it yielded an acceptable cake upon lyophilization without affecting labeling yields at varying concentrations. Another issue was the buffer composition. Here again, several buffers were examined, but we selected KHP because it gave the best yields in the absence of ascorbate. This result suggests it might have a role as a transfer ligand, albeit the possibility for this would need further investigation. We examined a range of KHP

concentrations, finding ~1 μmol to give better pH stabilization without reducing yields. It should be noted that while there has been some concern over phthalate esters used in plastics, the material safety data sheet for KHP indicates a $\text{LD}_{50} > 3200 \text{ mg/kg}$ when administered orally to rats. Although KHP performed well in the absence of ascorbate, we included ascorbate in the formulation buffer as a radioprotectant to minimize the chance for radiolytic processes that might occur during the labeling procedure, even if the kit were used with high amounts of ^{18}F (e.g., 10.8 GBq was used herein) at high temperatures. Finally, we reaffirmed previous studies performed in solution labeling that verified the optimal pH range for IMP485 and that the formulation buffer would maintain this pH after lyophilization, as well as affirming improvements in labeling yields with the addition of 1:1 EtOH/saline.

Using kits prepared with the optimal formulation buffer, additional studies were conducted to assess the necessary temperature and timing for the labeling procedure, and how the concentration of the peptide would affect labeling yields. These studies showed that labeling yields improved as the temperature increase, with maximum yields peaking at $\geq 90^\circ\text{C}$. Heating for 15 min provided high yields, with longer times possibly enhancing the yields slightly, but not sufficiently enough to warrant the loss in isotopic decay that would otherwise occur.

Naturally, the concentration of the peptide also affected yields. For example, in our initial testing of formulation conditions, we simply added $^{18}\text{F}^-$ in 200 μL of saline without EtOH, even though our prior experience in solution labeling with IMP485 showed higher yields.²⁶ Nevertheless, with kits containing 40 nmol of IMP485 (hence IMP485 concentration was 200 μM), yields between 75 and 85% were common with ~37 MBq of ^{18}F . However, to increase the specific activity, the amount of IMP485 in the kit was reduced to 20 nmol, where in the absence of EtOH, yields of only 50% were achieved, but with 1:1 EtOH, yields improved to ~80–85%, even though the peptide's molar concentration is reduced. Thus, the specific activity of a 40-nmol kit using 37 MBq of ^{18}F in only saline (0.2 mL) would be ~0.74 MBq/ μmol , a 20-nmol kit under the same conditions even with reduced yields would increase to ~0.925 MBq/ μmol , but the specific activity of a 20 nmol kit labeled with 37 MBq in saline (0.2 mL) and EtOH (0.2 mL) would be ~1.48 MBq/ μmol . Currently, the minimum volume of the $^{18}\text{F}^-$ in saline from our commercial source is 0.2 mL, but if reaction volumes could be decreased, the amount of peptide in the kit could also be decreased, and the specific activity would likely increase. It is important to note that higher levels of $^{18}\text{F}^-$ activity can be delivered in 0.2 mL, and thus the specific activity can be improved. A specific activity of 223 GBq/ μmol (6015 Ci/mmol) was achieved with a 20-nmol kit, and while the yields decreased to ~45%, it is likely that even higher specific activities could be achieved if more $^{18}\text{F}^-$ activity were added. For high-dose labeling, further kit optimization may improve the yields. However, depending on the desired specific activity, one could even achieve near quantitative yields with higher amounts of the peptide.

