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Good practices for ^{68}Ga radiopharmaceutical production

Bryce J. B. Nelson[1], Jan D. Andersson[1,2], Frank Wuest[1] and Sarah Spreckelmeyer[3*]

*Correspondence:
sarah.spreckelmeyer@charite.de

[1] Department of Oncology, University of Alberta, 11560 University Avenue, Edmonton, AB T6G 1Z2, Canada
[2] Edmonton Radiopharmaceutical Center, Alberta Health Services, 11560 University Ave, Edmonton, AB T6G 1Z2, Canada
[3] Department of Nuclear Medicine, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt Universität Zu Berlin, and Berlin Institute of Health, Augustenburger Platz 1, 13353 Berlin, Germany

Abstract

Background: The radiometal gallium-68 (^{68}Ga) is increasingly used in diagnostic positron emission tomography (PET), with ^{68}Ga-labeled radiopharmaceuticals developed as potential higher-resolution imaging alternatives to traditional ^{99m}Tc agents. In precision medicine, PET applications of ^{68}Ga are widespread, with ^{68}Ga radiolabeled to a variety of radiotracers that evaluate perfusion and organ function, and target specific biomarkers found on tumor lesions such as prostate-specific membrane antigen, somatostatin, fibroblast activation protein, bombesin, and melanocortin.

Main body: These ^{68}Ga radiopharmaceuticals include agents such as [^{68}Ga]Ga-macroaggregated albumin for myocardial perfusion evaluation, [^{68}Ga]Ga-PLED for assessing renal function, [^{68}Ga]Ga-*t*-butyl-HBED for assessing liver function, and [^{68}Ga]Ga-PSMA for tumor imaging. The short half-life, favourable nuclear decay properties, ease of radiolabeling, and convenient availability through germanium-68 (^{68}Ge) generators and cyclotron production routes strongly positions ^{68}Ga for continued growth in clinical deployment. This progress motivates the development of a set of common guidelines and standards for the ^{68}Ga radiopharmaceutical community, and recommendations for centers interested in establishing ^{68}Ga radiopharmaceutical production.

Conclusion: This review outlines important aspects of ^{68}Ga radiopharmacy, including ^{68}Ga production routes using a ^{68}Ge/^{68}Ga generator or medical cyclotron, standardized ^{68}Ga radiolabeling methods, quality control procedures for clinical ^{68}Ga radiopharmaceuticals, and suggested best practices for centers with established or upcoming ^{68}Ga radiopharmaceutical production. Finally, an outlook on ^{68}Ga radiopharmaceuticals is presented to highlight potential challenges and opportunities facing the community.

Keywords: ^{68}Ga-radiolabeling, Gallium-68, Automation, Cyclotron, Radiolabeling, ^{68}Ga-tracer, Radiopharmaceuticals

Background

The rise and increasingly widespread clinical use of positron emission tomography (PET) imaging with gallium-68 (^{68}Ga) radiopharmaceuticals motivates providing guidance on aspects of ^{68}Ga radiopharmaceutical production to aid the community in achieving consistent quality and reliable yields. Radiogallium isotopes have been extensively investigated, starting when gallium was first observed to accumulate at osteogenic activity in the late 1940s (Hayes 1978). Early clinical trials using reactor-produced ^{72}Ga ($t_{1/2} = 14.1$ h) for therapy and diagnostic evaluation of malignant bone lesions were

