Purification and Labeling Strategies for ^{68}Ga from ^{68}Ge/^{68}Ga Generator Eluate

Dirk Mueller, Ingo Klette and Richard P. Baum

Abstract

For successful labeling, ^{68}Ge/^{68}Ga generator eluate has to be concentrated (from 10 mL or more to less than 1 mL) and to be purified of metallic impurities, especially Fe(III), and ^{68}Ge breakthrough. Anionic, cationic and fractional elution methods are well known. We describe two new methods: (1) a combined cationic–anionic purification and (2) an easy-to-use and reliable cationic purification with NaCl solution. Using the first method, ^{68}Ga from 10 mL generator eluate was collected on a SCX cartridge, then eluted with 1.0 mL 5.5 M HCl directly on an anion exchanger (30 mg AG1X8). After drying with a stream of helium, ^{68}Ga was eluted with 0.4 mL water into the reaction vial. We provide as an example labeling of BPAMD. Using the second method, ^{68}Ga from 10 mL generator eluate was collected on a SCX cartridge, then eluted with a hydrochloric solution of sodium chloride (0.5 mL 5 M NaCl, 12.5 μL 5.5 M HCl) into the reaction vial, containing 40 μg DOTATOC and 0.5 mL 1 M ammonium acetate buffer pH 4.5. After heating for 7 min at 90°C, the reaction was finished. Radiochemical purity was higher than 95% without further purification. No ^{68}Ge breakthrough was found in the final product.

D. Mueller (✉) · I. Klette · R. P. Baum
Department of Nuclear Medicine, Centre for PETCT,
Zentralklinik Bad Berka, 99437 Bad Berka, Germany
e-mail: d.mueller.pet@zentralklinik-bad-berka.de

I. Klette
e-mail: ingo.klette@zentralklinik.de

R. P. Baum
e-mail: info@rpbaum.de

R. P. Baum and F. Rösch (eds.), *Theranostics, Gallium-68, and Other Radionuclides*, Recent Results in Cancer Research 194, DOI: 10.1007/978-3-642-27994-2_5,

Contents

1 Introduction

1.1 History

The first ^{68}Ga radiopharmaceuticals and ^{68}Ge/^{68}Ga radionuclide generators were developed at the beginning of the 1960s, in parallel with the creation of the first positron scintillation cameras, long before imaging with ^{18}F (Rösch 2011). The first ^{68}Ge/^{68}Ga generators were self-made by radiochemical laboratories, mostly being based on EDTA elution (Hnatowich 1975). ^{68}Ge breakthrough was relatively high, as well as other metallic impurities. For a number of years now, a new type of ^{68}Ge/^{68}Ga generator has been commercially available (Obninsk, Russia), followed by diverse improvements from other manufacturers as well.

1.2 Current State

Molecular imaging of tumors by PET/CT using peptides radiolabeled for receptors is on the rise (Prasad and Baum 2010; Baum et al. 2010; Sainz-Esteban et al. 2010). The popularity of ^{68}Ga-labeled radiopharmaceuticals is rising due to their utility for molecular imaging, as well as the potential for on-demand production of ^{68}Ga for radiolabeling in the absence of cyclotron facilities. As generator technologies have matured to more consistently deliver high-purity ^{68}Ga over many months, the availability of highly adaptable postprocessing radiochemistry modules has also increased.

These developments suggest a rich future for generator-produced ^{68}Ga as an attractive candidate for radiolabeling of not only peptides but also other molecular targeting vectors such as carbohydrates, proteins, and oligonucleotides (Tolmachev et al. 2011; Fellner et al. 2010). Numerous methods for ^{68}Ga generator elution, purification, and radiolabeling of peptide radiopharmaceuticals have been advanced, being primarily based on three thematic ^{68}Ga eluate purification procedures found in the literature.

