Optimization of Hapten-Peptide Labeling for Pretargeted ImmunoPET of Bispecific Antibody Using Generator-Produced ^{68}Ga

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Bispecific antibody pretargeting is highly sensitive and specific for cancer detection by PET. In this study, the preparation of a high-specific-activity ^{68}Ga-labeled hapten-peptide, IMP288, was evaluated. **Methods:** IMP288 (DOTA-D-Tyr-D-Lys(histamine-succinyl-glycine [HSG])-D-glu-D-Lys(HSG)-NH_2) was added to buffered ^{68}Ga and then heated in boiling water and purified on a reversed-phase cartridge. Tumor-bearing nude mice were used for biodistribution and tumor localization studies. **Results:** ^{68}Ga-IMP288 was prepared at a starting specific activity up to 1.78 GBq/nmol, with final yields of 0.74 GBq/nmol (decay-corrected) and less than 1% unbound ^{68}Ga. Purification was essential to remove unbound ^{68}Ga and ^{68}Ge breakthrough. Pretargeted animals showed a high ^{68}Ga-IMP288 uptake (27.5 ± 5.8 percentage injected dose per gram), with ratios of 13.6 ± 4.8, 66.8 ± 14.5, and 325.9 ± 61.9 for the kidneys, liver, and blood, respectively, at 1.5 h after peptide injection. **Conclusion:** High-specific-activity labeling of DOTA-hapten-peptide was obtained from the ^{68}Ga/^{68}Ge generator for approximately 1 y, yielding products suitable for immunoPET.

Key Words: bispecific antibody; pretargeting; ^{68}Ga; ^{68}Ge/^{68}Ga generator

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The PET radionuclide ^{68}Ga can be obtained from commercially available ^{68}Ge generators with no carrier added, allowing compounds to be prepared at high specific activity. The half-life of ^{68}Ge (270.8 d) allows the generator to be used for an extended period, which reduces the unit-dose cost, and the half-life of ^{68}Ga (67.6 min) is suitable for rapidly clearing molecules, such as peptides. Interest in ^{68}Ga-labeled compounds has been growing over the past 10 y, with promising initial clinical results (*1–11*).

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We are interested in using a small radiolabeled hapten-peptide as part of a bispecific antibody (bsmAb) pretargeting procedure for PET. Previous studies reported high tumor uptake with minimal accretion in normal tissues, producing targeting superior to that of directly radiolabeled antibody fragments and greater sensitivity and specificity than ^{18}F-FDG (*12–14*). Like directly radiolabeled peptides, the hapten-peptide used in pretargeting would benefit from high-specific-activity labeling. Thus, the main objective of the study was to evaluate the suitability of ^{68}Ga for pretargeting.

MATERIALS AND METHODS

Reagents

Humanized tri-Fab bsmAb TF2 and IMP288 (DOTA-D-Tyr-D-Lys(histamine-succinyl-glycine [HSG]-D-Glu-D-Lys(HSG)-NH_2) were described previously (*12,15*). A 1.3 mM stock solution of IMP288 was diluted to 6.5×10^{-5} M in 1 M *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), pH 6.9, and stored at −20°C.

^{68}Ga Generator

The IGG-100, 1.85-GBq (50-mCi) ^{68}Ga generator was purchased from Eckert-Ziegler Isotope Products Eurotope GmbH and eluted according to the manufacturer's recommendations, using 0.1 M HCl. Three fractions (1.5, 1.0, and 2.5 mL) were isolated, with fraction 2 containing the highest concentration of ^{68}Ga.

Within 2–17 d of the ^{68}Ga elution, aliquots of fraction 2 and a sample of the radiolabeled product before and after purification were counted in an open window using a Wizard 3″ automatic γ-counter (Perkin Elmer). To approximate the amount of the ^{68}Ge counts in fraction 2, we assumed 100% counting efficiency and expressed the activity as Becquerels of ^{68}Ge/37 MBq of ^{68}Ga. The ^{68}Ge counts in the labeled product were normalized as a percentage of the activity in fraction 2 used to prepare the labeled product.

