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What's New for ^{68}Ga in the World of Molecular Imaging?

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Abstract

During the last decade, the utilization of 68Ga for the development of imaging agents has increased considerably due to the increased accessibility of ^{68}Ge/^{68}Ga generators, straightforward labeling procedures, and the recent approval of 68Ga-DOTATATE by the FDA for routine clinical practice. This article is intended to provide an overview on current imaging applications of 68Ga-based radiopharmaceuticals in terms of their clinical significance and the alternative solution to overcome the innate limitation from a 68Ge/68Ga generator.

Keywords: ^{68}Ga; Molecular Imaging; Radiopharmaceutical; Cyclotron

Introduction

Molecular imaging was first introduced in 2001 but its concept actually began way back in the 1950s when 131I was used to image recurrent thyroid carcinoma [1, 2]. As the term is defined as the visual representation, characterization, and quantification in biological processes at the cellular or molecular level of living organisms with negligible perturbation, molecular imaging can be considered as an extension of nuclear medicine in which radioactive compounds are used in diagnostic/theranostic purposes [3, 4].

Among all conventional imaging techniques, positron emission tomography (PET) has played a critical role in the field of molecular imaging over the last decade due to its capability of imaging quantification and superb sensitivity without the limitation in tissue penetration. While ^{11}C, ^{13}N, ^{15}O, and ^{18}F are commonly used as "standard PET radionuclides", the requirement of an onsite cyclotron or shipment from a closely located commercial source decreases the availability and flexibility of the radiopharmaceuticals labeled with these radionuclides. On the contrary, because of the increased accessibility of ^{68}Ge/^{68}Ga generators, straightforward labeling procedures, and the recent approval of ^{68}Ga-DOTATATE by the FDA for routine clinical practice [5], PET imaging with 68Ga-labeled tracers has seen a dramatic increase over the past five years.

Gallium-68 is a short-lived radionuclide (T1/2:68 min) and primarily decays through positron emission (87.94%) with a maximum energy of 1.9 MeV (mean energy: 0.89 MeV). Because cationic ^{68}Ga can form stable complexes with various chelators and macromolecules, this characteristic allows for the development of radiopharmaceutical kits. This article is intended to provide an overview on current imaging applications of ^{68}Ga-labeled tracers and recent breakthroughs in ^{68}Ga production.

^{68}Ga-radiopharmaceuticals for PET imaging

It is common to consider [18F]-fluorodeoxyglucose ([^{18}F]FDG)-PET as part of a routine post treatment surveillance tool; however, the clinical applications of 68Ga actually begin long before [^{18}F]FDG was introduced in 1978 [6] and was used to localize brain tumors [7]. Because 68Ga was initially required to be eluted in complex with ethylenediaminetetraacetic acid (EDTA) from early ^{68}Ga generators [8], destruction of the complex was needed before preparation of the radiopharmaceuticals could be done. Due to this time-consuming process, the radiochemical yields of ^{68}Ga-labeled molecules were poor and thus resulted in very limited clinical applications.

When Obninsk first made a commercially available ^{68}Ge/^{68}Ga generator that could be eluted by diluted hydrochloric acid to provide cationic ^{68}Ga in 1996 [9], it opened new possibilities of ^{68}Ga in preclinical and clinical applications. As cationic ^{68}Ga is able to form stable complexes with chelators (e.g. diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), etc.) that have already been widely used to the agents for magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT), the amount of ^{68}Ga related research has greatly increased since 2000, especially after the first successful clinical trial of ^{68}Ga-DOTATOC in 2001[10]. Currently most ^{68}Ga clinical studies can be found in the areas of neuroendocrine tumors (NET), prostate cancer, and infection/inflammation.

^{68}Ga-based radiopharmaceuticals for neuroendocrine tumors (NET)

Neuroendocrine tumors (NET) begin with a heterogeneous group of neoplasms that arise from cells of the endocrine and nervous systems. Although the population of patients with NET remains relatively small, recent epidemiologic study has shown an increasing incidence [11]. In addition to common laboratory tests and biopsy as typical diagnostic tools to confirm patients with NET, conventional imaging techniques such as computed

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tomography (CT), ultrasound (US), MRI, SPECT, and PET also play an important role in diagnosing and staging/restaging; however, only images from SPECT and PET are attributed to specific biological information (e.g. cell metabolic condition, particular receptor expression, etc.) of tumors.

The importance of somatostatin receptors (SSTR) as the target for imaging NET was recognized when Krenning et al. performed SSTR scintigraphy with more than 1,000 patients using either [^{123}I-Tyr^{3}]- or [^{111}In-DTPA-D-Phe^{1}]-octreotide (111In-octreoscan) [12], in which most NET carry a high density of somatostatin receptors (SSTR). Even though SSTR can be found in some normal tissues, an increased density of SSTR in NET make them visible with radiolabeled somatostatin or its analogues; however, due to several drawbacks of using ^{123}I, (such as a relatively time-consuming and difficult labeling procedure compared to ^{111}In), ^{111}In-octreoscan has consequently been broadly used to visualize NET expressing SSTR.