ineffective, with investigation largely stopping by 1952 due to unsatisfactory patient benefits (Hayes 1978). A primary factor contributing to the negative diagnostic results was the poor detection equipment available at the time, while any further attempts exploit ^{72}Ga for therapy would have been limited by the high energy and intensity beta particle and gamma ray emissions depositing excess radiation dose in healthy tissue surrounding the tumor sites. Subsequently, accelerator-produced ^{67}Ga ($t_{1/2}$ = 3.3 d) was investigated for clinical use, and determined to be an effective tumor and abscess locating agent, with annual usage reaching nearly 250,000 patients by 1977 (Hayes 1978). In 1961, the first ^{68}Ga generator system was developed, using decay of germanium-68 (^{68}Ge) to provide a continuous supply of ^{68}Ga for clinical studies (Gleason 1960). ^{68}Ga was viewed as particularly attractive due to its short half-life permitting large activities to be administered for diagnostic imaging, with its rapid decay and clearance preventing excess patient radiation dose. Additionally, ^{68}Ga nuclear decay exhibits a high positron branching ratio (88.9%) with minimal co-emitted gamma rays, positioning it favorably compared to other radiometals with respect to dose (https://www.nndc.bnl.gov/nudat2/reCenter.jsp?z=56&n=77). Alongside advances in ^{67}Ga, ^{68}Ga was initially considered for potential use in PET imaging, however there was insufficient instrumentation at the time to achieve this application. The advent of ^{99m}Tc for single photon emission computed tomography (SPECT) imaging and ^{18}F for PET imaging delayed the application of ^{68}Ga diagnostic imaging owing to widespread ^{99m}Tc generator commercial distribution, and the longer half-life of ^{18}F compared to ^{68}Ga providing ease of production and clinical application. Additionally, early ^{68}Ge/^{68}Ga generators precluded direct radiolabeling by providing ^{68}Ga eluate complexed with EDTA, further slowing the development and utilization of ^{68}Ga radiopharmaceuticals (Banerjee and Pomper 2013). With the recent emergence of more advanced PET cameras, and the next generation of GMP-grade commercially available ^{68}Ge/^{68}Ga generators that reliably provide ^{68}Ga in chemically convenient dilute hydrochloric acid, ^{68}Ga use for research and clinical application became more widespread. Development and production of many ^{68}Ga radiopharmaceuticals ensued for various purposes including myocardial perfusion, renal and liver function, and tumor imaging. Somatostatin (DOTATOC/DOTATATE/DOTANOC) (Bauwens et al. 2010; Decristoforo et al. 2007), prostate-specific membrane antigen (PSMA) (Fuscaldi et al. 2021; Hennrich and Eder 2021), fibroblast activation protein (FAP) (Spreckelmeyer et al. 2020; Loktev et al. 2018), bombesin (Schuhmacher et al. 2005; Richter et al. 2016) and melanocortin 1 (Froidevaux et al. 2004) targeting ^{68}Ga radiotracers have been developed (Fig. 1), with their pharmacokinetics often well matched to the short physical half-life of ^{68}Ga (Banerjee and Pomper 2013).

With an increasing number of centers using ^{68}Ga on a regular basis for research and clinical application, several challenges have been maintaining consistency of reported parameters and providing sufficient process information for preclinical and production data of new ^{68}Ga radiopharmaceuticals. This review will present a set of common guidelines and standards would be useful for the ^{68}Ga community to report data in a uniform and reliable format. This review aims to outline key aspects of ^{68}Ga radiopharmacy, including means of ^{68}Ga production and purification via ^{68}Ge/^{68}Ga generators or medical cyclotrons, standard techniques for radiolabeling compounds with ^{68}Ga, and established quality control procedures for clinical grade ^{68}Ga radiopharmaceuticals. It

