Cyclotron-Based Production of ^{68}Ga, $[^{68}Ga]GaCl_3$, and [^{68}Ga]Ga-PSMA-11 from a Liquid Target

Melissa E Rodnick
University of Michigan
Carina Sollert
GE Healthcare
Daniela Stark
Royal Prince Alfred Hospital
Mara Clark
University of Michigan
Andrew Katsifis
Royal Prince Alfred Hospital
Brian G Hockley
University of Michigan
D. Christian Parr
GE Healthcare
Jens Frigell
GE Healthcare
Bradford D Henderson
University of Michigan
Monica Abghari-Gerst
University of Michigan
Morand R Piert
University of Michigan
Michael J Fulham
Royal Prince Alfred Hospital
Stefan Eberl
Royal Prince Alfred Hospital
Katherine Gagnon
GE Healthcare
Peter J. H. Scott (pjhscott@umich.edu)
University of Michigan https://orcid.org/0000-0002-6505-0450

Research article

Keywords: Gallium-68, cyclotron targetry, positron emission tomography, PSMA

Posted Date: June 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38981/v1

Version of Record: A version of this preprint was published at EJNMMI Radiopharmacy and Chemistry on November 12th, 2020. See the published version at https://doi.org/10.1186/s41181-020-00106-9.

Abstract

Purpose: To optimize the direct production of ^{68}Ga on a cyclotron, via the ^{68}Zn(p,n)^{68}Ga reaction using a liquid cyclotron target. We Investigated the yield of cyclotron-produced ^{68}Ga, extraction of [^{68}Ga]$GaCl_3$ and subsequent [^{68}Ga]Ga-PSMA-11 labeling using an automated synthesis module.

Methods: Irradiations of a 1.0 M solution of [^{68}Zn]$Zn(NO_3)_2$ in dilute (0.2-0.3 M) HNO_3 were conducted using GE PETtrace cyclotrons and GE ^{68}Ga liquid targets. The proton beam energy was degraded to a nominal 14.3 MeV to minimize the co-production of ^{67}Ga through the ^{68}Zn(p,2n)^{67}Ga reaction without unduly compromising ^{68}Ga yields. We also evaluated the effects of varying beam times (50-75 min) and beam currents (27-40 μA). Crude ^{68}Ga production was measured. The extraction of [^{68}Ga]$GaCl_3$ was performed using a 2 column solid phase method on the GE FASTlab Developer platform. Extracted [^{68}Ga]$GaCl_3$ was used to label [^{68}Ga]Ga-PSMA-11 that was intended for clinical use.

Results: The decay corrected yield of ^{68}Ga at EOB was typically >3.7 GBq (100 mCi) for a 60 min beam, with irradiations of [^{68}Zn]$Zn(NO_3)_2$ at 0.3 M HNO_3. Target/chemistry performance was more consistent when compared with 0.2 M HNO_3. Radionuclidic purity of ^{68}Ga was typically >99.8% at EOB and met the requirements specified in the European Pharmacopoeia (<2% combined $^{66/67}$Ga) for a practical clinical product shelf-life. The activity yield of [^{68}Ga]$GaCl_3$ was typically >50% (~1.85 GBq, 50 mCi); yields improved as processes were optimized. Labeling yields for [^{68}Ga]Ga-PSMA-11 were near quantitative (~1.67GBq, 45mCi) at EOS. Cyclotron produced [^{68}Ga]Ga-PSMA-11 underwent full quality control, stability and sterility testing , and was implemented for human use at the University of Michigan as an Investigational New Drug through the US FDA and also at the Royal Prince Alfred Hospital (RPA).

Conclusion: Direct cyclotron irradiation of a liquid target provides clinically relevant quantities of [^{68}Ga]Ga-PSMA-11 and is a viable alternative to traditional ^{68}Ge/^{68}Ga generators.

1 Introduction

The medicinal use of ^{68}Ga was first described over 4 decades ago albeit with a very small clinical footprint for much of that time (1–4). Over the past 15 years, there has been a surge in ^{68}Ga radiopharmaceutical development, exceeding that of other radiotracers, with a 100-fold increase in the number of ^{68}Ga publications. Over the last decade, there has also been a marked increase in the clinical use of ^{68}Ga that has been attributed to the ease of acquiring ^{68}Ga from ^{68}Ge/^{68}Ga generators and the development and approval of new theranostic tracers (5). The diagnostic applications of ^{68}Ga vary across jurisdictions/countries and include imaging of neuroendocrine tumors(1), infection/inflammation (4), prostate cancer (2, 3, 6), and most recently, fibroblast activation protein inhibitors (FAPI)(7) that was the Society of Nuclear Medicine and Molecular Imaging (SNMMI) 2019 image of the year. ^{68}Ga is usually produced from a ^{68}Ge/^{68}Ga generator, and thus can be readily implemented in PET facilities that do not

have a cyclotron. There are also many additional attributes of ^{68}Ga that make it a desirable PET radionuclide. As the first widely available PET radiometal for routine use globally, ^{68}Ga is a positron emitting (89% β+) radionuclide with a relatively short half-life ($t_{1/2}$=68 min). The $^{68}Ga^{3+}$ cation is small with an ionic radius of 0.62 Å, which behaves as a relatively hard Lewis acid with an affinity for binding ligands containing oxygen and nitrogen donors, and is suitable for conjugation to various biomolecular vectors using bifunctional chelators and various macromolecules including small molecules with rapid pharmacokinetic profiles, such as peptides and peptidomimetics (8–10). This synthetic diversity provides the ability for ^{68}Ga kit development.

A main contributor to the expansion of ^{68}Ga-based PET has been imaging of the prostate specific membrane antigen (PSMA) with [^{68}Ga]Ga-PSMA-11. Prostate cancer is the second most common cancer found in men in the United States and the second most prevalent cause of cancer death in men (11). Survival rates depend on the type of prostate cancer and the stage at diagnosis. Men with localized disease have a 5-year survival rate of nearly 100%. However, 20–40% of these patients develop biochemical recurrence (BCR) and the recurrent disease can be loco-regional or more widespread. Patients with metastatic disease have a markedly decreased 5-year survival rate of 30% (11). The early and accurate identification of tumor recurrence and metastatic disease is essential for optimal patient management, but this remains a major challenge for traditional imaging methods with anatomical imaging and bone scintigraphy.

The imaging of PSMA expression with [^{68}Ga]Ga-PSMA-11 and PET/CT has proven to be a highly effective and sensitive tool for patient management (8). While the primary use of [^{68}Ga]Ga-PSMA-11 has been for detecting recurrent disease, it has also been successful at staging primary prostate cancer, and useful for guiding biopsies to improve sample accuracy, guiding surgery, and monitoring treatment response (2). Additionally, [^{68}Ga]Ga-PSMA-11 has been used theranostically in conjunction with complementary ^{177}Lu (i.e. β-) or ^{225}Ac (i.e. α) therapeutic PSMA targeting agents. Such PSMA targeted therapies are currently undergoing evaluation in clinical trials in patients with castrate-resistant metastatic prostate cancer (12, 13). [^{68}Ga]Ga-PSMA-11 is rapidly becoming the most commonly used radiotracer for prostate cancer management and has higher accuracy and sensitivity in detecting metastatic disease than [^{18}F]fluorocholine, [^{11}C]choline, and CT (8, 14–16).