As PET has overall better spatial resolution than SPECT and the capability to perform quantitative analyses, the next generation of SSTR-targeted imaging for clinical applications was initiated by Hofmann et al. for ^{68}Ga-DOTATOC PET in 2001 [10]. Of note, the group found that tumor-to-normal tissue ratios based on ^{68}Ga-DOTATOC PET are superior to those achieved with ^{111}In-octreoscan SPECT (Figure 1), and more than 30% additional lesions were detected by 68Ga-DOTATOC PET. These encouraging results not only paved the way of this particular radiopharmaceutical to the clinical acceptance for imaging patients with NET, but also redefined the role of ^{68}Ga in modern nuclear medicine.

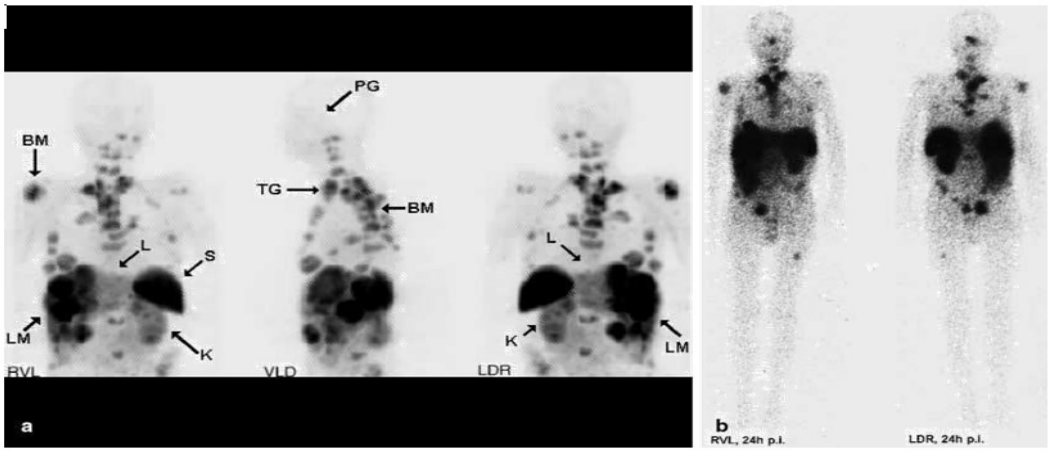

Figure 1:
a: Maximum-intensity-projections of a 54-year-old male patient, suffering from multiple liver, lung, abdominal and bone metastases of abdominal carcinoid. The scan was started 90 min following the intravenous injection of 220 MBq of ^{68}GaDOTATOC, and six bed positions were acquired. BM, Bone metastases; LM, liver metastases; K, kidney; S, spleen; L, liver; PG, pineal gland; TG, thyroid gland; VLD, anterior view; DRV, view from the right; LDR, posterior view.
b: The planar whole-body scintigraphy acquired 24 h after the injection of 110 MBq 111In-octreoscan does not reveal the true extent of liver involvement as visualization is impaired by intensive renal accumulation of the tracer. Multiple bone marrow and lung metastases are not as clearly delineated as in the ^{68}Ga-DOTATOC PET scan. RVL, Anterior view; LDR, posterior view. Reprinted with permission from European Journal of Nuclear Medicine, originally published by Hofmann et al [10].

Continuing the huge success of ^{68}Ga-DOTATOC, other octreotide-like analogues such as ^{68}Ga-DOTANOC and ^{68}Ga-DOTATATE (Figure 2) have also been studied extensively for imaging NET. The major difference among these tracers is their different binding affinities for the five subtypes of SSTR (SSTR1-5)[13]. A previous report indicates that ^{68}Ga-DOTATATE has a high affinity for SSTR2, whereas ^{68}Ga-DOTATOC binds to SSTR2 and SSTR5, and ^{68}Ga-DOTANOC to SSTR2, SSTR3 and SSTR5. Although over-expression levels of SSTR1-5 vary in different tumors, no significant impact on patient management with NET has been observed when applying these tracers in large clinical studies [14]. In June, 2016, the U.S. FDA approved the use of ^{68}Ga-DOTATATE as a PET diagnostic agent for patients with SSTR-positive NET [5].