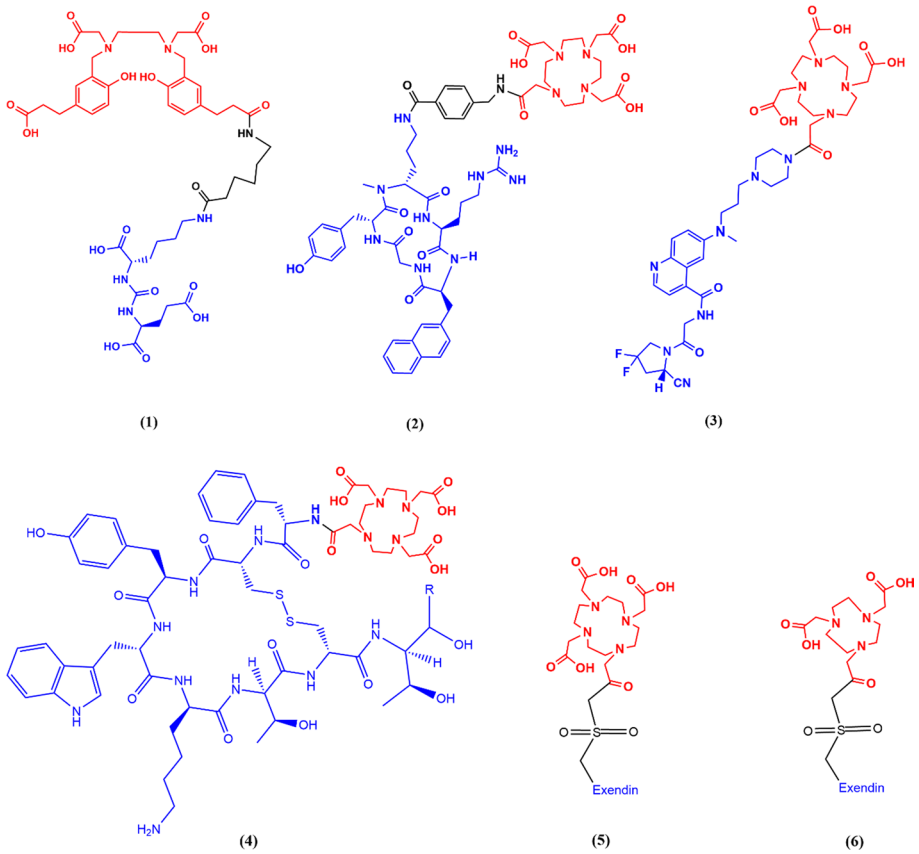

Fig. 1 Structures of several ^{68}Ga radiopharmaceuticals in clinical use (**1**) PSMA-11 (Fuscaldi et al.2021; Hennrich and Eder 2021) (**2**) PentixaFor (Sammartano et al. 2020; Spreckelmeyer et al. 2020) (**3**) FAPI-46 (Spreckelmeyer et al. 2020) (**4**) R = H DOTA-TOC (Bauwens et al. 2010; Decristoforo et al. 2007); R = Carbonyl DOTA-TATE (**5**) Exendin peptide sequence = HGEGTFTSDL SKQ M EEEAVR LFIEWLKNGG PSSGAPPPS C = Exendin-4-Cys40(DOTA) (Velikyan et al. 2017) (**6**) Exendin peptide sequence = HGEGTFTSDL SKQ M EEEAVR LFIEWLKNGG PSSGAPPPS K = Exendin-4-Lys40(NODAGA) (Velikyan et al. 2017; Migliari et al. 2021)

also suggests best practices for centers with existing or upcoming ^{68}Ga radiopharmaceutical production with respect to preparation of common ^{68}Ga tracers, and reporting key production parameters to the community. To conclude, an outlook on the future of ^{68}Ga radiopharmaceuticals is presented to highlight some of the upcoming challenges and opportunities presenting the community.

^{68}Ga production routes: generators and cyclotrons

^{68}Ga generator production

The most common method for obtaining ^{68}Ga is via a ^{68}Ge/^{68}Ga generator. Generators are convenient for many applications since the 270.93-day half-life of the parent nuclide, germanium-68 (^{68}Ge), guarantees an ongoing supply of ^{68}Ga sufficient for clinical use for up to a year. ^{68}Ga/^{68}Ge generators were first developed in the early 1960s, however early generators utilizing liquid–liquid extraction and EDTA eluant to obtain ^{68}Ga were not conducive to complex syntheses of ^{68}Ga radiopharmaceuticals, and the advent of ^{99m}Tc and ^{18}F radiopharmaceuticals slowed development of ^{68}Ga radiopharmaceuticals in the 1970s (Rösch 2013). Advances in radiochemistry led