There has been a positive clinical impact of [^{68}Ga]Ga-PSMA-11 at the University of Michigan with a change in patient care management in 70% of the scanned patient population. A similar high impact has been reported in a large Australian study that cited a 51% change in care management (62% for BCR patients and 21% for primary staging) (17). In 2016 a study from Belgium reported that in patients who underwent a [^{68}Ga]Ga-PSMA-11 scan there was a 76% impact in patient care management (18). A 2017 study from the University of California San Francisco reported a 53% change in patient management (19).

Since its FDA approval in 2012, [^{11}C]choline has been one of the most widely used radiotracers for the imaging of prostate cancer patients with suspected recurrence (20). The busiest cancer centers in the US reportedly perform 10–15 [^{11}C]choline scans daily for prostate cancer management (21). It has been possible to service this volume of patients given the high yielding [^{11}C]choline synthesis (> 200 mCi/dose) (22, 23), coupled with the ability to run multiple times per day, depending on the specific capabilities of the PET facility, and thus provides the proper framework to provide for 10–15 [^{11}C]choline scans daily. Many PET imaging sites in the US and Australia are moving to exclusively using [^{68}Ga]Ga-PSMA-11 rather than [^{11}C]choline, given the superior clinical performance (2, 14, 16). However, transferring that patient population to receive [^{68}Ga]Ga-PSMA-11 instead of [^{11}C]choline scans is not feasible using ^{68}Ge/^{68}Ga generators exclusively. While ^{68}Ge/^{68}Ga generators offer workflow simplicity for tracer production but there are a number of limitations: a) current GMP generators have a maximum activity of 50 mCi and are restricted to elutions every 3-4-hour increments, which in practice typically means 2 production runs per day with 2–4 doses per day; b) two or more generators increase the number of patients does to 6 or more, but still less than the requirements of busy cancer centers; c) commercial supply has not kept pace with the clinical demand and lead times for generator delivery can be up to 18 months in some markets (24); d) the eluted activity constantly declines over time and so to ensure a regular clinical supply of [^{68}Ga]Ga-PSMA-11, multiple sequential and overlapping generators must be purchased throughout the year and; e) there is the potential for long lived parent ^{68}Ge contamination and/or breakthrough. To this end, an additional source of ^{68}Ga needs to be explored and implemented into the clinical setting to meet the current and future patient demand (24).

An attractive alternative to diversifying the supply of ^{68}Ga is the direct production of ^{68}Ga on a cyclotron, via the ^{68}Zn(p,n)^{68}Ga reaction. This alternative approach has garnered significant interest by the community, including the drafting of a European Pharmacopeia monograph for the direct accelerator-based production of [^{68}Ga]$GaCl_3$ which was published late 2018 (25) and a technical document published by the IAEA in support of direct production of ^{68}Ga via liquid and solid targets (26). There are two strategies for producing ^{68}Ga via the ^{68}Z(p,n)^{68}Ga reaction on a cyclotron - namely, liquid (25–33) and solid targets (34–41). Liquid targets offer implementation simplicity for sites familiar with [^{18}F]FDG production as they present a similar workflow to production of [^{18}F]F^- and are compatible with laboratory set-ups in existing PET radiopharmaceutical production centers. Solid targets, however, typically impose increased requirements on infrastructure and/or local site expertise but offer more than order of magnitude higher ^{68}Ga yields (e.g. several Ci (37, 38). Regardless of opting for liquid or solid targets an efficient means for purifying the ^{68}Ga from the irradiated ^{68}Zn is required. The limitations of cyclotron produced ^{68}Ga are obviously: a) a cyclotron with suitable targets, b) the co-production of ^{67}Ga and ^{66}Ga and, c) the potential for residual levels of ^{68}Zn and other metal impurities affecting labeling efficiencies. These factors place stringent demands on the proton energy, the target material and reagent quality, and finally ^{68}Zn/^{68}Ga separation methods.

We present results of the liquid target-based production of ^{68}Ga on GE PETtrace cyclotrons, with focus on yield of ^{68}Ga and extraction of [^{68}Ga]$GaCl_3$ using the GE FASTlab Developer platform. Furthermore, to demonstrate the clinical relevance of this direct production method, a single FASTlab cassette was used to perform the ^{68}Zn/^{68}Ga purification and subsequent labeling of [^{68}Ga]Ga-PSMA-11. The cyclotron produced [^{68}Ga]Ga-PSMA-11 underwent full quality control, stability and sterility testing, and has been used in humans at the UM (University of Michigan, Michigan, USA) via an IND (FDA) and at RPA (Royal Prince Alfred Hospital, Sydney, Australia) under exemption of the Therapeutic Goods Act in a TGA GMP-licensed facility. The results from UM, GEMS (GE Healthcare Uppsala, Sweden) and RPA are presented.

2 Materials And Methods

2.1 Liquid target irradiations

The GE ^{68}Ga PETtrace Liquid Target (Fig. 1) is a water-cooled, gridded target without requiring He cooling of foils, designed specifically for ^{68}Ga production. The target comprises a 200 µm thick aluminum energy degrader, a 25 µm Havar foil for support, and a 25 µm niobium foil for chemical inertness with the target media, thus rendering a nominal 14.3 MeV incident proton energy on the target media. Including the target lines/dead volume, the total target fill volume is approximately 2.2 mL.

The target media was prepared from isotopically enriched [^{68}Zn]ZnO (Isoflex, USA) with addition of water (Ultrapur or 18 MΩ-cm) and 70% nitric acid (> 99.999% trace metal basis) to yield a 1.0 M solution of [^{68}Zn]$Zn(NO_3)_2$ with an excess 0.2 M or 0.3 M HNO_3 (both concentrations tested). All irradiations at UM and GEMS and the majority of irradiations at RPA employed the same lot of enriched ^{68}Zn – namely: ^{64}Zn (0.03%), ^{66}Zn (0.16%), ^{67}Zn (0.62%), ^{68}Zn (99.16%), and ^{70}Zn (0.03%), and from a chemical perspective, comprised 1 ppm iron. Recent irradiations at RPA used a different lot of enriched ^{68}Zn – namely: ^{64}Zn (0.1%), ^{66}Zn (0.18%), ^{67}Zn (0.96%), ^{68}Zn (98.20%), and ^{70}Zn (0.56%), and Fe 3.1 ppm.

Irradiations were performed on GE PETtrace cyclotrons using the ^{68}Ga Liquid Target and were typically 50–70 minutes in duration with beam currents of ~ 30–40 µA. Whenever possible, within the routine daily production schedule, a “cleaning” irradiation at 30–35 µA of typically 10–60 minutes was performed with dilute nitric acid (0.6 M) after irradiation of the [^{68}Zn]$Zn(NO_3)_2$ solution.