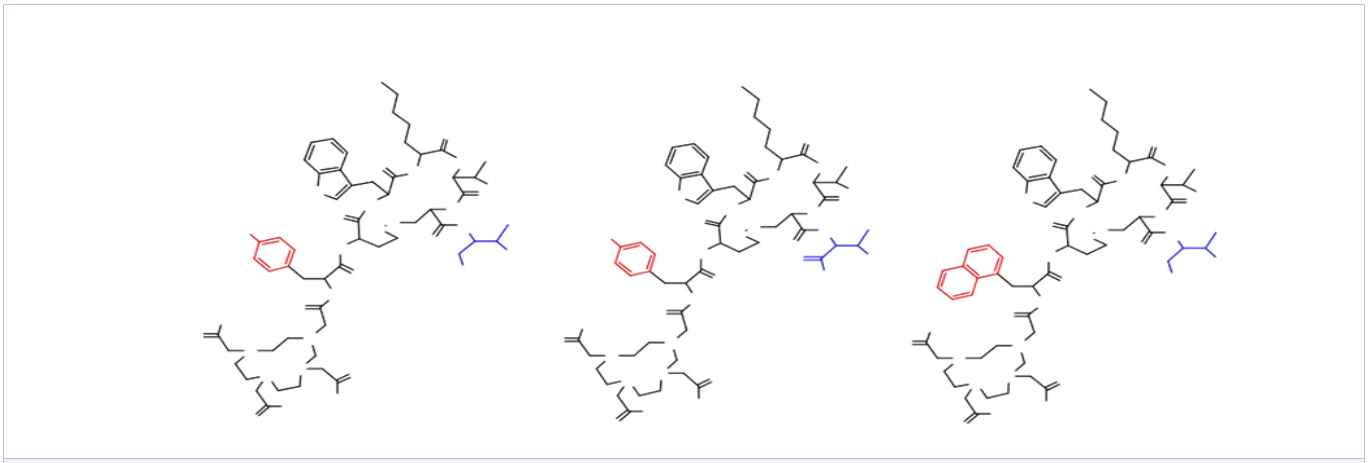

Figure 2: Chemical structures of ^{68}Ga-DOTATOC, ^{68}Ga-DOTATATE, and ^{68}Ga-DOTANOC.The differences in structures are highlighted

^{68}Ga-based radiopharmaceuticals for prostate cancer

Prostate cancer is the most common cancer in men in the United States [15] and the second most common cancer in men worldwide [16]. It is typically diagnosed with biopsy and its risk is classified by the concentration of prostate-specific antigen (PSA) in serum, Gleason score, and the clinical stage [17]. Conventional imaging of prostate cancer is currently limited in staging, restaging after cancer recurrence, and assessment for therapeutic response. Although structural imaging techniques such as MRI are advantageous in assessing intraprostatic progression of the disease, PET is superior in the detection of extraprostatic metastases.

^{11}C-choline and ^{18}F-fluciclovine are currently the major PET tracers used for the imaging of prostate cancer on a routine basis. It was not until Afshar-Oromieh et al. reported the first evaluation of Glu-urea-Lys-(Ahx)-[^{68}Ga(HBED-CC)] (^{68}Ga-PSMA-11) as a novel peptide-based PET tracer for prostate cancer that great attention began for applying ^{68}Ga for imaging prostate cancer [18]. Unlike ^{11}C/^{18}F-choline and ^{18}F-fluciclovine that differentiate tumor cells from normal tissues through involving biological metabolic pathways, the uptake of ^{68}Ga-PSMA-11 is proportional to the cellular expression level of prostate-specific membrane antigen (PSMA).

PSMA is a type II transmembrane glycoprotein that is expressed in all types of prostatic tissue. The expression of PSMA is low in normal prostatic tissue; however, its expression level increases in both localized and metastatic prostate cancer and highly correlates with tumor grade [19]. Because PSMA is also expressed in the neovasculature of many solid tumors other than prostate cancer, PSMA can be an excellent target not only for prostate cancer, but also for other malignancies [20]. The concept of utilizing PSMA as the target to reveal patients with prostate cancer and its metastases was first realized by using the ^{111}In-labeled monoclonal antibody, capromab pendetide (ProstaScint®, Cytogen Corporation, and Princeton, NJ). Nevertheless, the diagnostic capability of ProstaScint® in tumors within the prostate gland and seminal vesicles is very limited [21]. This is likely due to the fundamental issue of ProstaScint® that only binds to an intracellular domain of PSMA and fails to recognize viable cancer cells.

In order to overcome the fundamental issue that ProstaScint® has encountered, several small molecule-based PSMA inhibitors, differing slightly in chemical structure, have been developed to target external epitopes of PSMA with hopes of improving lesion detectability. Among these molecules, ^{68}Ga-PSMA-11 is perhaps the most widely used PET agent for imaging prostate cancer. Afshar-Oromieh et al. initiated the evaluation of ^{68}Ga-PSMA-11as a novel PET agent for prostate cancer [18]. The researchers found that ^{68}Ga-PSMA-11 detects recurrent and metastatic prostate cancer through binding to the extracellular domain of PSMA and through internalization of the compound/agent into the cell. Since then, multiple studies have been conducted to compare ^{68}Ga-PSMA-11 with other conventional PET tracers for imaging patients with prostate cancer.

A recent prospective study conducted by Caroli et al. [22], which involved 314 patients with recurrent prostate cancer, showed an overall positive ^{68}Ga-PSMA-11 PET/CT detection rate of 62.7%. The accuracy was increased with increasing PSA values: 42% for 0-0.2 ng/ml, 58% for 0.2-1 ng/ml, 75% for 1–2 ng/ml, and 94.8% for >2 ng/ml. In addition, the researchers found that of the 88 patients with negative ^{18}F-choline PET/CT scans, 59 (67%) were positive on ^{68}Ga-PSMA-11 PET/CT. Of these positive scans, 57% had a PSA value <2 ng/ml and 81% had a Gleason score of ≥7. In agreement to these findings, a positive ^{11}C-choline PET/CT detection rate was also reported with poor correlation when patients with a PSA value <2 ng/ml [23]. Furthermore, while a prospective trial is still underway, a preliminary comparison from 10 patients with recurrent prostate cancer also suggests improved detection rates for ^{68}Ga-PSMA-11 PET/CT when compared with ^{18}F-fluciclovine PET/CT[24] (Figure 3).