In this instance the specific activity of Al^{18}F -IMP485 used in the pretargeting study was 20.4 GBq/ μmol (550 Ci/mmol), which was sufficient to obtain excellent tumor targeting in our pretargeting model. In general, 37 GBq/ μmol (1000 Ci/mmol) is considered suitable for most receptor targeting agents. We have shown that we can obtain a specific activity as high as 223 GBq/ μmol , which would allow over three half-lives (~6 h) to deliver the Al^{18}F -IMP485 for injection into the patient and still have a usable specific activity. In the method described here we do not separate IMP485 and the unlabeled Al -IMP485 (IMP486) from the Al^{18}F -IMP485. This is analogous to the many Tc-99m or In-111 kits available where only a portion of the targeting agent actually contains the radioisotope but the specific activity is still high enough to achieve targeting. In many cases, HPLC purification can be used to separate the unreacted peptide and the Al -peptide from the Al^{18}F -peptide if desired. We have developed HPLC purification methods for both Al^{18}F -IMP485²⁶ and Al^{18}F -

IMP466.^{27,28} We believe that HPLC purification will not be necessary as long as the specific activity of the labeled peptide relative to the sum of the unlabeled targeting components is greater than 37 GBq/μmol.

Because the number of receptor sites is usually limited, most often it is desirable to have a high specific activity peptide to avoid blocking target receptors with the excess unlabeled peptide. While the minimal specific activity required for receptor targeting will likely depend on the target, in some cases excess peptide is present during the labeling procedure to improve yields, and then very often the final labeled peptide is isolated from the unlabeled peptide by HPLC. At least in one case, a radiolabeled peptide was generated with a high specific activity and an excellent yield (79%) without the need for HPLC purification.³⁷ With the AIF procedure, a SPE-purification is all that is necessary to remove unbound ¹⁸F. We previously used HLB purification, which first binds the peptide to the resin in the cartridge, and after washing to remove unbound materials, the desired product is eluted in 50% EtOH. Shetty et al.³² recently reported excellent separation of unbound ¹⁸F from the (Al¹⁸F)²⁺-complex using an Alumina N cartridge. We confirmed that with this procedure, the unwanted products are bound to the Alumina N resin, and thus the purification is simplified to a single step, further reducing the overall labeling/purification time.

These studies confirmed the excellent labeling yields with the new NODA-MPAA ligand over the NOTA derivatives we had described previously.²⁶ While adding the NODA-MPAA ligand to a receptor-targeting peptide could alter the affinity of the peptide for the receptor, it is usually possible to modify the receptor-targeting peptide to compensate for the effect of an added metal-binding ligand. In order to assess the robustness of our formulation conditions, we examined a second peptide, IMP466. This somatostatin-binding peptide was prepared with the simple NOTA ligand, and even though we suspected labeling yields would be lower than that found with the NODA-MPAA ligand, our goal was simply to show the value of formulating chelate-conjugated peptides in a lyophilized kit form. With just a minor adjustment to the final pH, the IMP466 kits were shown to label reproducibly and with just a simple filtration step for purification. High specific activity (60 GBq/μmol) Al¹⁸F-NOTA-IMP466 could be obtained in about 20 min, including the labeling and purification steps. Indeed, no HPLC-purification was required to obtain the high specific activity peptide. Our previous work showed that the tumor targeting and biodistribution of the Al¹⁸F complex was similar to the ⁶⁸Ga complex with the same NOTA-octreotide analog.²⁷

Many ¹⁸F-labeling processes are being adapted for use by remotely-operated automated machines, which reduce radiation exposure to workers and are highly amenable for developing a reproducible, GMP process. The introduction of a pre-formulated AIF kit will serve only to further improve the simplicity and reproducibility of preparing fluorinated peptides. We have experienced that the AIF-labeling method is reliable, giving similar labeling yields from day to day, and after over 100-kit labels, we have not encountered a single failure. The formulation of peptides into kits can remove uncertainties of obtaining a successful labeled product, and because of its simplicity, additional studies can be easily performed in a single day. For example, for the studies reported in Figure 2, 25 kits were labeled and purified in about 4 h. Given the successes that are starting to be reported in the literature from others who have evaluated the AIF procedure,^{29–32} we believe many other peptides can be formulated and labeled in a similar way and without the need for expensive equipment. We note that some of these early reports by others have not utilized some of the enhancements that we have developed, and thus their yields have been lower. However, in all these reports, the labeled products were prepared simply, quickly and often at higher specific activity than with other methods. Undoubtedly, some peptides will be challenged by

the insertion of a metal-binding ligand in their structure, but often the peptide's structure can be modified without seriously affecting its receptor binding. For those peptides or other compounds that might be affected by the high temperatures required for (Al¹⁸F)²⁺ insertion in the ligand, we recently presented an alternative 2-step procedure that allows for rapid fluorination of an antibody Fab' fragment.³⁸ Thus, we are confident that the AIF procedure can be adapted for use with many different agents, and thus expand the introduction of more ¹⁸F-labeled compounds for molecular imaging.