3 Chemical Isolation On The Fastlab

3.1 Delivery to the FASTlab

To facilitate use of the same FASTlab for both ^{68}Ga and ^{18}F processing and the dilution of the delivered ^{68}Ga target solution, the irradiated target media was delivered from the cyclotron into an external 10 mL V-vial with connections to the FASTlab (Fig. 2). Thus, delivery of ^{68}Ga target material completely bypasses the incoming activity plunger of the FASTlab module avoiding potential cross contamination

between $^{18}F^-$ and ^{68}Ga target deliveries when the module is used for both types of targets. In this activity receiving vial, the ^{68}Ga target solution was automatically diluted with water from the synthesis unit to achieve a nitric acid concentration of < 0.1 M required for subsequent processing. The diluted target solution is automatically loaded onto the cassette by nitrogen overpressure.

3.2 Chemical isolation of $[^{68}Ga]GaCl_3$

A primary goal of this effort was to develop a FASTlab cassette which allowed for $[^{68}Ga]GaCl_3$ extraction in a formulation comparable with existing generators. Additionally, on-line column conditioning, the use of minimum quantities of acid, and the exclusion of organic solvents or base-mediated pH adjustments were desired. Chemical isolation of $[^{68}Ga]GaCl_3$ was implemented on the GE FASTlab Developer platform. The process described here is based on the 2-column approach we have presented previously for liquid targets (30) and recently repeated by Riga et al in Italy (42). A graphical representation of the process is shown in Fig. 3. Initial separation of ^{68}Ga from ^{68}Zn is performed by trapping the ^{68}Ga on a hydroxamate-based resin (ZR resin, Triskem) cartridge. Further purification, concentration and acid reduction is realized by using a TOPO-based resin (TK200 resin, Triskem) cartridge.

In our initial efforts, elution of the TK200 resin with water (Scheme A in Table 1) resulted in a $[^{68}Ga]GaCl_3$ solution containing approximately 0.6 M HCl due to residual HCl content in the cartridge. Implementation of a NaCl/HCl rinse (43) for reduction of residual acid achieved a final $[^{68}Ga]GaCl_3$ formulation of 0.1 M HCl in 5 mL (Scheme B in Table 1). This formulation is directly comparable to commercially available $^{68}Ge/^{68}Ga$ generators and is compatible with formulations required for pharmaceutical cold kit labeling.

Process steps:

1. Trapping of ^{68}Ga on a hydroxamate-based resin (2 mL [~ 700 mg] ZR resin, Triskem)
2. Rinsing of the resin to remove residual zinc
3. Elution onto a TOPO-based resin (2 mL [~ 700 mg] TK200 resin, Triskem)
4. Wash to decrease residual acid content, and
5. Final elution with water and dilute hydrochloric acid, volumes of which can be varied, to yield $[^{68}Ga]GaCl_3$ in the desired formulation (e.g. 5 mL of 0.1 M HCl)

The process was optimized over time (with regards to flow rates, volumes, cassette rinsing, etc), thus not all runs were identical with regards to time lists on the FASTlab. Nevertheless, the chemical process can be categorized into two primary schemes (as noted in Table 1). Building on Scheme A, Scheme B includes a wash step of the TK200 resin in order to reduce the residual acid content in the $[^{68}Ga]GaCl_3$ eluate. The purification time is approximately 30 minutes.

Table 1

High level schemes of $[^{68}Ga]GaCl_3$ purifications.

	Scheme A*	Scheme B
▯ ZR Load	< 0.1 M HNO_3	
▯ ZR Wash	15 mL 0.1M HNO_3	
▯ ZR Elution / Trapping on TK200	5–6 mL ~ 1.75 M HCl	
▯ TK Wash	–	3.5 mL 2.0 M NaCl in 0.13 M HCl
▯ TK Elution	H_2O	1–2 mL H_2O followed by dilute HCl to formulate
*Process as reported previously(30)		

In comparison to recent work, this method requires less acid and does not involve organic solvents or base-mediated pH adjustments, which is highlighted in Table 2.

Table 2

Comparison of FASTlab $[^{68}Ga]GaCl_3$ purification vs. recent literature

Reference	HNO_3* [mmol]	HCl [mmol]	Organic solvents	Base-mediated pH adjustment?
This work	2.4	16	No	No
[Oehlke et al (*28*)]	-	886	Yes (Methanol)	No
[Alves et al (*44*)]	-	265	Yes (HBr/acetone)	No
[Pandey et al(*33*)]	0.25	38	Yes (Acetonitrile)	Yes
*Does not account for HNO_3 in the liquid target.				

The cassette layout for the automated $[^{68}Ga]GaCl_3$ separation on the FASTlab is given in Fig. 4, noting that the $[^{68}Ga]GaCl_3$ chemistry is reserved to the right-hand side of the cassette. The left-hand side was kept vacant to enable subsequent on-cassette labeling (e.g. PSMA, NET tracers, etc), including C18 cartridge purification, see Fig. 5. The line labeled “to activity source” is connected to the activity receiving vial (Fig. 2). Where applicable during the process, the vials of 0.6 M HNO_3, 4 M HCl, and 3 M NaCl were automatically diluted and/or mixed to the desired concentrations by the FASTlab.

In advance of receipt of the activity the columns were automatically conditioned on the FASTlab, the ZR resin was conditioned with 0.1 M HNO_3 (7 mL) and the TK200 was conditioned with both water (7 mL)

followed by 1.75 M HCl (4 mL). Recycling of ^{68}Zn is not presently being performed given the current availability and cost of ^{68}Zn (approximately US$100 per target fill), however, the ^{68}Zn solution is collected separately to facilitate future recycling.

3.3 Synthesis of [^{68}Ga]Ga-PSMA-11

The direct cyclotron-based production of [^{68}Ga]Ga-PSMA-11 was executed at the UM, GEMS and RPA using a single FASTlab cassette in a continuous process to perform both the [^{68}Ga]$GaCl_3$ isolation chemistry and subsequent PSMA-11 labeling, including C18 purification. Initial labeling tests (UM) employed [^{68}Ga]$GaCl_3$ separation scheme “A”, with 10 µg PSMA-11 precursor in 1.5 M Hepes (1 mL) and 3M NaOAc (1.3 mL) buffer. Scheme “B” developed and implemented at GEMS and RPA used 10 µg PSMA-11 precursor in 1.5 mL/1.0 M NaOAc (GEMS) or 1.3 mL/1.5 M NaOAc buffer (RPA) adjusted to pH 4.5–4.8. Approximately 3–4 mg L-ascorbic acid was also added (GEMS/RPA) to the precursor vial to minimize radiolysis during synthesis. An additional 20–21 mg of L-ascorbic acid (0.44 mL; 0.25 M) is also added directly into the product line at RPA as stabilizer of the final product. Labeling occurred for 5 min at 50 ^{o}C. At UM and RPA, the final product was formulated with Phosphate Buffered Saline (PBS). The cassettes were prepared at each institution based on the FASTlab developer cassettes and accessories.