Radiolabeling

IMP288 was radiolabeled with $^{111}InCl_3$, according to methods published previously (specific activity, 36.8 MBq/nmol [0.995 mCi/nmol]; <3% unbound by instant thin-layer chromatography) (*12*).

The ^{68}Ga-labeling procedure was modeled after that reported previously, with some modifications (*13*). IMP288 was added to

all or a portion of fraction 2, along with 1.0 M HEPES, pH 6.9. After being heated in a boiling water bath for 12 min, the vial was cooled in an ice bath to room temperature, and then 0.1 M ethylenediaminetetraacetic acid, pH 5.5, was added to a final concentration of 5 mM. The mixture was transferred to a reversed-phase polymeric-sorbent packed, 1-mL Oasis HLB cartridge for purification (Waters). After it was washed with three 1.0-mL aliquots of water, the product was eluted with two 200-μL aliquots of water:ethanol (1:1) into a vial containing 50 μL of ascorbic acid (300 mg/mL).

The peptide concentration ranged from 400 to 650 nM and from 200 to 250 nM for preparations that started with a specific activity of 0.888 and 1.776 GBq/nmol, respectively (116- and 56-fold molar excess to the ^{68}Ga activity, respectively). Final specific activity assumed full recovery of IMP288 from the HLB cartridge.

Unbound ^{68}Ga was determined by reverse-phase HPLC (RP-HPLC) (Nova-Pak C18, 4 μm, 8 × 100 μm Radial-Pak; Waters).

Additional details can be found in the supplemental data (available online only at http://jnm.snmjournals.org).

Animal Studies

Nude mice bearing subcutaneous implants of human colonic cancer cell lines were given intravenous injections of the radiolabeled product. Specific information regarding the doses administered and methods are provided in the "Results" section and supplemental data.

RESULTS

Generator Elution

Over a period of 350 d from generator calibration, 79.9% ± 3.1% of the expected ^{68}Ga activity was accounted for in the total elution volume (Fig. 1). Fraction 2 (1 mL) contained 63%–83% of the total eluted activity. An approximation of ^{68}Ge activity, based on an estimated counting efficiency of 100%, revealed that 0.05–0.22 Bq of ^{68}Ge/MBq of ^{68}Ga was present in fraction 2, except for 2 instances in which it was 0.64 and 0.49 Bq of ^{68}Ge/MBq of ^{68}Ga between days 160 and 350. Assays performed on samples taken before and after purification indicated the HLB purification assisted in the removal of unbound ^{68}Ga but also of ^{68}Ge. For example, most often ^{68}Ge was not detected in aliquots of purified ^{68}Ga-IMP288, but when present, more than 95% of the ^{68}Ge in the fraction 2 used for the labeling had been removed.

^{68}Ga-IMP288 Radiolabeling

^{68}Ga-IMP288 preparation was completed within 30–40 min. Variable product recovery was encountered when following the procedure of Schoffelen et al. (*13*), suggesting that colloidal forms of gallium were formed. Three labeling procedures were performed using IMP288 (0.444 GBq/nmol) with HEPES added at one half, one quarter, or one eighth of the ^{68}Ga volume. Mock mixtures found that the pH was 4.6 at one half, 3.7 at one quarter, and 3.1 at one eighth of the volume. Analysis without HLB purification showed that each label had approximately 0.9% unbound ^{68}Ga by RP-HPLC, but the RP-HPLC recoveries were 60%, 74%, and 87%, respectively, suggesting colloid formation at a higher pH. Therefore, all subsequent labeling procedures were performed with HEPES buffer at one eighth of the ^{68}Ga volume.