With encouraging results continuously shown in prospective studies and meta-analysis [22-29], several analogues based on PSMA-11 have also been evaluated as potential imaging agents for prostate cancer [30-34]. Among these molecules, PSMA-617 (Figure 4) has received the most clinical attention. Through pre-clinical studies, Benešová et al. observed that the binding

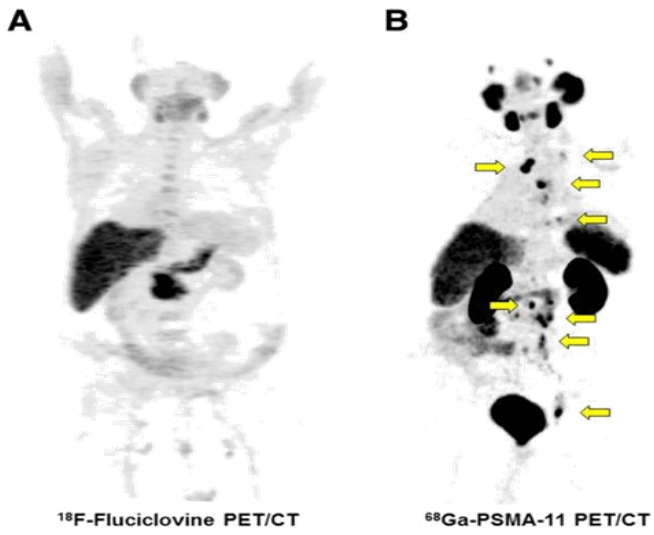

Figure 3: Maximum-intensity-projection of 18F-fluciclovine PET **A** and 68Ga-PSMA-11 PET **B** in a 74-year-old male patient. Arrows indicate intense uptake in pelvic, abdominal, thoracic, and supraclavicular LNs. Corresponding LNs on 18F-fluciclovine PET showed no uptake. Reprinted with permission from Journal of Nuclear Medicine, originally published by Calais et al.[24]

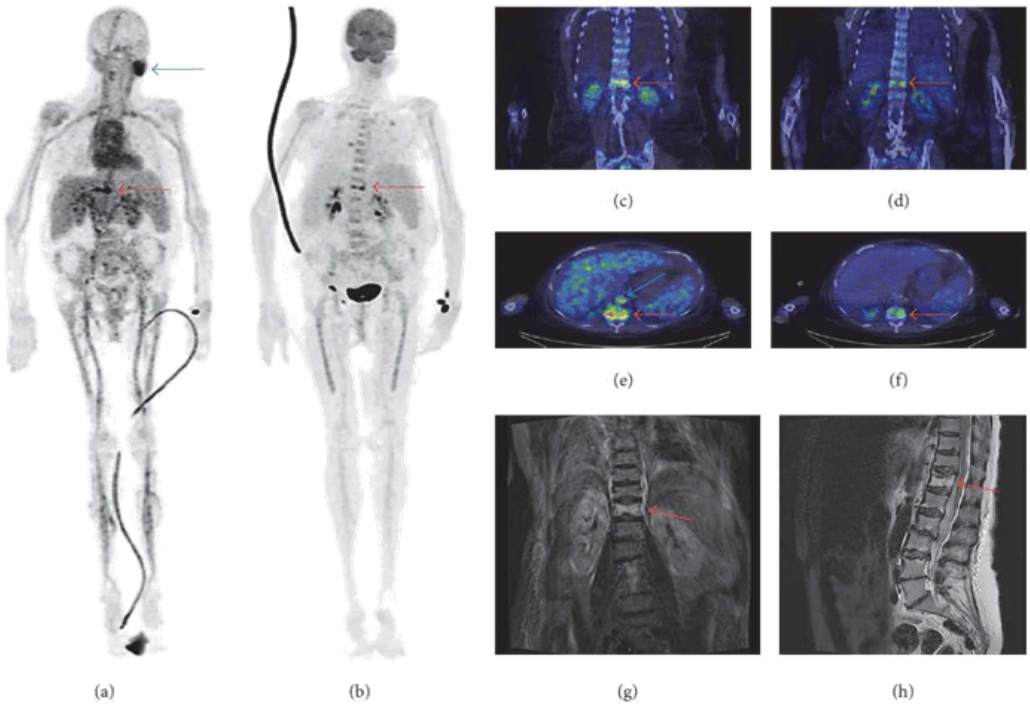

Figure 4: Patient was a 70-year-old woman (weight: 69 kg), with multiple background diseases, who was admitted to hospital because of back pain and high fever. Both ^{68}Ga-citrate (a, c, e) and 18F-FDG PET/CT (b, d, f) showed vertebral osteomyelitis (spondylodiscitis) in Th12 (red arrows) and pneumonia in both lungs. MRI showed oedema in Th12 (g, h). ^{68}Ga-citrate PET/CT also revealed uptake in the left parotid gland (unspecific; (a), blue arrow), neck lymph nodes (reactive), and inferior vena cava (thrombosis; (e), blue arrow). There was no ^{18}F-FDG uptake in these areas. The injected radioactivity dose of ^{18}F-FDG was 279 MBq and the PET acquisition started 50 min after injection. The injected radioactivity dose of ^{68}Ga-citrate was 199 MBq and the PET acquisition started 100 min after injection. MRI sequences were as follows: T2-weighted short inversion time inversion recovery (STIR) on the coronal view image (left) and T2-weighted on the sagittal view image (right). Reprinted with permission from Contrast Media & Molecular Imaging, originally published by Salomaki et al.[51]