4 Results And Discussion

4.1 ^{68}Ga Yields

Total ^{68}Ga yields from the target were assessed by: (a) downloading the total irradiated target contents into a vial placed in a dose calibrator without chemical purification and ensuring suitable decay time (90–120 min) or curve fitting to avoid any ^{13}N contribution, or (b) measurement of residual activity of cassette components and product post [^{68}Ga]$GaCl_3$ isolation or post [^{68}Ga]Ga-PSMA-11 labeling chemistry. For the data presented at GEMS, this includes an early series of 9 consecutive 60-minute irradiations from 30–40 µA (entire target contents), and 20 consecutive irradiations (post-chemistry) following a target rebuild.

Radioactivity yields exceeding 100 mCi (3.7 GBq) at EOB are typical (see Table 3) with irradiation of [^{68}Zn]$Zn(NO_3)_2$ at 0.3 M HNO_3 yielding more consistent target/chemistry performance. Albeit higher acid concentrations have been reported in the literature (29), we opted to maintain the excess nitric acid as low as possible to minimize corrosive wear on components and facilitate the subsequent chemistry (which requires < 0.1 M HNO_3 for ZR resin loading).

While it is theoretically possible to increase the target yields by increasing the ^{68}Zn concentration, the 1.0 M solution used here facilitates transfer to the hot cell (i.e. the solution is not too viscous). Should multi-Ci yields of ^{68}Ga be desired, adoption of the proposed method to solid targets as has been reported

previously by taking advantage of ^{68}Ga trapping on ZR resin in high HCl concentration loading conditions (38).

Table 3
Summary of ^{68}Ga productions and total ^{68}Ga radioactivity yield at EOB

Site	HNO_3 [M]	N	I [uA]	Beam time [min]	EOB activity [GBq]	EOB activity [mCi]	Measurement
UM	0.2	13	30	60	4.1 ± 0.6	112 ± 16	Entire target contents
	0.2	6	35	60	3.9 ± 0.6	106 ± 17	Entire target contents
	0.2	6	40	60	3.8 ± 0.4	102 ± 11	Entire target contents
	0.3	12	34 ± 4	60	4.6 ± 0.4	126 ± 12	Entire target contents
GEMS	0.2	9	36 ± 5	60	4.5 ± 0.3	120 ± 9	Entire target contents
	0.2	14	30	69 ± 7	3.5 ± 0.9	94 ± 24	□ of parts post chemistry
	0.3	6	29 ± 1	70 ± 13	4.3 ± 0.5	115 ± 14	□ of parts post chemistry
RPA	0.3	25	36 ± 2.2	60	4.0 ± 0.6	107 ± 17	Entire target contents
	0.3	53	35	60	3.8 ± 0.5	104 ± 14	□ of parts post chemistry

4.2 [^{68}Ga]$GaCl_3$, [^{68}Ga]Ga-PSMA-11 – Yields and Quality

Several hundred irradiations and purifications/labelings have been performed throughout the development efforts, however, for sake of brevity, we report herein on several representative subsets of experimental data. These data are summarized in Tables 4 to 6.

Table 4

High-level summary of ^{68}Ga runs reported herein for UM, GEMS and RPA.

	Site	N	comment
[^{68}Ga]$GaCl_3$	UM	27	60 min beam current
	GEMS	13	Consecutive productions, 0.2 or 0.3 M HNO_3
	RPA	20	60 min 35 µA beam, 0.3 M HNO_3
[^{68}Ga]Ga-PSMA-11	UM	3 + 35	Validation + clinical
	GEMS	3	Consecutive productions
	RPA	8	Validation + clinical

Table 5

Overview of [^{68}Ga]$GaCl_3$ productions (EOS)

Site	Chemistry Scheme	HNO_3	I	Beam time	N	Product activity	
		[mol/L]	[µA]	[min]		[GBq]	[mCi]
UM	A	0.2	30	60	15	2.0 ± 0.3	54 ± 8
			35		6	2.0 ± 0.3	55 ± 8
			40		6	1.9 ± 0.2	50 ± 5
GEMS	B	0.2	30	64 ± 6	10	1.7 ± 0.5	46 ± 13
		0.3	29 ± 1	73 ± 6	3	2.5 ± 0.1	67 ± 3
RPA	B	0.3	35	60	20	2.0 ± 0.2	55 ± 6

Table 6

Overview of [^{68}Ga]Ga-PSMA-11 productions (EOS)

Site	HNO_3	I	Beam time	N	Product activity		Notes
	[mol/L]	[µA]	[min]		[GBq]	[mCi]	
UM	0.2	30–40	60	3	1.6 ± 0.3	43 ± 9	Validation runs
UM	0.2	30–40	60	35	1.7 ± 0.2	45 ± 6	Clinical
GEMS	0.3	30	64 ± 4	3	2.1 ± 0.4	57 ± 10	R&D efforts
RPA	0.3	35	60	14	1.6 ± 0.1	44 ± 3	Final validation and clinical (5) runs

Table 5 clearly demonstrate a robust routine production of ~ 50 mCi of [^{68}Ga]$GaCl_3$ via the liquid target cyclotron route. This compares favorably with approximately 40 mCi of [^{68}Ga]$GaCl_3$ from a brand new, highest commercially available activity GMP generator with 50 mCi of ^{68}Ge. Furthermore, the eluted ^{68}Ga activity steadily decreases over time due to the decay of the ^{68}Ge.

[^{68}Ga]Ga-PSMA-11 activity yields at EOS varied slightly across sites (Table 6) which may be at least partly attributed to beam parameters, state of target and slightly different labeling conditions used. At RPA, 3 patients can be readily scanned from a single batch of [^{68}Ga]Ga-PSMA-11 using 2 scanners, which is the same number of patients which can be scanned with [^{68}Ga]Ga-PSMA-11 produced from 2 staggered ^{68}Ge/^{68}Ga generators. As more than 2 productions runs can potentially be performed with the target, the number of patients able to be scanned per day is potentially is increased.

Although activity yield is an important parameter to measure process performance, the quality of the cyclotron-produced [^{68}Ga]$GaCl_3$ is of even greater importance as high quality [^{68}Ga]$GaCl_3$ is critical to enable efficient labeling. Therefore, in addition to yield measurement and periodic quality control (QC) assessment, validation studies for [^{68}Ga]$GaCl_3$ and [^{68}Ga]Ga-PSMA-11 were carried out at UM and for [^{68}Ga]Ga-PSMA-11 at RPA. The test methods performed (half-life, radiochemical purity, pH, radionuclidic purity, metal analysis) were in accordance with the EUP monograph for [^{68}Ga]$GaCl_3$ (25), with an exception for Fe and Zn, for which semi-quantitative colorimetric test strips (e.g. EM-Quant, Merck) and/or ICP-MS were used.

Table 7 reports on the QC results for the [^{68}Ga]$GaCl_3$ validation runs carried out at UM. QC testing of [^{68}Ga]Ga-PSMA-11 is shown in Table 8 for UM and Table 9 for RPA, with RCP assessment by radio-TLC

and HPLC. Endotoxin, 4-hour stability (data not shown), and sterility testing were also performed for the three validation runs. The 3 validation runs and all 4-hour stability time points passed all QC parameters.