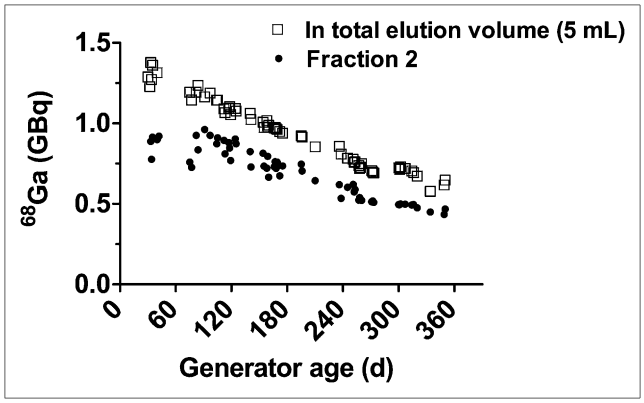

FIGURE 1. ^{68}Ga generator was eluted over 350 d with 0.1 M HCl in 3 fractions, starting 28 d after its calibration date. Activity in 1-mL fraction 2 and total in all fractions are shown.

The starting specific activity was increased to as high as 1.776 GBq/nmol using peptide concentrations ranging from approximately 200 to approximately 650 nM. High specific activity could be achieved when the generator was 350 d old using a starting ratio of 0.888 GBq/nmol (unbound ^{68}Ga, ~1%).

Initially, ascorbic acid was added only to the HLB-purified product, but later several products were prepared by adding ascorbic acid to the reaction mixture before heating at a final concentration of 6.7 mg/mL. This step improved recovery without compromising product quality (e.g., at initial specific activity of 0.88 GBq/nmol, ^{68}Ga-IMP288 recovery improved from 59.1% ± 8.8% [n = 11] to 70.4% ± 16.8% [n = 6]). A mock-labeling mixture showed that the addition of ascorbic acid had little effect on the pH. Cenolate (Hospira Worldwide, Inc.), pH 6.18, ascorbic acid for human use, could not be added during radiolabeling, presumably because of the presence of excessive amounts of aluminum or the higher pH.

Seven products, all including ascorbic acid in the reaction mixture, were prepared at the initial specific activity of 1.7 GBq/nmol. The overall recovery of the purified product without decay correction was 38.3% ± 8.9% (54.1% ± 10.5% decay-corrected; starting activity was 407–444 MBq). These 7 labeled products were stable for 2 h at room temperature (i.e., RP-HPLC indicated no change in unbound or the molecular character of IMP288, and size-exclusion HPLC showed that HSG-binding was retained) (data not shown).

Biodistribution

One animal injected with ^{68}Ga-IMP288 before HLB purification (3.5% unbound by RP-HPLC and 14% colloidal ^{68}Ga, as indicated by the activity retained on the RP-HPLC) had 14.1 percentage injected dose per gram (%ID/g), 4.5 %ID/g, and 2.1 %ID/g, respectively, in the liver, spleen,