1.3 Basic Methods

1.3.1 Anionic Purification

In the first of these basic methods, Meyer et al. described ^{68}Ga labeling using an anionic purification step. In this procedure, ^{68}Ga chloride in the ^{68}Ge/^{68}Ga generator eluate is converted to the ^{68}Ga gallate anion by addition of concentrated HCl and then trapped on an anionic exchanger cartridge. After drying the cartridge with a stream of inert gas or air, the ^{68}Ga is eluted with water into the reaction vessel with chelator-modified peptide (Meyer et al. 2004). Although the obtained ^{68}Ga eluate has high chemical purity, an alternative to the intermediate conversion step using concentrated HCl may have advantages.

1.3.2 Cationic Purification

In a second approach, a labeling procedure based on a purification step of the ^{68}Ga eluate using a cation exchange resin was described by Zhernosekov et al. (2007). In this method, ^{68}Ga eluted from the generator is trapped by a cation exchange resin and then washed and eluted with two different hydrochloric acid/acetone solutions. The majority of the acetone is removed during the radiolabeling step, which is carried out at 100°C. This method has formed the basis for numerous effective strategies for purification of ^{68}Ga generator eluates and radiolabeling of peptides. To ensure that the final product is of sufficient radiochemical purity (>95%), a variation on this basic approach to ^{68}Ga purification and radiolabeling applies the same principles (Meyer et al. 2004) but includes also a final product purification using a C-18 cartridge, from which the final product is extracted with ethanol. The use of acetone/HCl may lead to detectable presence of mesityl oxide (4-methyl-3-penten-2-on) in the final product, although this can be avoided by using fresh preparations of the acetone/HCl eluent.

1.3.3 Fractional Elution

The third fundamental approach to ^{68}Ga labeling procedures uses the generator eluate directly based on fractional elution of the ^{68}Ge/^{68}Ga generator. In this method, early and late fractions of the 0.1 M HCl eluent containing ^{68}Ga are discarded, and an intermediate volume of eluted ^{68}Ga is collected and buffered to an appropriate pH for radiolabeling (e.g., with HEPES). Theoretically, a significant fraction of impurities are removed in early fractions of eluent (Breeman et al. 2005),

and the fraction of eluent containing the highest concentration of pure ^{68}Ga is used for preparation of the radiopharmaceutical. Following the radiolabeling step, impurities such as free ^{68}Ga are removed by use of a C-18 cartridge and extraction with ethanol (as described above). The potential disadvantages of this method lie in that the procedure uses only a fraction of the elutable ^{68}Ga activity, which may reduce the achievable specific activity of the final radiopharmaceutical product, as well as the need for gas chromatographic analysis to establish the concentration of ethanol as an excipient in the final product.

2 Combined Cationic–Anionic Purification of ^{68}Ge/^{68}Ga Generator Eluate for Labeling of Fragile Peptides and Proteins

2.1 Aim

The combination of cationic and anionic purification of ^{68}Ga eluate should reduce the amount of concentrated HCl as well as the eluate volume. On the other hand, in the case of larger and fragile peptides or proteins such as DOTA-conjugated Affibody, the cationic concentration with the help of acetonic HCl followed by labeling with ^{68}Ga (Zhernosekov et al. 2007) requires high temperature to evaporate the acetone. The basic idea for this procedure is trapping of ^{68}Ga using a cation exchanger in a first step followed by elution of the precleaned ^{68}Ga with a minimal amount of 5.5 M HCl. The resulting ^{68}Ga tetrachlorogallate can then be collected using an anion exchanger in a second purification step (Mueller et al. 2009). This purification procedure for the ^{68}Ge/^{68}Ga eluate also eliminates acetone or other organic solvents.

2.2 Description

The ^{68}Ge/^{68}Ga generator was eluted with a total of 10 mL 0.1 M HCl, and the ^{68}Ga was collected on a SCX cartridge (Varian, Bond Elut-SCX, 100 mg, 1 mL). The activity was then eluted with 1.0 mL 5.5 M HCl and directly collected again on an anion exchanger cartridge (AG1X8, 30 mg, preconditioned with 1 mL 5.5 M HCl). After drying of the cartridge with a stream of helium (or air) for 1 min, 60% of the ^{68}Ga was eluted with 0.4–1.0 mL water into the reaction vial with 0.4 mL 1.5 M HEPES buffer and 100 μg DOTA-Affibody (DOTA-$Z_{Her2:342\text{-}pep2}$). The reaction mixture was heated to 80°C for 5 min.