affinity of PSMA-617 is significantly improved toward PSMA (Ki for PSMA-11: 12 ± 2.8 nM, PSMA-617: 2.3 ± 2.9 nM) as well as its internalized ratio into prostate cancer cells (internalized ratio for PSMA-11: 9.47% ± 2.56% injected activity/10^6LNCaP cells, PSMA-617: 17.67% ± 4.34% injected activity/10^6 LNCaP cells) [35]. As DOTA is used as a chelator for PSMA-617, this tracer can be labeled with ^{68}Ga, ^{111}In, 177Lu, 90Y thus served as a theranostic agent.; however, when comparing with 68Ga-PSMA-11, though both tracers showed the highest uptake in the kidneys and salivary glands [32, 36], the pharmacokinetics of ^{68}Ga-PSMA-617 is slower in patients. Because most nuclear medicine clinical workflow is designed to conduct PET/CT scans within 1 h after injection of ^{68}Ga-labeled tracers, it remains unclear if ^{68}Ga-PSMA-617, even with its higher PSMA binding affinity and internalization nature, could detect more prostate cancer lesions than ^{68}Ga-PSMA-11. Therefore, ^{68}Ga-PSMA-11 with its putatively faster clearance provides a clear advantage over ^{68}Ga-PSMA-617.

Besides imaging patients with recurrent prostate cancer, ^{68}Ga-PSMA-11 PET/CT can also be a valuable tool for evaluating primary prostate cancer and detecting lymph node and bone metastases. Kabasakal et al. imaged 28 prostate cancer patients with ^{68}Ga-PSMA-11 PET/CT either 5 or 60 min post injection and found that images acquired on the early time point (5 min p.i.) can help better distinguish between urinary bladder and tumor lesions [37]. Perveen et al. further confirmed this observation by performing dynamic ^{68}Ga-PSMA-11 PET/CT in 15 prostate cancer patients within a time frame of 1 to 10 min, and then compared these images to the static ones that were acquired between 45 and 60 min after injection from the same patients [38].

^{68}Ga-based radiopharmaceuticals for infection/inflammation

Infection is usually triggered by the invasion of disease-causing agents such as bacteria and virus. Inflammation is part of the immune response to harmful stimuli; however, because autoimmune (e.g. lupus Erythematosus, rheumatoid arthritis, and etc.) and cancerous diseases can both result in chronic inflammation, infection is not the only source that leads to inflammatory responses. It continues to be a major cause of morbidity and mortality worldwide [39].

X-ray, US, CT, and MRI are conventional imaging techniques to reveal infectious/inflammatory lesions. Although they are generally helpful, diagnoses based on these techniques are not specific to neither inflammation nor infection type since they heavily depend on the presence of anatomic abnormalities; however, nuclear medicine techniques that provide functional images hold great promise to evaluate disease progression before noticeable changes in anatomical structure [40].

In spite of strong medical need, the research and development of ^{68}Ga-labeled tracers for the diagnosis and discrimination of inflammation and infection was only accelerated during the past decade [41-45]. Among all published reports, ^{68}Ga-citrate seems to have a great potential of becoming a radiopharmaceutical for routine clinical practice.

The concept of using ^{68}Ga-citrate to image infections and inflammations was originated from the routine clinical application of ^{67}Ga-citrate. ^{67}Ga-citrate has been well known for decades as an infection/inflammation imaging agent and its production, quality control as well as its value in the evaluation of various infections has been well documented [46-50]. After the development of ^{68}Ge/^{68}Ga generators, the initial idea to produce ^{68}Ga-citrate was discarded since images are usually obtained after 24-72 h injection of ^{67}Ga-citrate which is far beyond the half-life of ^{68}Ga. After the first attempt by Nanni et al. to explore the possible advantages of ^{68}Ga-citrate PET/CT over ^{67}Ga-citrate SPECT for evaluating patients with infections of the bone [45], interest of using ^{68}Ga-citrate as an alternative infection/inflammation imaging agent began to rise. Salomäki et al. recently performed a direct comparison between ^{68}Ga-citrate and ^{18}F-FDG PET/CT for the detection of infectious foci in four consecutive patients with *Staphylococcus aureus* bacteraemia [51]. The researchers found that both tracers are comparable for the imaging of osteomyelitis (Figure 4). Encouraging results have also been reported by Vorster et al. when the group evaluated the use of ^{68}Ga-citrate in the imaging of patients with confirmed tuberculosis or pulmonary fibrosis [52]. These preliminary results demonstrate the potential of ^{68}Ga-citrate in the evaluation of various infectious and inflammatory diseases.

Other potential ^{68}Ga-based radiopharmaceuticals and research

Since 2008 major generator manufacturers (iThemba LABS and Eckert & Ziegler Radiopharma GmbH) entered the market, thus the utilization of ^{68}Ga for the development of imaging agents focused in oncology has considerably increased [9]. In addition to the scope of using ^{68}Ga in imaging NET, prostate cancer, and infection/inflammation, ^{68}Ga-labeled tracers have been developed to target various cells and their receptors. Some examples of ^{68}Ga-labeled tracers as potential imaging agents are summarized in Table 1.