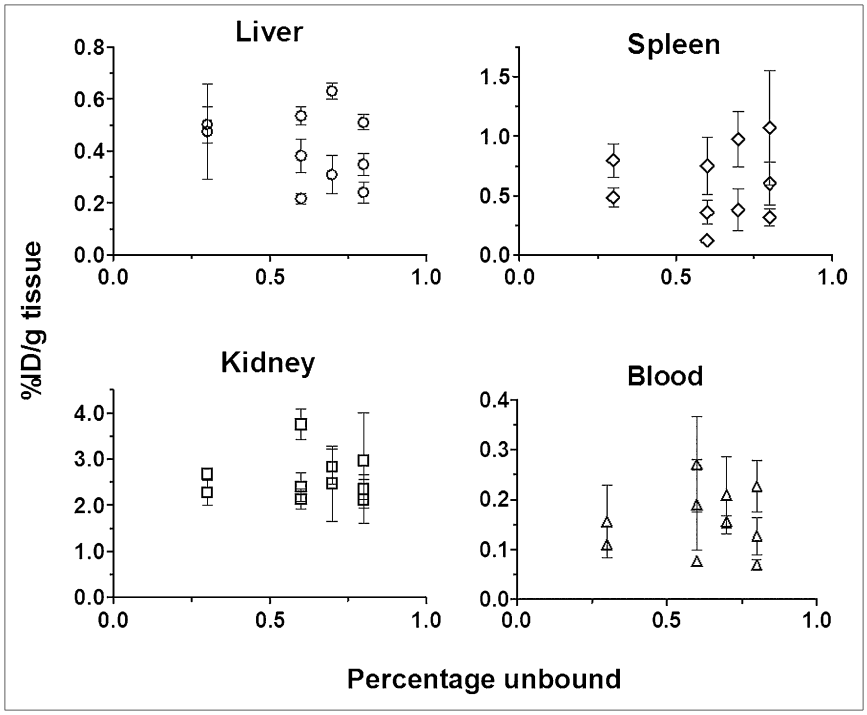

FIGURE 2. Evaluation of ^{68}Ga-IMP288 tissue uptake in 10 separate studies. Each study consisted of groups of 2–5 mice that were given 25 pmole (7.77–11.1 MBq [210–300 μCi]) of ^{68}Ga-IMP288 and necropsied 1 h later.

and bone at 1 h after injection. Most of the purified products had less than 1% unbound ^{68}Ga by RP-HPLC, and in 10 separate studies with purified product prepared without ascorbic acid, uptake in the liver, spleen, kidneys, and blood at 1 h after injection averaged 0.42 ± 0.05, 0.59 ± 0.13, 2.60 ± 0.29, and 0.16 ± 0.04 %ID/g, respectively (Fig. 2). Thus, purification was considered essential to minimize normal-tissue uptake.

TF2 anti-CEACAM5 pretargeting was assessed in mice bearing either subcutaneous LS 174T or HT-29 xenografts. A TF2 dose-finding study was performed with a fixed amount of ^{111}In-IMP288 given at 16 h after the TF2 injections, using 10:1, 20 or 25:1, and 40:1 TF2:^{111}In IMP288 mol ratios. In each model, 25:1 to 40:1 was preferred (Fig. 3).

Pretargeted ^{68}Ga-IMP288 tumor uptake averaged 27.5 ± 5.8 %ID/g in LS 174T, with low tissue uptake providing high tumor-to-nontumor ratios at 1.5 h after injection (Table 1). ^{68}Ga-IMP288 alone was 0.33 ± 0.07 %ID/g. Tumor and tissue uptake of the ^{68}Ga-IMP288 compared favorably to LS 174T–bearing animals that had been necropsied at 1.0 h after being given TF2-pretargeted ^{111}In-IMP288; however, there was a suggestion that liver and spleen uptake was higher for the ^{68}Ga group ($P < 0.05$) and a suggestion of

TABLE 1
Tissue Uptake of ^{68}Ga-IMP288 in Mice Bearing Subcutaneous LS 174T Human Colon Cancer Xenografts

Tissue	TF2/^{68}Ga-IMP288 (n = 5)	^{68}Ga-IMP288 (no TF2) (n = 5)	TF2/^{111}In-IMP288 (n = 5)
Tumor	27.5 ± 5.8	0.33 ± 0.07	23.6 ± 5.4
Tumor weight (g)	0.40 ± 0.10	1.11 ± 0.37	0.37 ± 0.18
Liver	0.41 ± 0.03	0.38 ± 0.06	0.11 ± 0.03
Spleen	0.62 ± 0.24	0.36 ± 0.10	0.13 ± 0.02
Kidney	2.08 ± 0.25	2.40 ± 0.31	2.99 ± 0.79
Lung	0.26 ± 0.04	0.22 ± 0.04	0.28 ± 0.06
Blood	0.09 ± 0.01	0.19 ± 0.09	0.09 ± 0.02
Stomach	0.07 ± 0.09	0.09 ± 0.08	0.22 ± 0.17
Small intestine	0.17 ± 0.02	0.22 ± 0.10	0.36 ± 0.16
Large intestine	0.20 ± 0.36	0.10 ± 0.07	0.14 ± 0.11
Scapula	0.06 ± 0.02	0.06 ± 0.03	0.19 ± 0.17

Tissue uptake (%ID/g) data are mean ± SD. TF2 anti-CEACAM5 bsmAb (100 μg, 6.34×10^{-10} mol) was given intravenously; then, 16 h later, ^{68}Ga-IMP288 (2.53×10^{-11} mol, 9.065 MBq [245 μCi]; specific activity, 0.397 GBq/nmol; prepared in absence of ascorbic acid) was given. Another group received only ^{68}Ga-IMP288 (2.53×10^{-11} mol, 9.99 MBq [270 μCi]). Mice were necropsied 1.5 h after peptide injection. In separate study, mice were given TF2 (100 μg, 6.34×10^{-10} mol) followed 16 h later by ^{111}In-IMP288 (2.53×10^{-11} mol, 0.925 MBq [25 μCi]), and then were necropsied 1 h afterward.