For the anionic purification step, the authors developed a combined cartridge. For this purpose a 100 mg SAX cartridge (Alltech SAX Extract Clean SAX, 100 mg, 1.5 mL) was covered with 70 mg AG1X8 (BIO-RAD, 200–400 Mesh). If this cartridge is used, up to 80% of ^{68}Ga is elutable.

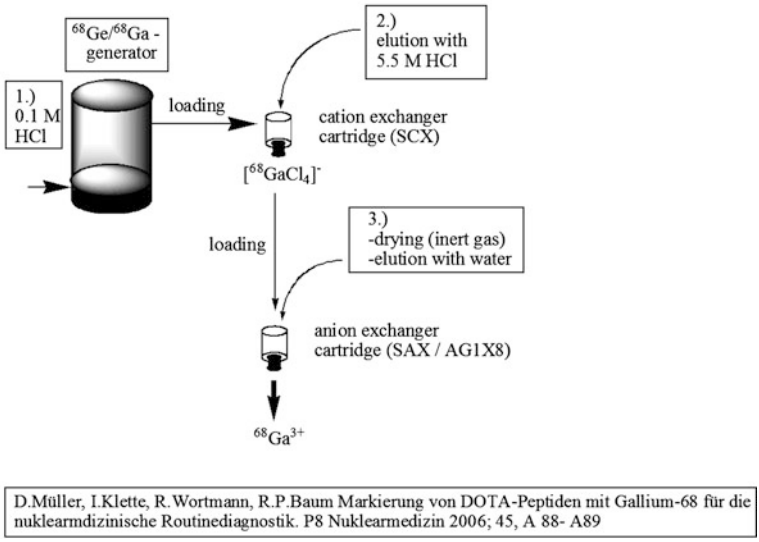

Fig. 1 Purification procedure, schematic drawing

2.3 Advantages

This procedure leads to a final product with radiochemical purity greater than 95% without further purification steps. The described purification delivers ^{68}Ga in high chemical and radiochemical purity. Use of acetone or ethanol during the labeling procedure is not necessary, so that GC investigation is not essential for release. This method allows labeling of fragile peptides with molecular mass higher than DOTA-D-Phe1-Tyr3-octreotide (DOTATOC) such as DOTA-Affibody (DOTA-$Z_{Her2:342\text{-}pep2}$) or proteins (Mueller et al. 2006, 2009) (Figs. 1, 2, 3).

2.4 Example: Synthesis of ^{68}Ga-BPAMD

2.4.1 Introduction

For the ^{68}Ga-labeled bisphosphonate monoamide derivative of DOTA (BPAMD) for patient studies, the combined cationic/anionic ^{68}Ga eluent purification was successfully used in our department. The widely used cationic labeling procedure (Zhernosekov et al. 2007) leads to a nonphysiologic final product with pH lower than 2. Neutralization of the final product with sodium hydrogencarbonate (8.4%) is not possible, because of the decomposition of ^{68}Ga-BPAMD. Furthermore, the final product contains nonreacted and free $^{68}Ga^{3+}$ in different concentrations, so that a purification step is necessary. The combined purification method avoids these problems.