Table 7

Quality Control Data for three [^{68}Ga]$GaCl_3$ validation runs (UM)

TEST	1	2	3	Avg & SD	Release Criteria (EUP)
Radiochemical Purity [^{68}Ga]$GaCl_3$ (iTLC-SG)	99	98	98	98.3 ± 0.3	≥ 95
Rf [^{68}Ga]$GaCl_3$ (TLC)	< 0.2	< 0.2	< 0.2	< 0.2	≤ 0.2
Rf Ref B* (TLC)	> 0.7	> 0.7	> 0.7	> 0.7	≥ 0.7
pH	< 2	< 2	< 2	< 2	< 2
Visual Inspection	Passed	Passed	Passed	N/A	Clear, colorless, no visible particulate
Radionuclidic Identity ($t_{½}$)	67.2	68.8	69.1	68.4 ± 0.8	64.6–71.4 min
Endotoxin Analysis	< 2	< 2	< 2	< 2	≤ 58.3 EU/mL
Fe µg/GBq	< 5	< 5	< 5	< 5	≤ 10 ug/GBq
Zn µg/GBq	< 1.25	< 1.25	< 1.25	< 1.25	≤ 10 ug/GBq
RNP at EOB (MCA)	99.8	99.8	99.8	99.8	≥ 98% (at time of use)
*Ref B-Pentetic acid solution					

Table 8

Quality Control Data for three [^{68}Ga]Ga-PSMA-11 validation runs (UM)

Tests	1	2	3	Avg & SD	Release Criteria (UM)
Radiochemical Purity (via TLC)	99.5	99.4	99.3	99.4 ± 0.1	≥ 90%
Relative Retention time (via HPLC)	1.004	1.005	1.005	1.0046 ± 0.0003	RRT: 0.9–1.1
pH	7.0	7.0	7.0	7.0	4.0–8.0
Visual Inspection	Passed	Passed	Passed	N/A	Clear, colorless, no visible particulate
Radionuclidic Identity ($t_{½}$)	67.61	68.45	67.20	67.75 ± 0.53	64.6–71.4 min
Endotoxin Analysis	< 2	< 2	< 2	< 2	≤ 10.9 EU/mL
Bubble Point (PSI)	51	52	53	52 ± 1	≥ 50 PSI
Sterility	Passed	Passed	Passed	Passed	Complies with USP < 71> (*45*)
RNP at EOB (MCA)	99.8	99.8	99.8	99.8	≥ 98% (at time of use)

Table 9
Quality Control Data for three [^{68}Ga]Ga-PSMA-11 validation runs (RPA)

Tests	1	2	3	Avg & SD	Release Criteria (RPA)
Radiochemical Purity (via TLC)	99.94	99.99	99.94	99.96 ± 0.03	≥ 95%
Radiochemical Purity (via HPLC)	99.94	99.97	100.0	99.97 ± 0.03	≥ 95%
pH	5.0	5.5	5.5	5.3 ± 0.3	4.0–8.0
Visual Inspection	Passed	Passed	Passed	N/A	Clear, colorless, no visible particulate
Radionuclidic Identity ($t_{½}$)	67.9	68.1	67.7	67.9 ± 0.20	62–74 min
Endotoxin Analysis	< 1	< 1	< 1	< 1	≤ 17.5 EU/mL
Bubble Point (bar)	4.1	4.2	4.1	4.1 ± 0.06	≥ 3.5 bar
Sterility	Passed	Passed	Passed	Passed	Sterile – no growth
RNP at EOS (Well Counter)	99.7	99.8	99.8	99.8 ± 0.06	≥ 98% (at time of use)

The validation data shown here demonstrates the high quality of cyclotron-produced [^{68}Ga]$GaCl_3$ and [^{68}Ga]Ga-PSMA-11 and highlights the reliability and reproducibility of both processes. Notably, the reported RNP satisfied the proposed EU Pharmacopoeia limits (radioactivity maximum 2% of combined ^{66}Ga + ^{67}Ga) (25). The dosimetry of such a limit has been previously reported using worst-case assumptions, such as no biological clearance and rapid organ uptake (46). For this scenario, a relative dose increase up to 20% is reported but is typically less than 10% when compared with “pure” ^{68}Ga (i.e. not comparing with generator ^{68}Ga which may contain ^{68}Ge). Overall, the obtained results provided a solid basis for the clinical evaluation of cyclotron-produced [^{68}Ga]Ga-PSMA-11.

4.3 Clinical production and use of [^{68}Ga]Ga-PSMA-11

To date, over 700 patients have been scanned with [^{68}Ga]Ga-PSMA-11 at UM under IND. Initially, this was with generator-based [^{68}Ga]Ga-PSMA-11, though amended to include cyclotron-based [^{68}Ga]Ga-PSMA-11, with the first clinical production of cyclotron-based [^{68}Ga]Ga-PSMA-11 from a single FASTlab cassette in February 2019. As of March 2020, 50 clinical batches of cyclotron-produced [^{68}Ga]Ga-PSMA-11 were used to scan more than 90 patients (see Table 6) and the image from the first patient scanned with cyclotron-produced [^{68}Ga]Ga-PSMA-11 in Fig. 6). There were no differences noted in the quality of studies where ^{68}Ga was produced from a cyclotron when compared to a generator. At RPA, cyclotron-based ^{68}Ga used for clinical [^{68}Ga]Ga-PSMA-11 began in 2020.

5 Conclusions And Outlook

A process for isolating high purity [^{68}Ga]$GaCl_3$ from cyclotron-produced ^{68}Ga and subsequent labeling of PSMA-11 on the GE FASTlab synthesizer with both steps being performed on a single cassette has been developed. The cyclotron-based method offers a reliable source of ^{68}Ga and delivers consistently higher yields than currently available commercial 50 mCi ^{68}Ge/^{68}Ga generators. Furthermore, in contrast to generators, for which ^{68}Ga activity falls over time due to ^{68}Ge decay, cyclotron-based ^{68}Ga activity is consistent with time thereby simplifying patient scheduling. The FASTlab-derived [^{68}Ga]$GaCl_3$ solution for radiolabeling met the requirements in the European Pharmacopeia (EUP) with the purity of reagents and ^{68}Zn enrichment and purity used at these sites, and validation of [^{68}Ga]$GaCl_3$ and [^{68}Ga]Ga-PSMA-11 for clinical application has been demonstrated by the UM and RPA. Over 90 patients have been scanned using cyclotron-based [^{68}Ga]Ga-PSMA-11 to date, and the process is in routine use to meet the growing demands for PSMA-based PET imaging at UM. Studies to broaden the applicability of the [^{68}Ga]$GaCl_3$ process for labeling with other commonly used chelators such as DOTA have been performed successfully at RPA, with [^{68}Ga]Ga-DOTA-TATE labeled with cyclotron-produced ^{68}Ga being used clinically. Similar studies are also currently ongoing at other sites.

Declarations

Acknowledgements

The authors thank Dr. Ali Afshar-Oromieh, MD, PhD, Prof. Dr. Klaus Kopka and their colleagues at the University Hospital of Heidelberg and the German Cancer Research Center (DKFZ) Heidelberg for the valuable assistance in qualifying the University of Michigan for clinical production of [^{68}Ga]Ga-PSMA-11. In addition, the authors thank Steffen Happel at Triskem for valuable suggestions, feedback, and initial resin samples.