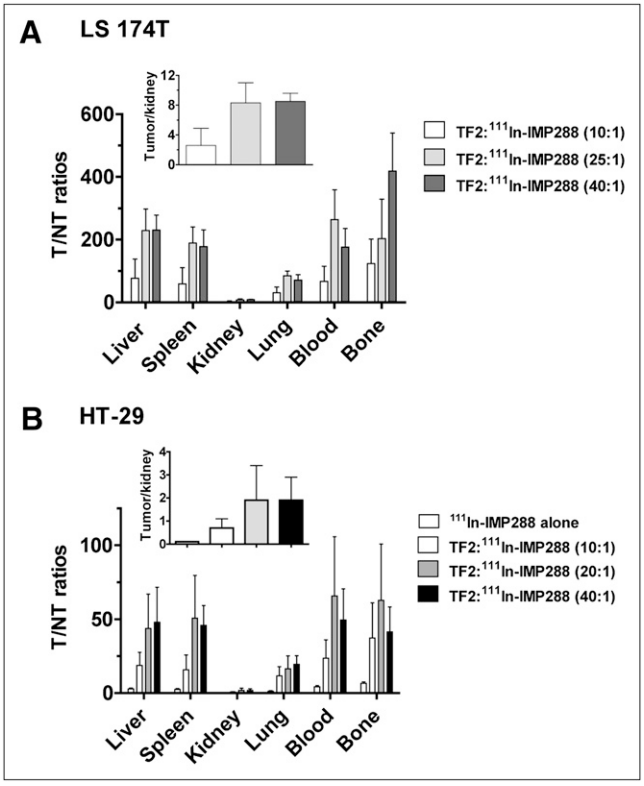

FIGURE 3. Tumor-to-nontumor ratios of ^{111}In-IMP288 in mice bearing subcutaneous LS 174T (A) and HT-29 (B) human colon cancer xenografts. For pretargeting, TF2 anti-CEACAM5 bsmAb was given intravenously at 3 doses and then, 16 h later, ^{111}In-IMP288 (2.53×10^{-11} mol, 0.925 MBq [25 μCi]) was given to all groups. For LS 174T, TF2 dose was 40 μg (2.53×10^{-10} mol) for 10:1, 100 μg (6.34×10^{-10} mol) for 25:1, and 160 μg (1.01×10^{-9} mol) for 40:1 groups. For HT-29, TF2 doses were 40, 80, and 160 μg for 10:1, 20:1, and 40:1 groups, respectively. Mice were necropsied 1.5 h after peptide injection. Inserts show tumor-to-kidney ratios. Tumor-to-kidney ratio for ^{111}In-IMP288 (without TF2) was 0.08 ± 0.03. T/NT = tumor to nontumor.

somewhat higher renal uptake for the ^{111}In-IMP288 ($P = 0.04$).

HT-29 accretion in the TF2–^{68}Ga-IMP288 pretargeted group was 8.6 ± 1.3 %ID/g (0.20 ± 0.03 %ID/g with ^{68}Ga-IMP-288 alone). Tumor-to-nontumor ratios were again favorable (e.g., tumor-to-liver, tumor-to-spleen, tumor-to-kidney, and tumor-to-blood ratios were 27 ± 11, 22 ± 13, 2.3 ± 0.3, and 28 ± 9, respectively) (Fig. 4). Tumor-to-liver, tumor-to-blood, and tumor-to-kidney ratios were 1.3 ± 0.1, 1.6 ± 0.3 and 0.065 ± 0.003, respectively, for ^{68}Ga-IMP-288 alone.