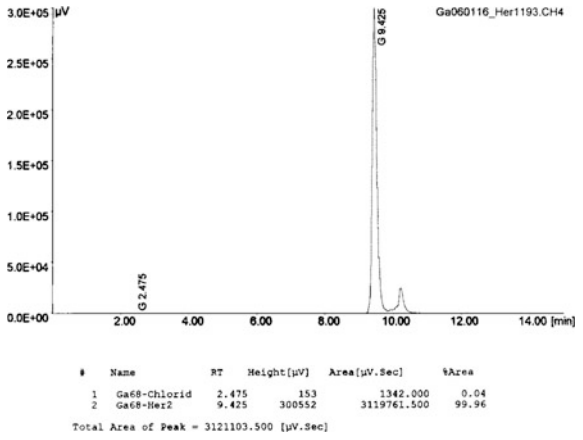

Fig. 2 HPLC of the final product. Column: RP-18, LiChroCART 250-4, LiChrospher 100, RP-18e (5 µm); solvent A: acetonitrile solution in water (5%), 0.1% TFA; solvent B: 95% acetonitrile solution in water, 0.1% TFA; flow rate: 1.2 mL/min; gradient: from 0–2 min 100% A, 3–15 min to 100% B. $^{68}GaCl_3$: (R_t = 2.5 min) 0.04%, ^{68}Ga-Affibody: (R_t = 9.4 min) 99.96%

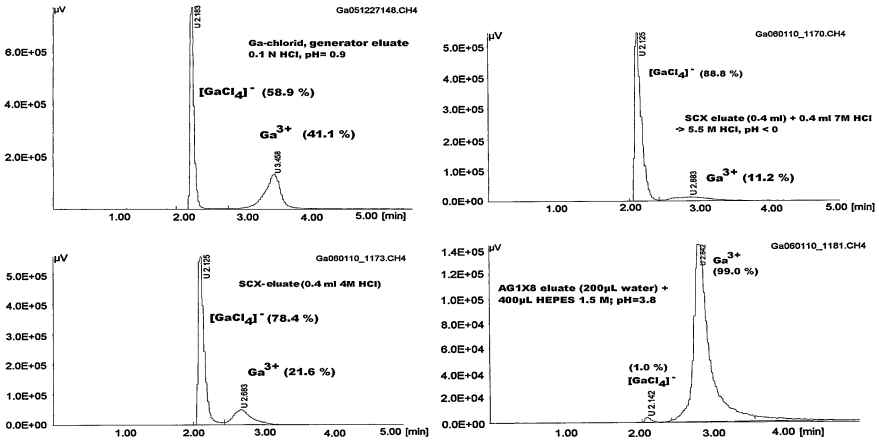

Fig. 3 HPLC of $^{68}GaCl_3$ samples at different HCl concentrations. Column RP-18, LiChroCART 250-4, LiChrospher 100, RP-18e (5 µm); solvent A: acetonitrile solution in water (5%), 0.1% TFA; solvent B: 95% acetonitrile solution in water, 0.1% TFA; flow rate: 1.2 mL/min; gradient: from 0–2 min 100% A, 3–5 min to 30% B

2.4.2 Labeling Procedure

The ^{68}Ga generator was eluted with a total of 10 mL 0.1 M HCl, and the ^{68}Ga was collected on a SCX cartridge (Varian, Bond Elut-SCX, 100 mg, 1 mL). This cartridge was then eluted with 5.5 M HCl directly through an anion exchanger cartridge.

For this anionic purification step we used a combined SAX cartridge (Alltech SAX Extract Clean SAX, 100 mg/1.5 mL), covered with 70 mg AG1X8 (BIO-RAD,

Fig. 4 TLC of sterile filtered reaction solution synthesized by the combined cationic–anionic purification method (ITLC-SG; solvent: 0.1 M citric acid)

200–400 Mesh, hydroxide form), preconditioned with 1 mL 5.5 M HCl. The cartridge was dried with a stream of inert gas or air for 1 min, and ^{68}Ga was then eluted with 1 mL water followed by elution with 0.5 mL aqueous 1.5 M ammonium acetate solution into the reaction vial with 1 mL water, 0.5 mL aqueous 1.5 M ammonium acetate solution, and 20 μg BPAMD. Then, the reaction mixture was heated to 100°C for 12 min.