CONCLUSIONS

The NODA-MPAA-containing peptide, IMP485, can be formulated conveniently into a lyophilized kit and radiolabeled with ¹⁸F[−] in saline in high yield and specific activity, using only an inexpensive, disposable, Alumina N or a reverse-phase SPE cartridge (HLB) for purification. Importantly, the ligand used in IMP485 forms a single complex with (Al¹⁸F)²⁺. The Al¹⁸F-IMP485 peptide is stable *in vivo* and *in vitro* and has excellent tumor targeting in a pretargeting human colorectal cancer xenograft model. The same ligand and kit formulation can be applied to receptor-targeting peptides, such as somatostatin, which can tolerate heating to 100 °C. We believe that the development of these single-vial freeze-dried kits, which can be radiolabeled with a high degree of reproducibility, simplifies the ¹⁸F-labeling methodology, thereby making it amenable for use in a clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Lenka Muskova and Jayson Jebsen for their technical assistance. This project was supported in part by the National Center for Research Resources and the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health through Grant Number 5R44RR028018-03 to WJM.

REFERENCES

- (1). Miller PW, Long NJ, Vilar R, Gee AD. Synthesis of ¹¹C, ¹⁸F, ¹⁵O, and ¹³N radiolabels for positron emission tomography. *Angew. Chem. Int. Ed.* 2008; 47:8998–9033.
- (2). Olberg DE, Hjelstuen OK. Labeling strategies of peptides with ¹⁸F for positron emission tomography. *Curr. Top. Med. Chem.* 2010; 10:1669–1679. [PubMed: 20583991]
- (3). Cai L, Lu S, Pike VW. Chemistry with [¹⁸F]fluoride ion. *Eur. J. Org. Chem.* 2008:2853–2873.
- (4). Mamat C, Ramenda T, Weust FR. Recent applications of click chemistry for the synthesis of radiotracers for molecular imaging. *Mini-Rev. Org. Chem.* 2009; 6:21–34.
- (5). Schirmacher E, Wängler B, Cypriak M, Bradtmöller G, Schäfer M, Eisenhut M, Jurkschat K, Schirmacher R. Synthesis of *p*-(di-*tert*-butyl[¹⁸F]fluorosilyl)benzaldehyde ([¹⁸F] SiFA-A) with high specific activity by isotopic exchange: a convenient labeling synthon for the ¹⁸F-labeling of N-amino-oxy derivatized peptides. *Bioconjugate Chem.* 2007; 18:2085–2089.
- (6). Wängler C, Waser B, Alke A, Iovkova L, Buchholz H-G, Niedermoser S, Jurkschat K, Fottner C, Schirmacher R, Reubi J-C, Wester H-J, Wängler B. One-step ¹⁸F labeling of carbohydrate-conjugated octreotate-derivatives containing a silicon-fluoride-acceptor (SiFA): in vitro and in vivo evaluation as tumor imaging agents for positron emission tomography (PET). *Bioconjugate Chem.* 2010; 21:2289–2296.
- (7). Wängler B, Quandt G, Iovkova L, Schirmacher E, Wängler C, Boening G, Hacker M, Schmoekel M, Jurkschat K, Bartenstein P, Schirmacher R. Kit-like ¹⁸F-labeling of proteins: synthesis of 4-(Di-*tert*-butyl[¹⁸F]fluorosilyl)benzenethiol (Si[¹⁸F]FA-SH) labeled rat serum albumin for blood pool imaging with PET. *Bioconjugate Chem.* 2009; 20:317–321.