to availability of new generators providing $^{68}Ga^{3+}$ in hydrochloric acid eluate (Razbash et al. 2005). The eluted ^{68}Ga, in the form of $[^{68}Ga]GaCl_3$, can be used for radiolabeling and has led to significant advances in ^{68}Ga chemistry and the development of targeted PET radiopharmaceuticals. Modern commercially available $^{68}Ge/^{68}Ga$ generators utilize TiO_2, SiO_2, CeO_2, or SnO_2 solid phase matrixes to provide $[^{68}Ga]GaCl_3$ by elution with dilute HCl while the mother ^{68}Ge radionuclide remains on the matrix (Table 1). ^{68}Ge content is less than 0.001% of ^{68}Ga eluate throughout the life of the generator, with the eluate containing minimal metallic impurities (Rösch 2013; Chakravarty et al. 2016; Romero et al. 2020). A recent development is a 4.04 GBq $^{68}Ga/^{68}Ge$ generator, capable of producing significantly higher ^{68}Ga elution and drug product activities with a longer generator shelf-life compared to previous generators (Waterhouse et al. 2020). The ^{68}Ge generator parent radionuclide can be produced via several accelerator-based nuclear transformations, the most common being the $^{69}Ga(p,2n)^{68}Ge$ reaction. The cross section for this reaction peaks just under 20 MeV, which is within the range of many medical cyclotrons, however, to achieve reasonable commercial scale yields (> 37 GBq) irradiations of ^{69}Ga at 40–100 μA for several days are needed (IAEA PUB1436).

^{68}Ga can also be produced directly on the cyclotron via the $^{68}Zn(p,n)^{68}Ga$ nuclear reaction (Tieu et al. 2019; Alnahwi et al. 2020; Lin et al. 2018; Nelson et al. 2020; Thisgaard et al. 2021; Rodnick et al. 2020; Alves et al. 2017; Pandey et al. 2014, 2019; Riga et al. 2018; Jensen and Clark 2011), with various production routes and yields presented in Table 2. Depending on the production technique, cyclotron ^{68}Ga yields are typically one to several orders of magnitude greater than currently available $^{68}Ga/^{68}Ge$ generators. Significant development has been undertaken in the field of liquid targets for ^{68}Ga production. Aqueous solutions of isotopically enriched zinc-68 (^{68}Zn) were first subjected to proton bombardment in a regular niobium target mainly used for ^{18}F production (Jensen and Clark 2011) and later upgraded to use a niobium foil as a beam degrader, producing 1800 MBq at end of bombardment (EOB) (Riga et al. 2018). Subsequently, a modified target design using an aluminum foil as beam degrader was developed (Pandey et al. 2019) where zinc nitrate in nitric acid was irradiated at 20 μA, producing 9.85 ± 2.09 GBq at EOB. Alternatively, solid targets using electroplated or pressed metal ^{68}Zn powder have been used, where ^{68}Zn is electroplated or pressed onto metallic target backings. Post-irradiation, the metallic ^{68}Zn is dissolved for chemical separation and ^{68}Ga purification. An alternative target system combining irradiation and dissolution has recently been developed that aims to address the limitations of solid and liquid targetry.

Table 1 Commercially available $^{68}Ge/^{68}Ga$ generators

Manufacturer	GMP	Matrix	Elution	Size (GBq)
IRE Elit	Yes	TiO_2	0.1 M HCl	1.85
ITG	Yes	Octadecyl silica	0.05 M HCl	2/4.04 (Waterhouse et al. 2020)
Eckert & Ziegler	Yes	TiO_2	0.1 M HCl	3.7
iThemba Labs	No	SnO_2	0.6 M HCl	1.85
Obninsk Cyclotron Co Ltd	No	TiO_2	0.1 M HCl	3.7
Pars Isotopes	No	nano-SnO_2	1.0 M HCL	2.59 (Romero et al. 2020)