Table 1: Examples of ^{68}Ga-based imaging agents investigated preclinically and clinically

Receptor/mechanism	Imaging agent	Disease/Target (Study Type)
Integrins	^{68}Ga-PRGD2	Lung cancer (clinical) [71], Rheumatoid arthritis (clinical)[72]
Glucagon-like peptide 1 receptor (GLP1R)	^{68}Ga-Exendin-4	Insulinoma (clinical) [73]
Hypoxia	^{68}Ga-HP-DO3A-nitroimidazole	Tumor hypoxia (preclinical)[74]
Cholecystokinin-2 (CCK2)	^{68}Ga-minigastrin	Tumor with overexpressed CCK2 (preclinical) [75, 76]
Chemokine receptor-4 (CXCR4)	^{68}Ga-Pentixafor	Glioblastoma (clinical) [77]
Melanocortin-1 receptor (MC1-R)	^{68}Ga-NAPamide	Melanoma (preclinical) [78]
Glycolysis	^{68}Ga-DOTA-2-deoxy-D-glucosamine	Tumor metabolism (preclinical) [79]
Neurotensin Receptor 1 (NTS1)	^{68}Ga-neurotensin peptides	Tumor with overexpressed NTS1 (preclinical) [80]
Human epidermal growth factor receptor 2 (HER2)	^{68}Ga-DTPA anti-HER2	Breast cancer (clinical) [81]
lymphatic pathways	^{68}Ga-nanocolloid	Prostate cancer (clinical) [82]
Gastrin-Releasing Peptide Receptors (GRPRs)	^{68}Ga-Bombesin analogues	Tumor with overexpressed with GRPRs (preclinical) [83]

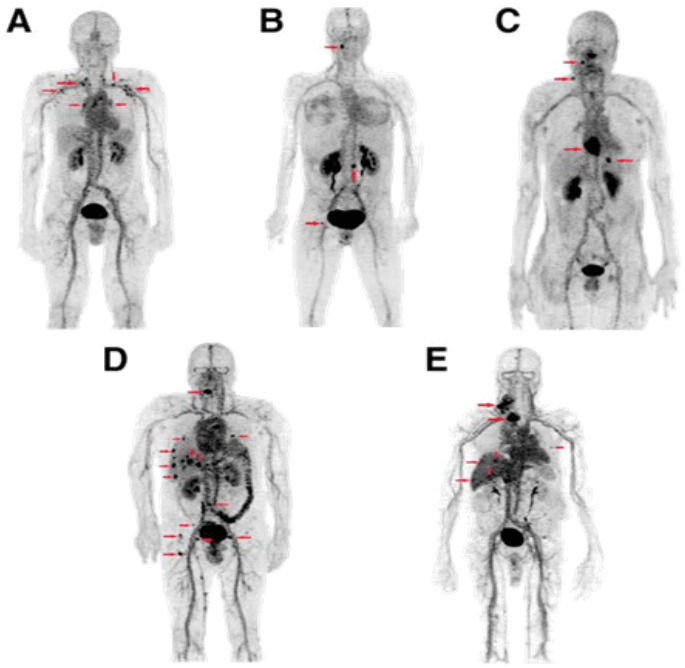

Figure 5: Maximum-intensity-projection of pre-targeted immuno-PET images recorded in one patient of each cohort using TF2 BsMAb and ^{68}Ga-IMP288 peptide. Arrows showed foci considered as pathologic by immuno-PET: supradiaphragmatic nodes in C1 (A) cervical node; lumbar and femoral bones foci in C2 (B); supradiaphragmatic nodes and liver and heart lesions in C3 (C); supradiaphragmatic nodes, lung, liver, and bone foci in C4 (D); and supradiaphragmatic nodes and liver foci in C5 (E). Reprinted with permission from Journal of Nuclear Medicine, originally published by Bodet-Milin et al.[55]

Other than the ^{68}Ga-labeled tracers that are developed to directly target particular cells or receptors, interest in the application of ^{68}Ga for pre-targeted imaging has also gradually emerged. The concept of pre-targeted imaging began as early as three decades ago and is comprised of two major steps: 1) a functionalized antibody that is first administered for target localization and clearance from blood and normal tissue and 2) a radiolabeled small molecule that is capable of binding to the functionalized antibody then administered [53, 54]. Considering the short half-life of ^{68}Ga, pre-targeted imaging provides an alternative way to overcome this limitation, especially when it comes to applying ^{68}Ga in immuno-PET imaging studies.

The first-in-human feasibility of ^{68}Ga-based pre-targeted imaging was recently demonstrated by Bodet-Milin et al. using the trivalent humanized TF2 bispecific monoclonal antibody (TF2 BsMAb) and ^{68}Ga-IMP288 peptide [55]. TF2BsMAb can specifically bind to carcinoembryonic antigen (CEA)-expressing tumor cells, histamine-succinyl-glycine (HSG) motif, and IMP288 which is the hapten molecule that comprises HSG motif through a DOTA chelator for complexation with ^{68}Ga. Although further studies are needed to compare with conventional imaging techniques, excellent tumor uptake and contrast have been obtained when using this strategy to image patients with recurrent medullary thyroid carcinoma (MTC) (Figure 5).