- (8). Iovkova L, Wängler B, Schirmacher E, Schirmacher R, Quandt G, Boening G, Schürmann M, Jurkschat K. *Para*-Functionalized aryl-di-*tert*-butylfluorosilanes as potential labeling synthons for ^{18}F radiopharmaceuticals. *Chem. Eur. J.* 2009; 15:2140–2147. [PubMed: 19156812]
- (9). Ting R, Harwig C, Keller UAD, McCormick S, Austin P, Overall CM, Adam MJ, Ruth TJ, Perrin DM. Toward [^{18}F]-labeled aryltrifluoroborate radiotracers: in vivo positron emission tomography imaging of stable aryltrifluoroborate clearance in mice. *J. Am. Chem. Soc.* 2008; 130:12045–12055. [PubMed: 18700764]
- (10). Honer M, Mu L, Stellfeld T, Graham K, Martic M, Fischer CR, Lehmann L, Schubiger PA, Ametamey SM, Dinkelborg L, Srinivasan A, Borkowski S. ^{18}F -labeled bombesin analog for specific and effective targeting of prostate tumors expressing gastrin-releasing peptide receptors. *J. Nucl. Med.* 2011; 52:270–278. [PubMed: 21233180]
- (11). Becaude J, Mu L, Schubiger PA, Ametamey SM, Graham K, Stellfeld T, Lehmann L, Borkowski S, Berndorf D, Dinkelborg L, Srinivasan A, Smits R, Koks B. Direct one-step ^{18}F -Labeling of peptides via nucleophilic aromatic substitution. *Bioconjugate Chem.* 2009; 20:2254–2261.
- (12). Meares CF, Wensel TG. Metal chelates as probes of biological systems. *Acc. Chem. Res.* 1984; 17:202–209.
- (13). Scheinberg DA, Strand M, Gansow OA. Tumor imaging with radioactive metal chelates conjugated to monoclonal antibodies. *Science.* 1982; 215:1511–1513. [PubMed: 7199757]
- (14). Fani M, Pozzo LD, Abiraj K, Mansi R, Tamma ML, Cescato R, Waser B, Weber WA, Reubi JC, Maecke HR. PET of receptor-positive tumors using ^{64}Cu - and ^{68}Ga -somatostatin antagonists: the chelate makes the difference. *J. Nucl. Med.* 2011; 52:1110–1118. [PubMed: 21680701]
- (15). Tewson TJ. Procedures, pitfalls and solutions in the production of [^{18}F]2-deoxy-2-fluoro-D-glucose: a paradigm in the routine synthesis of fluorine-18 radiopharmaceuticals. *Nucl. Med. Biol.* 1989; 16:533–551.
- (16). Martin RB. Ternary complexes of Al^{3+} and F^- with a third ligand. *Coordination Chem. Rev.* 1996; 141:23–32.
- (17). McBride WJ, Sharkey RM, Karacay H, D'Souza CA, Rossi EA, Laverman P, Chang C-H, Boerman OC, Goldenberg DM. A novel method of ^{18}F radiolabeling for PET. *J. Nucl. Med.* 2009; 50:991–998. [PubMed: 19443594]
- (18). Gold DV, Goldenberg DM, Karacay H, Rossi EA, Chang C-H, Cardillo TM, McBride WJ, Sharkey RM. A novel bispecific, trivalent antibody construct for targeting pancreatic carcinoma. *Cancer Res.* 2008; 68:4819–4826. [PubMed: 18559529]
- (19). Sharkey RM, Cardillo TM, Rossi EA, Chang C-H, Karacay H, McBride WJ, Hansen HJ, Horak ID, Goldenberg DM. Signal amplification in molecular imaging by pretargeting a multivalent, bispecific antibody. *Nature Medicine.* 2005; 11:1250–1255.
- (20). McBride WJ, Zanzonico P, Sharkey RM, Norén C, Karacay H, Rossi EA, Losman MJ, Brard P-Y, Chang C-H, Larson SM, Goldenberg DM. Bispecific antibody pretargeting PET (immunoPET) with an ^{124}I -labeled hapten-peptide. *J. Nucl. Med.* 2006; 47:1678–1688. [PubMed: 17015905]
- (21). Sharkey RM, Karacay H, Vallabhajosula S, McBride WJ, Rossi EA, Chang C-H, Goldsmith SJ, Goldenberg DM. Metastatic human colonic carcinoma: molecular imaging with pretargeted SPECT and PET in a mouse model. *Radiology.* 2008; 246:497–507. [PubMed: 18227543]
- (22). Schoffelen R, Sharkey RM, Goldenberg DM, Franssen G, McBride WJ, Rossi EA, Chang C-H, Laverman P, Disselhorst JA, Eek A, van der Graaf WTA, Oyen WJG, Boerman OC. Pretargeted immunoPET imaging of CEA-expressing tumors with a bispecific antibody and a ^{68}Ga and ^{18}F -labeled hapten-peptide in mice with human tumor xenografts. *Mol. Cancer Ther.* 2010; 9:1019–1027. [PubMed: 20354120]
- (23). Rossi EA, Goldenberg DM, Cardillo TM, McBride WJ, Sharkey RM, Chang C-H. Stably tethered multifunctional structures of defined composition made by the dock and lock method for use in cancer targeting. *Proc. Natl. Acad. Sci. USA.* 2006; 103:6841–6846. [PubMed: 16636283]
- (24). McBride WJ, D'Souza CA, Sharkey RM, Sharkey RM, Karacay H, Rossi EA, Chang C-H, Goldenberg DM. Improved ^{18}F labeling of peptides with a fluoride-aluminum-chelate complex. *Bioconjugate Chem.* 2010; 21:1331–1340.