Compliance with ethical standards

Conflict of interest KG, JF, DCP, and CS are employees of GE Healthcare. SE, AK, DS, MJF, MER, MC, BGH, BDH, MA-G, MRP and PJHS declare no conflict of interest.

Ethical Approval This article does not contain any original studies with human or animal subjects performed by any of the authors.

Funding

PJHS acknowledges financial support from Michigan Medicine.

References

1. Graham MM, Gu X, Ginader T, Breheny P, Sunderland JJ. Ga-DOTATOC Imaging of Neuroendocrine Tumors: A Systematic Review and Metaanalysis. *J Nucl Med*. 2017;58:1452-1458.
2. Lenzo N, Meyrick D, Turner J, Lenzo NP, Meyrick D, Turner JH. Review of Gallium-68 PSMA PET/CT Imaging in the Management of Prostate Cancer. *Diagnostics*. 2018;8:16.
3. Eder M, Neels O, Müller M, et al. Novel Preclinical and Radiopharmaceutical Aspects of [68Ga]Ga-PSMA-HBED-CC: A New PET Tracer for Imaging of Prostate Cancer. *Pharmaceuticals*. 2014;7:779-796.
4. Velikyan I. Prospective of 68Ga Radionuclide Contribution to the Development of Imaging Agents for Infection and Inflammation. *Contrast Media Mol Imaging*. 2018;2018:1-24.
5. Baum RP, Kulkarni HR. THERANOSTICS: From Molecular Imaging Using Ga-68 Labeled Tracers and PET/CT to Personalized Radionuclide Therapy - The Bad Berka Experience. *Theranostics*. 2012;2:437-47.
6. Ruangma A, Kijprayoon S, Ngokpol S. PSMA FOR PET IMAGING OF PROSTATE CANCER. *Bangkok Med J*. 2018;14:95-100.
7. Kratochwil C, Flechsig P, Lindner T, et al. 68Ga-FAPI PET/CT: Tracer Uptake in 28 Different Kinds of Cancer. *J Nucl Med*. 2019;60:801-805.
8. Blower JE, Cooper MS, Imberti C, et al. The Radiopharmaceutical Chemistry of the Radionuclides of Gallium and Indium. In: Lewis J, Windhorst A, Zeglis B, eds. Radiopharmaceutical Chemistry. Cham: Springer, Cham; 2019:255-271.
9. Smith DL, Breeman WAP, Sims-Mourtada J. The untapped potential of Gallium 68-PET: The next wave of 68Ga-agents. *Appl Radiat Isot*. 2013;76:14-23.
10. Martiniova L, De Palatis L, Etchebehere E, Ravizzini G. Gallium-68 in Medical Imaging. *Curr Radiopharm*. 2016;9:187-207.
11. Prostate Cancer: Statistics | Cancer.Net. https://www.cancer.net/cancer-types/prostate-cancer/statistics.
12. Fendler WP, Rahbar K, Herrmann K, Kratochwil C, Eiber M. 177Lu-PSMA Radioligand Therapy for Prostate Cancer. *J Nucl Med*. 2017;58:1196-1200.
13. Kratochwil C, Bruchertseifer F, Rathke H, et al. Targeted a-therapy of metastatic castration-resistant prostate cancer with 225 Ac-PSMA-617: Swimmer-Plot Analysis Suggests efficacy regarding duration of tumor control. *J Nucl Med*. 2018;59:795-802.
14. Schwenck J, Rempp H, Reischl G, et al. Comparison of 68 Ga-labelled PSMA-11 and 11 C-choline in the detection of prostate cancer metastases by PET/CT. *Eur J Nucl Med Mol Imaging*. 2017;44:92-101.

15. Afshar-Oromieh A, Zechmann CM, Malcher A, et al. Comparison of PET imaging with a 68Ga-labelled PSMA ligand and 18F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging*. 2014;41:11-20.
16. McCormick B, Mahmoud A, Williams S, Davis J. Biochemical recurrence after radical prostatectomy: Current status of its use as a treatment endpoint and early management strategies. *Indian J Urol*. 2019;35:6-17.
17. Roach PJ, Francis R, Emmett L, et al. The impact of 68 Ga-PSMA PET/CT on management intent in prostate cancer: Results of an australian prospective multicenter study. *J Nucl Med*. 2018;59:82-88.
18. Albisinni S, Artigas C, Aoun F, et al. Clinical impact of 68 Ga-prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET/CT) in patients with prostate cancer with rising prostate-specific antigen after treatment with curative intent: preliminary analysis . *BJU Int*. 2017;120:197-203.
19. Hope TA, Aggarwal R, Chee B, et al. Impact of 68Ga-PSMA-11 PET on management in patients with biochemically recurrent prostate cancer. *J Nucl Med*. 2017;58:1956-1961.
20. Evans JD, Jethwa KR, Ost P, et al. Basic Original Report Prostate cancer-specific PET radiotracers: A review on the clinical utility in recurrent disease. *Pract Radiat Oncol*. 2018;8:28-39.
21. Lowe VJ, Kwon ED. PET in Prostate Cancer: A Focus on C-11 Choline. *Clin Trials Netw Newsl*. 2015;January.
22. Shao X, Hockley BG, El Hoareau R, Schnau PL, Scott PJH. Fully automated preparation of [11 C]choline and [18 F]fluoromethylcholine using TracerLab synthesis modules and facilitated quality control using analytical HPLC. *Appl Radiat Isot*. 2010;69:403-409.
23. Shao X, Hoareau R, Runkle AC, et al. Highlighting the versatility of the Tracerlab synthesis modules. Part 2: fully automated production of [11C]-labeled radiopharmaceuticals using a Tracerlab FXC-Pro. *J Label Compd Radiopharm*. 2011;54:819-838.
24. Cathy Cutler. Shortage of Germanium68/gallium68 generators in the United States.; 2018.
25. Gallium (68Ga) Chloride (accelerator produced) solution for radiolabelling. *Pharmeuropa*. 2018;30.4.
26. Gallium-68 Cyclotron Production IAEA-TECDOC-1863. Vienna; 2019.
27. Jensen M, Clark JC. Direct production of Ga-68 from proton bombardment of concentrated aqueous solutions of [Zn-68] Zinc Chloride. In: 13th Workshop on Targetry and Target Chemistry. Roskilde, Denmark; 2010.
28. Oehlke E, Hoehr C, Hou X, et al. Production of Y-86 and other radiometals for research purposes using a solution target system. *Nucl Med Biol*. 2015;42:842-9.
29. Pandey MK, Byrne JF, Schlasner KN, Schmit NR, DeGrado TR. Cyclotron production of 68 Ga in a liquid target: Effects of solution composition and irradiation parameters. *Nucl Med Biol*. 2019.
30. Nair M, Happel S, Eriksson T, Pandey M, DeGrado T, Gagnon K. Cyclotron production and automated new 2-column processing of [68Ga]GaCl3. *Eur J Nucl Med Mol Imaging* . 2017;44:S119-S956.