2.4.3 Results

The $^{68}Ga^{3+}$ was completely bonded, and after sterile filtration the radiochemical yield was about 55% (n.d.c.). This procedure leads to a final product with radiochemical purity greater than 95% without further purification steps. The pH of the final solution is about 4. Determination of other impurities by gas chromatography is not necessary. No organic solvents were added. The use of our developed combined SAX/AG1X8 cartridge increased the yield significantly. The instant thin layer chromatography (ITLC) quality control (Fig. 4) determines free $^{68}Ga^{3+}$ as well as potentially formed ^{68}Ga hydroxide.

We thereby developed a reproducible and applicable labeling procedure for synthesis of ^{68}Ga-BPAMD. The reaction delivers the product with radiochemical purity higher than 95%. Subsequent purification is not necessary, and the pH of the reaction solution is about 4.

3 A New Highly Efficient NaCl-Based Cationic ^{68}Ge/^{68}Ga Generator Eluate Purification: The Basis for Effective ^{68}Ga Labeling

3.1 Aim

The aim is to develop efficient ^{68}Ga labeling procedures for routine application in clinical practice. The purification procedure for the ^{68}Ge/^{68}Ga eluate should reduce handling with concentrated HCl, and should form the labeled final product in high

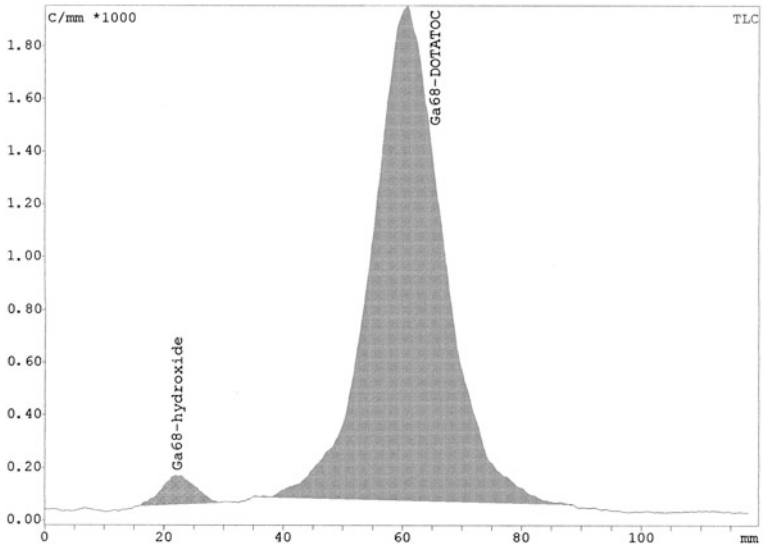

Fig. 5 ITLC-SG of the final reaction mixture; eluent: acetonitrile/water 1:1; ^{68}Ga-hydroxide/-chloride ($R_f = 0$): 2.4% ^{68}Ga-DOTATOC ($R_f = 0.5$): 97.6%

yield with high purity. Use of acetone or other organic solvents or compounds such as HEPES should be avoided. Handling should be reduced to a minimum of simple steps and should allow transfer to an automated system.

3.2 Labeling Strategy

Our strategy was influenced in part by the methods mentioned above, with the finer points of the new approach inspired by a reported procedure in which a rinse step for an OASIS WAX cartridge with 5 M NaCl solution was used to remove the 5.5 M HCl (de Blois et al. 2011). The use of only one ion exchange cartridge, but without use of organic solvents and high purification ability, were part of this method, combined with a search for a suitable buffer system. For DOTA-conjugated peptides, we exemplarily used DOTATOC.

3.3 Description of the Method

All reagents were purchased from commercial sources and used as received. The mentioned cartridges are commercially available. For all experiments a ^{68}Ga generator from Obninsk (Eckert and Ziegler Europe) and IGG100 ^{68}Ga generator (Eckert and Ziegler Europe) were used. The generator was eluted with 10 mL 0.1 M HCl (Merck, Germany). The ^{68}Ga of the generator eluate was collected on a SCX cartridge (VARIAN, Bond Elut-SCX, 100 mg, 1 mL; preconditioned with