- (25). Chong H-S, Garmestani K, Ma D, Milenic DE, Overstreet T, Brechbiel MW. Synthesis and biological evaluation of novel macrocyclic ligands with pendent donor groups as potential yttrium chelators for radioimmunotherapy with improved complex formation kinetics. *J. Med. Chem.* 2002; 45:3458–3464. [PubMed: 12139456]
- (26). D'Souza C, McBride WJ, Sharkey RM, Todaro L, Goldenberg DM. High-yielding aqueous ^{18}F -labeling of biomolecules via Al^{18}F chelation. *Bioconjugate Chem.* 2011; 22:1793–1803.
- (27). Laverman P, McBride WJ, Sharkey RM, Eek A, Joosten L, Oyen WJG, Goldenberg DM, Boerman OC. A novel facile method of labeling octreotide with ^{18}F -fluorine. *J. Nucl. Med.* 2010; 51:454–461. [PubMed: 20150268]
- (28). Laverman P, D'Souza CA, Eek A, McBride WJ, Sharkey RM, Oyen WJG, Goldenberg DM, Boerman OC. Optimized labeling of NOTA-conjugated octreotide with F-18. *Tumor Biol.* 2011 DOI: 10.1007/s13277-011-0250-x.
- (29). Leyton J, Iddon L, Perumal M, Indrevall B, Glaser M, Robins E, George AJT, Cuthbertson A, Luthra SK, Aboagye EO. Targeting somatostatin receptors: preclinical evaluation of novel ^{18}F -fluoroethyltriazole-tyr³-octreotate analogs for PET. *J. Nucl. Med.* 2011; 52:1441–1448. [PubMed: 21852355]
- (30). Liu S, Liu H, Jiang H, Xu Y, Zhang H, Cheng Z. One-step radiosynthesis of ^{18}F -AIF-NOTA-RGD₂ for tumor angiogenesis PET imaging. *Eur. J. Nucl. Med. Mol. Imaging.* 2011; 38:1732–1741. [PubMed: 21617974]
- (31). Lang L, Li, W, Guo N, Ma Y, Zhu L, Kiesewetter D, Shen B, Niu G, Chen X. Comparison study of [^{18}F]FAI-NOTA-PRGD₂ for PET imaging of U87MG tumors in mice. *Bioconjugate Chem.* 2011 DOI: 10.1021/bc200197h.
- (32). Shetty D, Choi SY, Jeong JM, Lee JY, Hoigebazar L, Lee Y-S, Lee DS, Chung J-K, Lee MC, Chung YK. Stable aluminum fluoride chelates with triazacyclononane derivatives proved by X-ray crystallography and ^{18}F -labeling study. *Chem. Comm.* 2011; 47(34):9732–9734. [PubMed: 21776539]
- (33). Technetium-99m radiopharmaceuticals: manufacture of kits. International Atomic Energy Agency; Vienna, Austria: 2008. Technical reports series no. 466
- (34). Griffiths GL, Goldenberg DM, Jones AL, Hansen HJ. Radiolabeling of monoclonal antibodies and fragments with technetium and rhenium. *Bioconjugate Chem.* 1992; 3:91–99.
- (35). Rossi EA, Goldenberg DM, Cardillo TM, McBride WJ, Sharkey RM, Chang C-H. Stably tethered multifunctional structures of defined composition made by the dock and lock method for use in cancer targeting. *Proc. Natl. Acad. Sci. USA.* 2006; 103:6841–6846. [PubMed: 16636283]
- (36). Ferguson D, Orr P, Gillanders J, Corrigan G, Marshall C. Measurement of long lived radioactive impurities retained in the disposable cassettes on the tracerlab MX system during the production of [^{18}F]FDG. *Appl. Radiat. Isot.* 2011; 69:1479–1485. [PubMed: 21641809]
- (37). Marik J, Sutcliffe JL. Click for PET: rapid preparation of [^{18}F]fluoropeptides using Cu^{I} catalyzed 1,3-dipolar cycloaddition. *Tetrahedron Lett.* 2006; 27:6681–6684.
- (38). McBride WJ, D'Souza CA, Sharkey RM, Goldenberg DM. The radiolabeling of proteins by the [^{18}F]AIF method. *Appl. Radiat. Isot.* 2012; 70:200–204. [PubMed: 21890371]