Current limitation of ^{68}Ge/^{68}Ga generators and production of 68Ga by cyclotron

The dramatic rise in PET imaging with ^{68}Ga-labeled tracers during the last decade has been a result of increased interest in applying ^{68}Ga-radiopharmaceuticals for theranostic purposes. The theranostic concept is when a diagnostic scan is first performed using a ^{68}Ga-labeled molecule then the same molecule is labeled with a therapeutic radionuclide (e.g. 90Y, 177Lu, or 188Re) for treatment [56-58]. Currently ^{68}Ga is commonly provided through ^{68}Ge/^{68}Ga generators. There are multiple methodologies developed to produce ^{68}Ge [59]. The production in general is sophisticated and time-consuming. Furthermore, commercially available ^{68}Ge/^{68}Ga generators nominally deliver up to 3.7 GBq (100 mCi) when fresh, but the lifespan of generators do not usually parallel with the long half-life of ^{68}Ge. This fact is a result of decreasing elution efficiencies of ^{68}Ga [60], which in turn lead to limited production and distribution of ^{68}Ga-labeled tracers to a few daily doses per generator. Importantly, the use of ^{68}Ge/^{68}Ga generators is inevitably accompanied by the concern of ^{68}Ge breakthrough in the eluate [60]. This parameter is known to increase as the generator ages. With these regards, the development of an alternative way to meet the increasing demand for ^{68}Ga is warranted.

Because of high cost and limited activity per elution from a ^{68}Ge/^{68}Ga generator, several attempts have been made to produce ^{68}Ga via ^{68}Zn(p,n) ^{68}Ga reaction using cyclotron. Jensen and Clark reported the first attempt to produce ^{68}Ga using a cyclotron with a liquid target filled with a ^{68}ZnCl2 solution [61]. Since then, other groups have tried to optimize 68Ga production through liquid targetry [62, 63]. Based on recent study performed by Alves et al., a liquid targetry containing 200 mg of ^{68}Zn resulted in 0.3 GBq/μA•h at End of Bombardment (EOB) when the target was irradiated by 14.2 MeV proton [62]. Although producing ^{68}Ga via a liquid target does ease the process of target preparation and avoid the need of target dissolution during separation procedure after cyclotron irradiation, the available activity of ^{68}Ga at end of processing is not significantly higher than generator produced isotope.

The possibility of producing ^{68}Ga with a solid target was first explored by Engle et al. using natZn as the target material [64]. Derived from cross section measurements, the theoretical production yield can be up to 5.81 GBq/μA•h when an enriched ^{68}Zn solid target is irradiated by 15 MeV proton [65]; however, high zinc and HCl acid contents in the final ^{68}Ga solution are two potential challenges for cyclotron-produced ^{68}Ga. Although most ^{68}Zn can be removed by using the AG50W cation resin alone [62-64], additional processing is usually required to reconstitute.

^{68}Ga solution with a low concentration of HCl for radiolabeling use. Considering the relatively short half-life of ^{68}Ga, a simple, fast, and cost-efficient processing procedure of ^{68}Ga post target irradiation is required.

At MD Anderson Cancer Center, we have recently developed a reliable and cost-efficient procedure for cyclotron production using solid target and chemical separation of ^{68}Ga [66]. To optimize target thickness for maximum yield, targets with a diameter of 7 mm and a mass of 68Zn between 60-120 mg were irradiated with 14.5 MeV protons. We found that the 68Ga activity at EOB correlated well with irradiation time and amount of ^{68}Zn metal present. Targets containing 104.1 ± 2.7 mg of 68Zn (n = 3) irradiated for 1 hr at a beam current of 30 μA resulted in 44.5 ± 1.4 GBq of ^{68}Ga with the production yield as 2.72 ± 0.08 GBq/μA•h, which is close to 10 times the value reported by Alves et al. using a liquid target [62]. In addition, by applying a dual-column system that was originally used for processing ^{68}Ga from a ^{68}Ge/68Ga generator [67], we are not only able to recover most of cyclotron-produced 68Ga activity (75-85%) and minimize zinc content (0.004 ± 0.002 μg/GBq) to the level that is lower than the generator-produced ^{68}Ga (0.37 ± 0.09 μg/GBq) determined by ICP-MS analysis, but also allows the reconstitution of the final ^{68}Ga solution in a low concentration of HCl at the same time. Most importantly, the whole separation process takes less than 10 min, which is especially important for ^{68}Ga production.

To explore the potential applications of cyclotron-produced ^{68}Ga in radiopharmaceutical compounding, we further used PSMA-11 as the model molecule for our labeling tests. In like fashion to the ^{68}Ge/^{68}Ga generator-produced ^{68}Ga, we found that the cyclotron-produced ^{68}Ga was also able to label these molecules directly in the range of curie level when the reaction was performed in FDA approved buffer solutions such as sodium acetate; however, because a large scale of activity (18.5-37 GBq) was used, decomposition of ^{68}Ga-PSMA-11 has been observed during or soon after the labeling reaction (Figure 6B). In order to overcome this issue that is likely caused by radiolysis, we added L-ascorbic acid as the free radical scavenger during the reaction