31. Alves F, Alves VHP, Do Carmo SJC, Neves ACB, Silva M, Abrunhosa AJ. Production of copper-64 and gallium-68 with a medical cyclotron using liquid targets. *Mod Phys Lett A*. 2017;32:1740013.
32. Pandey MK, Byrne JF, Jiang H, Packard AB, DeGrado TR. Cyclotron production of (68)Ga via the (68)Zn(p,n)(68)Ga reaction in aqueous solution. *Am J Nucl Med Mol Imaging*. 2014;4:303-10.
33. Pandey MK, DeGrado TR. Rapid Isolation of Cyclotron-Produced Gallium-68. 2019.
34. Zeisler S, Limoges A, Kumlin J, Siikanen J, Hoehr C. Fused Zinc Target for the Production of Gallium Radioisotopes. *Instruments*. 2019;3:10.
35. Engle JW, Lopez-Rodriguez V, Gaspar-Carcamo RE, et al. Very high specific activity $^{66}/^{68}$Ga from zinc targets for PET. *Appl Radiat Isot* . 2012;70:1792-6.
36. Sadeghi M, Kakavand T, Rajabifar S, Mokhtari L, Rahimi-Nezhad A. Cyclotron production of 68Ga via proton-induced reaction on 68Zn target. *Nukleonika*. 2009;54:25-28.
37. Lin M, Waligorski GJ, Lepera CG. Production of curie quantities of 68 Ga with a medical cyclotron via the 68 Zn(p,n) 68 Ga reaction. *Appl Radiat Isot*. 2018;133:1-3.
38. Schweinsberg C, Johayem A, Llamazares A, Gagnon K. The first curie-quantity production of [68Ga]Ga-PSMA-HBED-CC. *J Label Compd Radiopharm*. 2019;62:P121.
39. Gallium-68 Cyclotron Production. Vienna, Austria; 2019.
40. Boschi A, Martini P, Costa V, et al. Interdisciplinary Tasks in the Cyclotron Production of Radiometals for Medical Applications. The Case of 47 Sc as Example. *Molecules*. 2019;24:1-14.
41. Tolmachev V, Lundqvist H. Rapid separation of gallium from zinc targets by thermal diffusion. *Appl Radiat Isot*. 1996;47:297-299.
42. Riga S, Cicoria G, Pancaldi D, et al. Production of Ga-68 with a General Electric PETtrace cyclotron by liquid target. *Phys Medica*. 2018;55:116-126.
43. Mueller D, Klette I, Baum RP, Gottschaldt M, Schultz MK, Breeman WAP. Simplified NaCl based (68)Ga concentration and labeling procedure for rapid synthesis of (68)Ga radiopharmaceuticals in high radiochemical purity. *Bioconjug Chem*. 2012;23:1712-7.
44. Alves V, do Carmo S, Alves F, Abrunhosa A. Automated Purification of Radiometals Produced by Liquid Targets. *Instruments*. 2018;2:17.
45. USP 71 Microbiological Tests/Sterility Tests. In: The United States Pharmacopeial Convention. ; 2012.
46. Graves SA, Engle JW, Eriksson TE, Gagnon K. Dosimetry of cyclotron-produced [68Ga]Ga-PSMA-11, [68Ga]Ga-DOTA-TATE, and [68Ga]Ga-DOTA-TOC. *J Nucl Med*. 2018;59:1003.