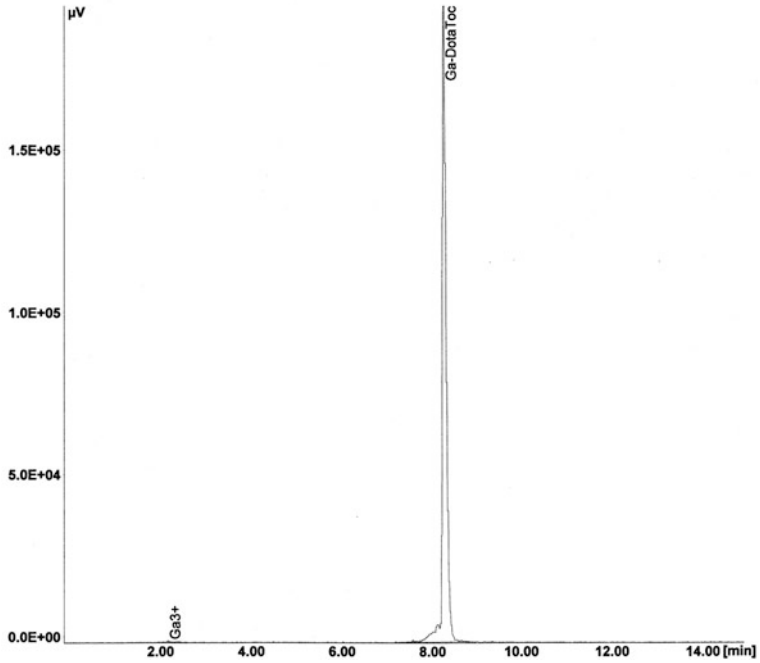

Fig. 6 HPLC of the final product. Column: RP-18, LiChroCART 250-4, LiChrospher 100, RP-18e (5 µm); solvent A: 5% acetonitrile in water, 0.1% TFA; solvent B: 95% acetonitrile in water, 0.1% TFA; flow rate: 1.2 mL/min; gradient: from 0–2 min 100% A, 3–15 min to 100% B. $^{68}Ga^{3+}$ (R_t = 2.3 min) 0.6%, ^{68}Ga-DOTATOC (R_t = 8.4 min): 99.4%

1 mL 5.5 M HCl, 10 mL water) and then eluted with hydrochloric solution of sodium chloride (0.5 mL 5.0 M NaCl, 12.5 µL 5.5 M HCl) into the reaction vial with 40 µg DOTATOC, 3 mL water, and 250 µL 1 M ammonium acetate buffer (3.9 g NH_4Ac, 50 mL water, 1 mL HCl conc., adjusted with acetic acid to pH 4.5). After heating the solution for 7 min at 90°C, the reaction was finished.

This method works very reliably on an automated synthesis module. The concentration of unbound ^{68}Ga is lower than 5%. The radiochemical purity of the labeled DOTATOC is higher than 95%. The reaction mixture contains no toxic substances or substances of concern, so subsequent purification is not required. After sterile filtration, the radiochemical yield is about 82% (n.d.c.).

3.4 Results

We developed a new, easy-to-handle, highly effective NaCl-based cationic method for ^{68}Ge/^{68}Ga generator eluate purification. With this procedure, the radiochemical yield for labeling of DOTATOC with ^{68}Ga is about 82%. A subsequent purification