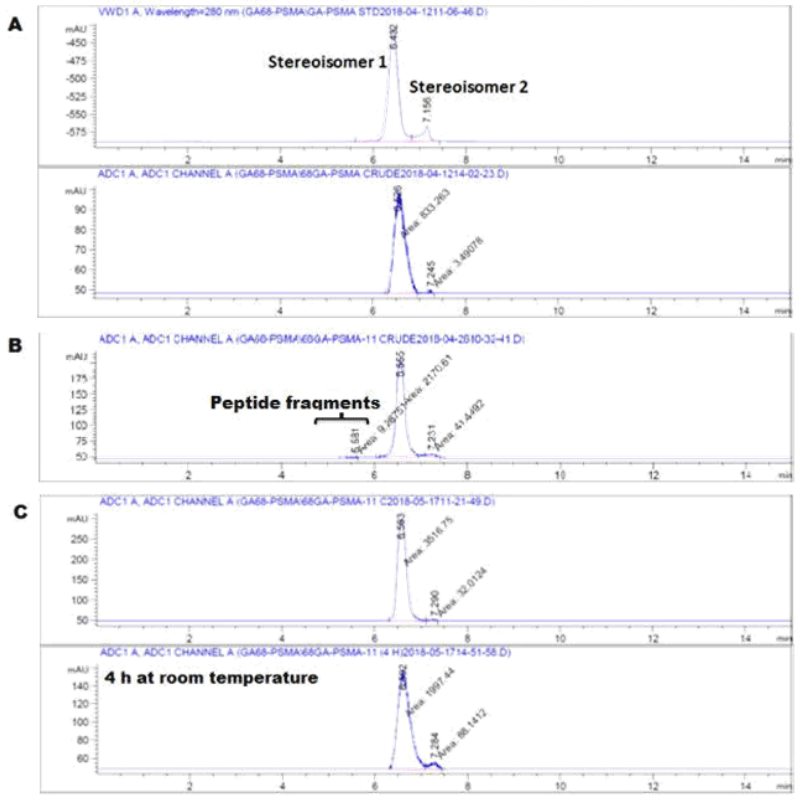

Figure 6: HPLC analyses of cold Ga-PSMA-11 and 68Ga-PSMA-11 using the activity (less than 1 GBq) from itG ^{68}Ge/68Ga generator A; ^{68}Ga-PSMA-11 using the activity (18.5-37 GBq) from cyclotron-produced ^{68}Ga B; 68Ga-PSMA-11 using the activity (18.5-37 GBq) from cyclotron-produced ^{68}Ga with L-ascorbic acid as the free radical scavenger C. ^{68}Ga-PSMA-11 remained intact after 4 h at room temperature

and found that ^{68}Ga-PSMA-11 remained intact after 4 h at room temperature (Figure 6C). Whereas the studies to demonstrate similarity between the compounds labeled by either ^{68}Ge/^{68}Ga generator or cyclotron-produced ^{68}Ga are currently underway, our preliminary data indicate the potential for large scale production of ^{68}Ga-labeled radiopharmaceuticals that can meet the increasing demand and facilitate regional distribution of these imaging agents.

Conclusions

The PET radiopharmaceuticals in nuclear medicine have gained a tremendous growth during the last decade as a result of considerable progress made in PET technologies and image analysis methodologies. In particular, ^{68}Ga-DOTATATE and ^{68}Ga-PSMA-11 are recognized to have great impact on the management of NET and prostate cancer, respectively; however, as more ^{68}Ga-labeled tracers have shown their promise in clinical application, streamlining the transition from bench to bedside becomes more important than ever. Whereas ^{68}Ga-DOTATOC (listed in European Pharmacopeia [68]) and ^{68}Ga-DOTATATE (approved by the FDA [5]) pave the way on the regulatory side for using ^{68}Ga in routine clinical settings, current ^{68}Ga-PET/CT imaging is still limited due to high cost and relatively small activity per elution from a ^{68}Ge/^{68}Ga generator.

Recently we have developed a reliable, on-demand and cost-effective method for the routine production of Curie amounts of ^{68}Ga with a medical cyclotron [66]. More importantly, our data suggests that the quality of cyclotron-produced ^{68}Ga is equal if not better when compared to that of its generator-produced counterpart. In addition, by successfully labeling PSMA-11 with our cyclotron-produced ^{68}Ga at 0.5-1.0 Ci level, we provide an alternative way to produce ^{68}Ga-labeled tracers not only for meeting the increasing market demand but also facilitating their regional distribution. Based on the recent report of ^{68}Ge/^{68}Ga generator licensing guidance from the Nuclear Regulatory Commission (NRC), we might be able to use cyclotron-produced ^{68}Ga to prepare radiopharmaceuticals under the same policy as ^{18}F (10 CFR 35.200) [69]. While further investigations are needed to meet possible regulatory requirements, minor metal impurities presented in the cyclotron-produced ^{68}Ga solution such as ^{67}Ga are likely to be reconciled through the specification of ^{67}Ga-citrate [70].

In this review article, we have summarized the application of ^{68}Ga-based radiopharmaceuticals in terms of their clinical significance and the alternative solution to overcome the innate limitation from a ^{68}Ge/^{68}Ga generator. As a direct result of the aforementioned and the superior performance of PET/CT compared to other conventional imaging techniques, we

anticipate that ^{68}Ga will play a significant role in the field of molecular imaging in the near future.

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