Table 2 Liquid and solid target ^{68}Ga cyclotron production routes

Target	Foil	Beam	Yield	References
[^{68}Zn]$ZnCl_2$	Niobium	15 MeV, 20 μA	1800 MBq EOB	Jensen and Clark (2011)
[^{68}Zn]$Zn(NO_3)_2$ (1.7 M) in HNO_3 (0.2 N)	Aluminum	14 MeV, 20 μA	192.5 ± 11.0 MBq/μA-hr EOB	Pandey et al. (2014)
[^{68}Zn]$Zn(NO_3)_2$ (1.7 M) in HNO_3 (0.2 N)	Niobium	12 MeV, 20 μA	4.3 ± 0.3 GBq	Riga et al. (2018)
1.4 M $^{68}Zn(NO_3)_2$ in 1.2 N HNO_3	Aluminum	14 MeV, 40 μA, 60 min	9.85 ± 2.09 GBq EOB	Pandey et al. (2019)
100 mg $^{68}Zn(NO_3)_2$	Niobium	14 MeV, 45 μA, 50 min	6 GBq EOB	Alves et al. (2017)
1.0 M $^{68}Zn(NO_3)_2$ in 0.3 N HNO_3	Niobium/Havar	14.3 MeV, 34 μA, 60 min	4.6 ± 0.4 GBq	Rodnick et al. (2020)
Pressed ^{68}Zn	Aluminum	13 MeV, 80 μA, 120 min	194 GBq EOB	Thisgaard et al. (2021)
Pressed ^{68}Zn	Aluminum	12.5 MeV, 30 μA, 73 min	37.5 GBq	Nelson et al. (2020)
Electrodeposited ^{68}Zn		14.5 MeV, 30 μA, 60 min	60.9 GBq	Lin et al. (2018)
Pressed ^{68}Zn		13 MeV, 35 μA, 90 min	145 GBq	Alnahwi et al. (2020)
Electrodeposited ^{68}Zn		14.5 MeV, 35 μA, 8.5 min	6.30 GBq	Tieu et al. (2019)

To effectively establish ^{68}Ga production, sites should select a liquid or solid target production route based upon their anticipated ^{68}Ga demand, available infrastructure, and existing technical expertise. The following sections outline the advantages and disadvantages of liquid and solid ^{68}Zn targetry and ^{68}Zn/^{68}Ga chemical separation techniques.

^{68}Ga solid and liquid cyclotron targetry

Liquid ^{68}Zn target solutions are prepared by dissolving isotopically enriched ^{68}Zn metal or ^{68}Zn oxide in nitric acid to produce [^{68}Zn]$Zn(NO_3)_2$ (Rodnick et al. 2020; Alves et al. 2017; Pandey et al. 2014, 2019; Riga et al. 2018). Alternatively, [^{68}Zn]$ZnCl_2$ can be employed (Jensen and Clark 2011), however [^{68}Zn]$Zn(NO_3)_2$ is preferred, as it was found that irradiating $ZnCl_2$ leads to a significant pressure buildup of hydrogen and oxygen resulting from beam-induced radiolysis of the target solution (Pandey et al. 2014). Target assemblies can utilize a combination of helium and water cooling to remove heat, with the target solution and cooling fluids separated by aluminum and niobium foils. Targets are typically irradiated at energies of 12–14 MeV up to 45 μA beam current (Rodnick et al. 2020; Alves et al. 2017; Pandey et al. 2014, 2019; Riga et al. 2018), with ^{68}Ga yields dependent on the target pressure and concentration of ^{68}Zn solution, yielding up to 9.85 GBq after a 60 min irradiation. While irradiating at higher beam energies increases ^{68}Ga yield, it increases production of the ^{67}Ga radionuclidic impurity, so irradiating at a lower energy of ~ 12 MeV improves radionuclidic purity through avoiding onset of the $^{68}Zn(p,2n)^{67}Ga$ reaction. However trace levels of undesired isotopic impurities (0.1% ^{66}Zn and 0.48% ^{67}Zn) present in highly enriched ^{68}Zn (99.3%) lead to unavoidable production of ^{66}Ga and ^{67}Ga from the $^{66}Zn(p,n)^{66}Ga$ and $^{67}Zn(p,n)^{67}Ga$ reactions, respectively (Nelson et al. 2020). To achieve higher beam currents on liquid targets, pressurized target assemblies are required due to cavitation of the target solution. Advantages of liquid targets include ease of solution loading and removal from the

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