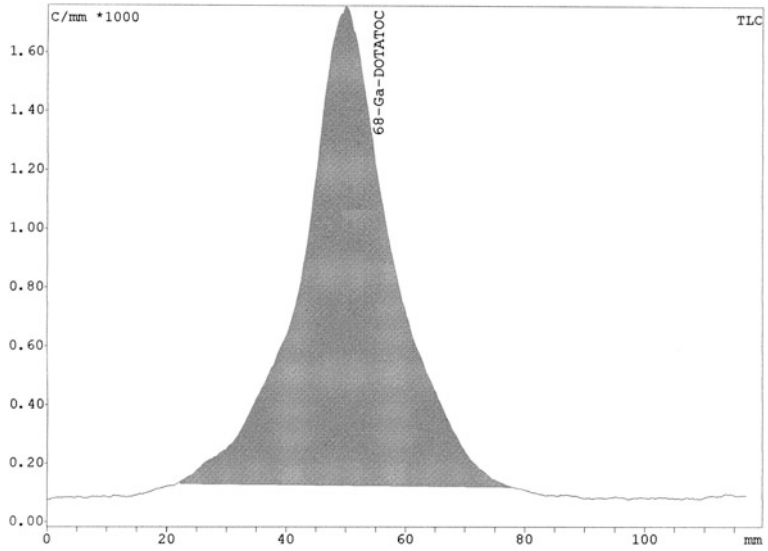

Fig. 7 ITLC-SG of the final product after sterile filtration; eluent: acetonitrile/water 1:1

step is not necessary. The final solution contains no organic solvents, and a GC investigation is not essential for release (Figs. 5, 6, 7).

References

Baum RP, Prasad V, Müller D, Schuchardt C, Orlova A, Wennborg A, Tolmachev V, Feldwisch J (2010) Molecular imaging of HER2-expressing malignant tumors in breast cancer patients using synthetic 111In- or 68 Ga-labeled affibody molecules. J Nucl Med 51:892–897

Breeman WA, de Jong M, de Blois E, Bernard BF, Konijnenberg M, Krenning EP (2005) Radiolabeling DOTA-peptides with 68 Ga. Eur J Nucl Med Mol Imaging 32:478–485

de Blois E, Sze Chan H, Naidoo C, Prince D, Krenning EP, Breeman WA (2011) Characteristics of SnO_2 based $^{68}Ge/^{68}Ga$ generator and aspects of radiolabeling DOTA-peptides. Appl Radiat Isot 69:308–315

Fellner M, Baum RP, Kubcek V, Hermann P, Lukes I, Prasad VFR (2010) PET/CT imaging of osteoblastic bone metastases with 68 Ga-bisphosphonates: first human study. Eur J Nucl Med Mol Imaging 37:834

Hnatowich DJ (1975) A method for the preparation and quality control of ^{68}Ga radiopharmaceuticals. J Nucl Med 16:764

Meyer GJ, Mäcke H, Schuhmacher J, Knapp WH, Hofmann M (2004) 68 Ga-labelled DOTA- derivatised peptide ligands. Eur J Nucl Med Mol Imaging 31:1097–1104

Mueller D, Klette I, Wortmann R, Baum RP (2006) Markierung von DOTA-peptiden mit gallium- 68 für die nuklearmedizinische Routinediagnostik. Nuklearmedizin 45:A88–A89

Mueller D, Klette I, Gottschaldt M, Baum RP (2009) Radiolabeling of fragile macroligands with ^{68}Ga. J Labelled Compd Radiopharm 52:477

Prasad V, Baum RP (2010) Biodistribution of the Ga-68 labeled somatostatin analogue DOTA- NOC in patients with neuroendocrine tumors: characterization of uptake in normal organs and tumor lesions. Q J Nucl Med Mol Imaging 54:61–67

Rösch F (2011) ^{68}Ge/^{68}Ga- generators – past, present and future. World J Nucl Med 10:26
Sainz-Esteban A, Prasad V, Baum RP (2010) Interesting image. Pancreatic neuroendocrine tumor with involvement of the inferior mesenteric vein diagnosed by Ga-68 DOTA-TATE PET/CT. Clin Nucl Med 35:40–41
Tolmachev V, Altai M, Sandström M, Perols A, Eriksson Karlström AH, Boschetti F, Orlova A (2011) Evaluation of a maleimido derivative of NOTA for site-specific labeling of affibody molecules. Bioconjug Chem 22:894–902
Zhernosekov KP, Filosofov DV, Baum RP, Aschoff P, Bihl H, Razbash AA, Jahn M, Jennewein M, Rösch F (2007) Processing of generator produced 68 Ga for medical application. J Nucl Med 48:1741–1748