DISCUSSION

The short half-life of ^{68}Ga is matched well for rapidly clearing molecules, cost, procedural simplicity, and the ability to prepare products at high specific activities, contributing to its appeal for PET.

Our ^{68}Ga-IMP288 labeling experience was favorable. The generator performed according to the manufacturer's specifications, with high elution efficiency remaining at approx-

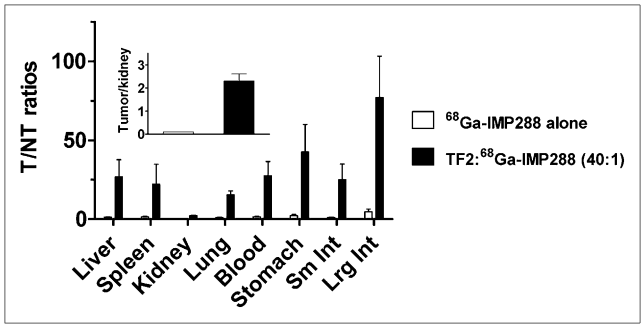

FIGURE 4. Tumor-to-nontumor ratios in nude mice bearing subcutaneous HT-29 human colon cancer xenografts (%ID/g). Mice ($n = 5$) were given TF2 (160 μg, 1.01×10^{-9} mol) intravenously, and after 16 h ^{68}Ga-IMP288 (2.53×10^{-11} mol, 11.1 MBq [300 μCi]) was given intravenously. Another group received the same ^{68}Ga-IMP288 alone (no bsmAb). Specific activity of purified ^{68}Ga-IMP288 was 0.516 GBq/nmol, and it was prepared with ascorbic acid. Mice were necropsied 1 h after peptide injection. Lrg Int = large intestine; Sm Int = small intestine; T/NT = tumor-to-nontumor.

imately 1 y after calibration (98 elutions). Isolating the peak amount of radioactivity in a 1.0-mL fraction minimized the total elution volume. A more concentrated form was not required for successful radiolabeling. Ascorbic acid added during the radiolabeling procedure appeared to enhance recovery without altering the pH, but we also routinely added it to the final product, which was stable for 2 h.

^{111}In-, ^{90}Y-, and ^{177}Lu-IMP288 have been prepared without purification, but ^{68}Ga-IMP288 required HLB purification. Purification removed unwanted forms of $^{68}Ga^{3+}$, but it also removed ^{68}Ge. Because gallium exists in various forms based on the pH and concentration (*16*), our goal was to retain the pH near 3.0, which is lower than most other ^{68}Ga-DOTA-peptides (*6,10,17–19*). For example, Bauwens et al. found labeling efficiencies or more than 90% for ^{68}Ga-DOTATOC using 1 M HEPES, pH 3.75, whereas with 0.5 M sodium succinate, pH approximately 4.0, labeling efficiencies suffered when 10 μg of the peptide was used (*19*). IMP288 labeling was always performed with less than 10 μg, and at pH 3.7 and 4.6, recovery from analytic RP-HPLC was low, but better recoveries were possible at the lower pH.

Because unbound forms of $^{68}Ga^{3+}$ can be difficult to quantify, and to ensure ^{68}Ga was retained sufficiently by DOTA-IMP288, we repeatedly administered randomly selected products into mice. Hepatic and splenic uptake was reduced substantially by HLB purification, but even then it was somewhat higher than with ^{111}In-IMP288, suggesting there may be a small amount of transchelation. A similar observation was reported, in a comparison study in rats, in which ^{68}Ga-DOTATOC showed higher uptake in the liver than did ^{111}In-DOTATOC (*6*).

CONCLUSION

^{68}Ga-IMP288 specific activities as high as 0.84 GBq/nmol (i.e., starting specific activity, 1.776 GBq/nmol) were achieved without any tedious handling of ^{68}Ga.^{68}Ga-IMP288 had excellent tumor localization properties in a bsmAb pretargeting setting and is easily and conveniently prepared, but a

final purification procedure using an HLB reversed-phase cartridge is necessary to remove unwanted unbound $^{68}Ga^{3+}$ forms. Acceptable products could be prepared over a 1-y period from the generator's calibration date.

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