Figure 1.
Structures of IMP485 and IMP486 as compared to hapten-peptide ligand reported previously IMP467.

Figure 2. Isolated % yield vs. reaction temperature at different IMP485 concentrations. The labeling reactions were performed in 400 μ L of 1:1 ethanol/saline for 15 min at the temperatures indicated.

Figure 3.

Biodistribution at 1 h (black bars) and 3 h (white bars) post injection of Al^{18}F -IMP485 in nude mice bearing sc LS174T human colon cancer xenografts. (**AandB**), Al^{18}F -IMP485 (n=7) percent uptake and T/NT ratios, respectively, at 1 and 3 h in mice pretargeted with TF2 anti-CEACAM5 bsMAb 16 h prior to injection of the peptide. (**C**) Percent uptake of Al^{18}F -IMP485 (n=6) alone at 1 h and 3 h.

Figure 4.
RP-HPLC of Al¹⁸F-IMP485 after purification (**A**) and activity recovered from mouse urine at 50 min PI (**B**).

Table 1Radiolabeling yields as a function of time and $^{18}\text{F}^-$ -activity.

Reaction Time, min	30MBq	2.5 GBq	
	% Yield	% Yield	Specific Activity GBq/ μmol
5	51.1	ND	----
10	77.1	ND	----
15	86.4	74.3	76.5
20	ND	77.3	78.5
30	91.4	79.2	74.0
60	83.1	ND	----

^{18}F in 200 μL of saline was added to a 20-nmol IMP485 kit with 200 μL of ethanol and heated to 108–110 $^{\circ}\text{C}$ for the times indicated. Purification by Alumina N.

Table 2

Comparison of high-dose $^{18}\text{F}^-$ labeling in kits containing 20 nmol of IMP466.

Reaction time (min)	Starting activity GBq	Isolated activity GBq	Specific activity GBq/ μmol	Isolated % yield
15	2.51	1.21	60.5	54.6
20	2.51	1.17	58.5	55.7
30	2.39	1.08	54.0	58.1

Each 20-nmol kit received ~ 2.5 GBq of $^{18}\text{F}^-$ in 200 μL of saline plus 200 μL of ethanol. After incubation at 110 $^{\circ}\text{C}$ for the times indicated, the products were purified on an Alumina N cartridge.