MATERIALS and METHODS

Reagents

Trace-metal grade hydrochloric acid was purchased from Fisher Scientific (Fair Lawn, NJ). Water was obtained from Milli-Q Synthesis system (Billerica, MA). HEPES, Sigma-ultra grade, chelex-100 and L-ascorbic acid (ACS reagent grade) were purchased from Sigma-Aldrich (St. Louis, MO). Clear polypropylene, self-standing 2 mL screw-cap tubes with "O" rings were purchased from Axygen Scientific (Union City, CA). Oasis® HLB (reverse phase) 1cc cartridges were obtained from Waters (Milford, MA).

Radiolabeling

^{111}In-IMP288 was prepared as described previously *(1)*, with excess DTPA (diethylenetriaminepentaacetic acid) added at the completion of the labeling procedure. ITLC indicated <3% unbound radionuclide, and final specific activity was 36.8 MBq/nmol (0.995 mCi/nmol).

^{68}Ga-IMP288 was prepared by adding IMP288 to all or a portion of the second fraction isolated from the generator, along with 1.0 M HEPES, pH 6.9, with early studies showing improved recoveries using a ratio of one-eighth of the total ^{68}Ga volume, and therefore most of the radiolabeled preparations reported used this ratio. For example, to prepare ^{68}Ga-IMP288 at a starting specific activity of 888 MBq/nmol (24 mCi/nmol), 6.4 μL of IMP288 (6.5×10^{-5} M) and 90 μL of 1.0 M HEPES, pH 6.9, were added to 720 μl of $^{68}GaCl_3$ (9.9 mCi). The reaction vial was repeatedly centrifuged to ensure the contents were collected in the bottom. After heating in a boiling water bath for 12 minutes, the vial was cooled in an ice bath to room temperature, the vial, and then 0.1 M EDTA, pH 5.5, was added to a final concentration of 5 mM. The mixture was transferred to an Oasis HLB cartridge for purification 2 min later. After collecting the flow

through, the cartridge was washed with 3 x 1.0-mL aliquots of water. The labeled peptide was eluted with 2 x 200-μL aliquots of water:ethanol (1:1) mixture into a vial containing 50 μL of 300 mg/mL ascorbic acid and further diluted with saline to the desired concentration.

The peptide concentration at the onset ranged from 400-650 nM, with the peptide being in 116-fold mole excess of the ^{68}Ga-activity for preparations that started with a specific activity of 0.888 GBq/nmol, and 200-250 nM and a 56:1-fold mole excess for preparations that started with a specific activity of 1.776 GBq/nmol (48 mCi/nmol). All calculations of final specific activity assumed full recovery of IMP288 from the HLB column.

Reverse-phase HPLC (RP-HPLC) analyses were performed on a Waters Nova-Pak C18 4 μm, 8 x 100 μm Radial-Pak cartridge (Milford, MA). The column was eluted over 15 min at 1.5 mL/min with a linear gradient of 100% A (0.075% trifluoroacetic acid in water) to 45% B, where B was 0.075% trifluoroacetic acid in 75% acetonitrile and 25% water. At 15 min, the solvent was switched to 100% B and maintained for 5 min before re-equilibration to initial conditions. Size-exclusion HPLC (SE HPLC; BioSil SE 250 SE column and guard column equipped with an in-line UV and radiation detector) analyses were performed to assess binding of the HSG portion of IMP288 by mixing the labeled IMP288 with the bsMAb TF2 in 20-fold mole excess to the peptide.

Animal Studies

LS 174T and HT-29 (American Tissue Culture Collection, Manassas, VA) human colonic cancer cell lines were implanted subcutaneously (1 x 10^7 cells) in 5- to 6-week old female athymic NCr nu-m nude mice (Taconic Farms, Germantown, NY). The radiolabeled product, diluted in saline, was administered intravenously in ≤0.2 mL. For pretargeting, the doses of TF2

and IMP288 were adjusted to yield a prescribed mole ratio (i.e., moles of TF2 to IMP288 administered, typically 25:1). At 1 or 3 h post peptide injection, mice were anesthetized, bled by cardiac puncture, and then euthanized by cervical dislocation. Tissues were isolated, weighed and counted in a gamma counter along with a standard representing a dilution of the injected product.

RESULTS

Generator Elution

Generator elutions were initiated 28 days after its calibration date. From there on, it was eluted 98 times for a total activity of 2465 mCi over a period of 350 days from the calibration date. Most of the elutions were performed once a day with ~18- to 24-h interval between each elution over 5 days.

The amount of ^{68}Ge in ^{68}Ga (Fraction #2) was only 2-8.1 Bq per 37 MBq of ^{68}Ga, except for two instances, where it was 23.7 and 18.3 Bq per 37 MBq ^{68}Ga between Day 160 to Day 350**.** We also determined the presence of ^{68}Ge in HLB-purified ^{68}Ga-IMP288, the flow-through, and water washes from HLB, and on the HLB cartridge. The results showed: (A) ^{68}Ge was present primarily in the flow-through and in the water washes, indicating the HLB-purification assisted not only in the removal of any unbound ^{68}Ga, but also ^{68}Ge; (B) some ^{68}Ge was retained on the HLB cartridge; and more importantly, (C) ^{68}Ge was most often not detected in aliquots of purified ^{68}Ga-IMP288, or when present, >98% of the ^{68}Ge found in the ^{68}Ga used for radiolabeling was removed, except for one instance where the removal of ^{68}Ge was only 95%.

Reference

1. McBride WJ, Zanzonico P, Sharkey RM, et al. Bispecific antibody pretargeting PET (immunoPET) with an ^{124}I-labeled hapten-peptide. *J Nucl Med.* 2006;47:1678-1688.