Figures

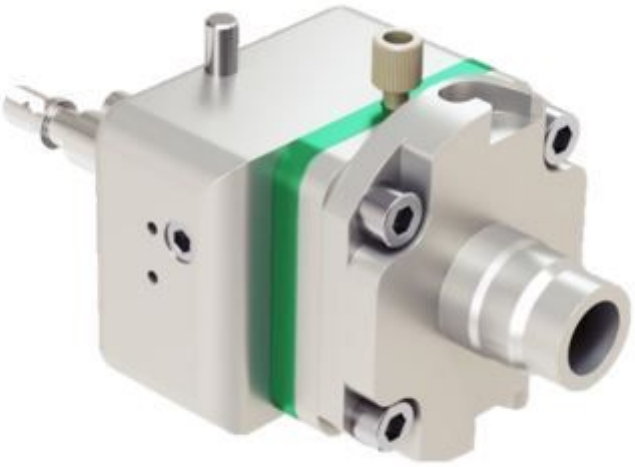

Figure 1

GE Gallium-68 Liquid Target

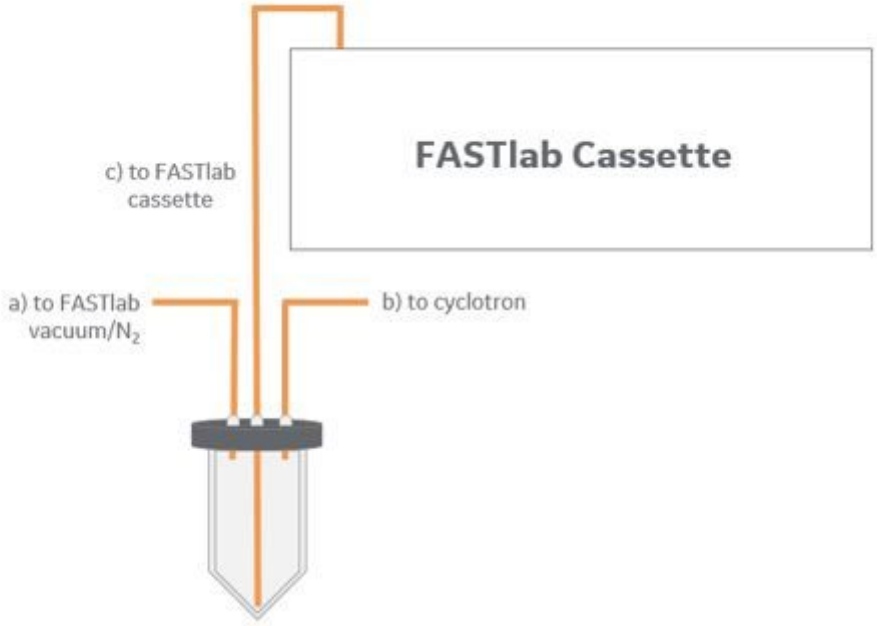

Figure 2

Activity receiving vial for collection of the irradiated 68Zn solution

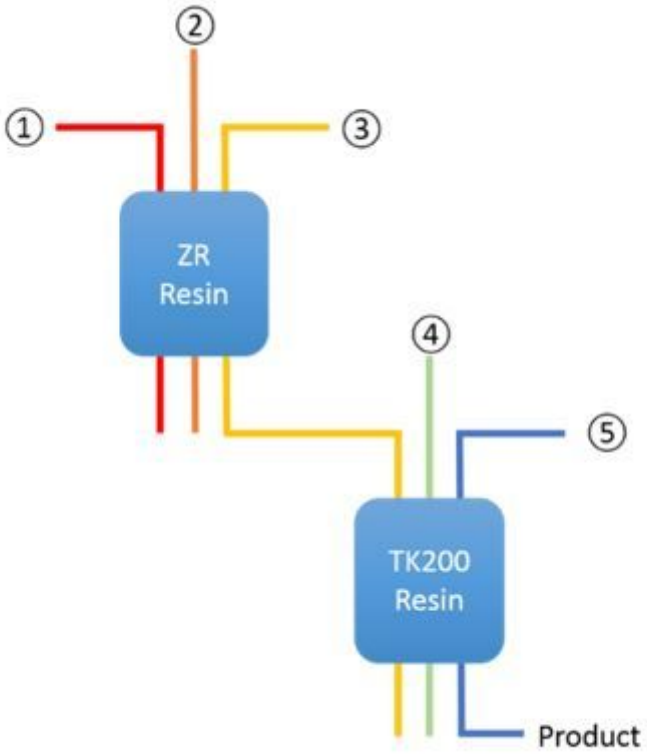

Figure 3

Two-column approach for 68Ga chemical separation

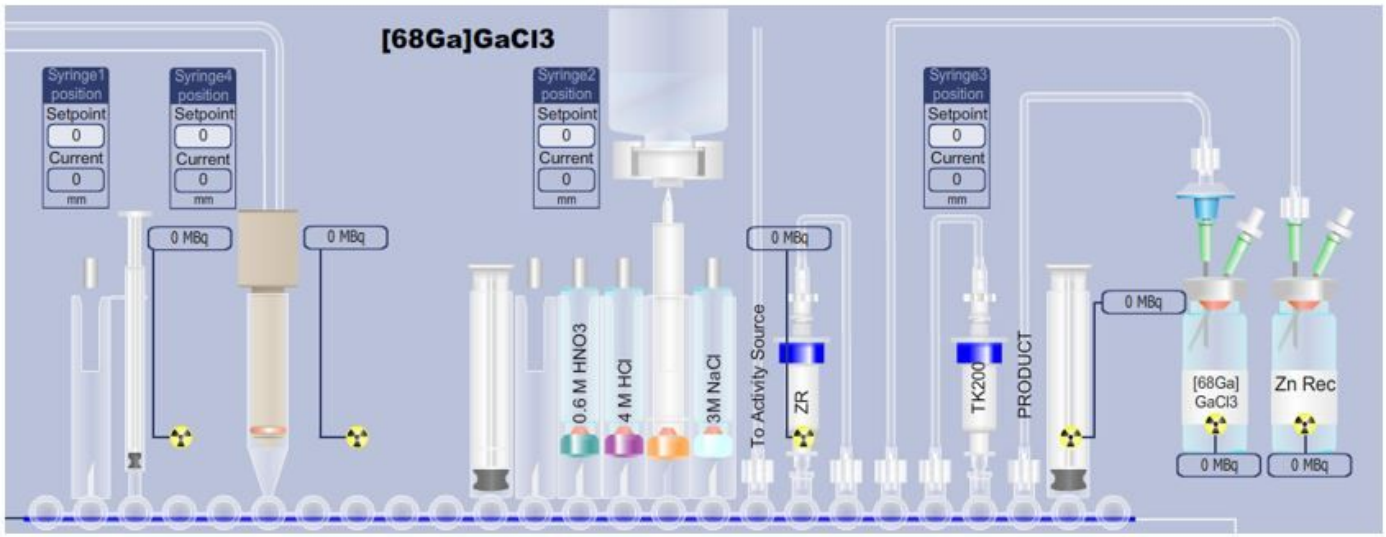

Figure 4

FASTlab cassette layout – applicable to Schemes “A&B” of Table 3.

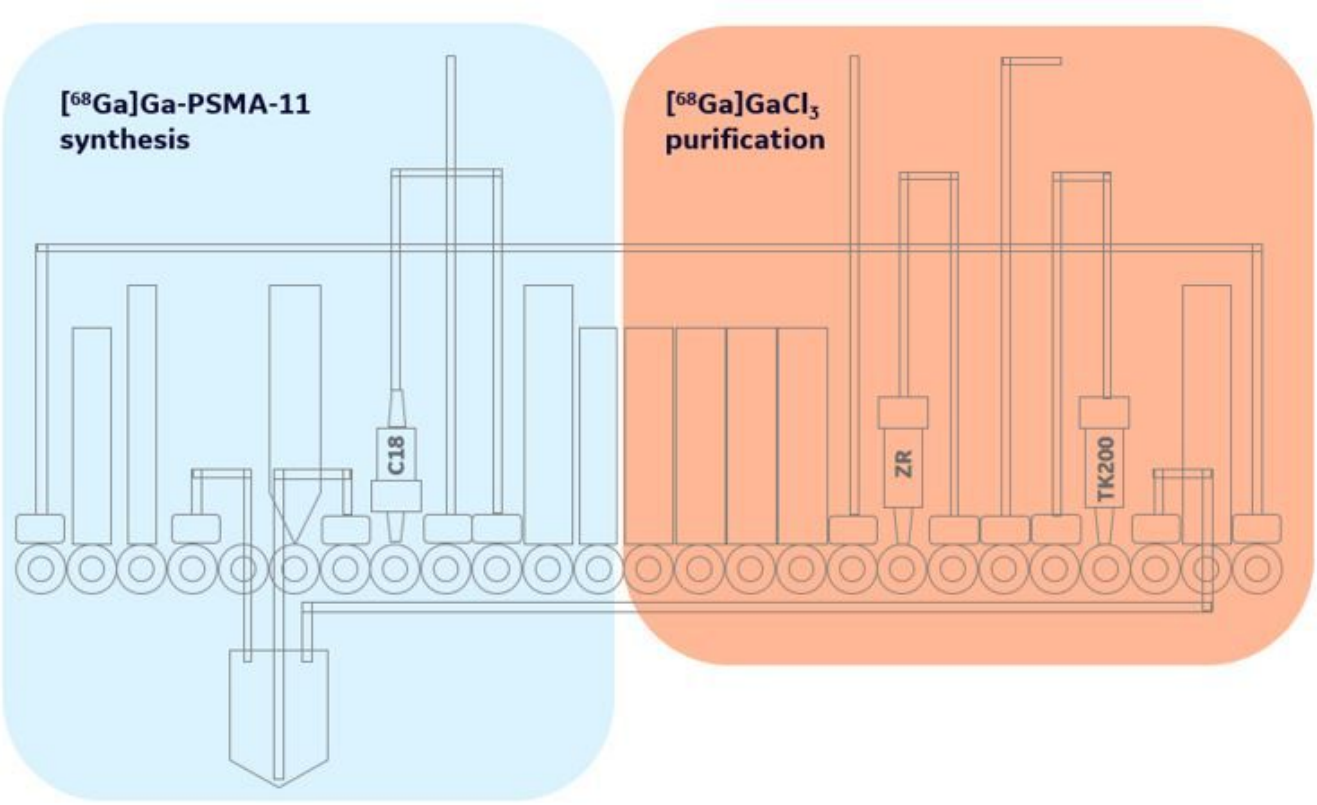

Figure 5

Partitioning of FASTlab cassette: Right-hand side is reserved for [68Ga]GaCl3 purification, and the left-hand side accommodates the [68Ga]Ga-PSMA-11 synthesis and C18 cartridge based purification.

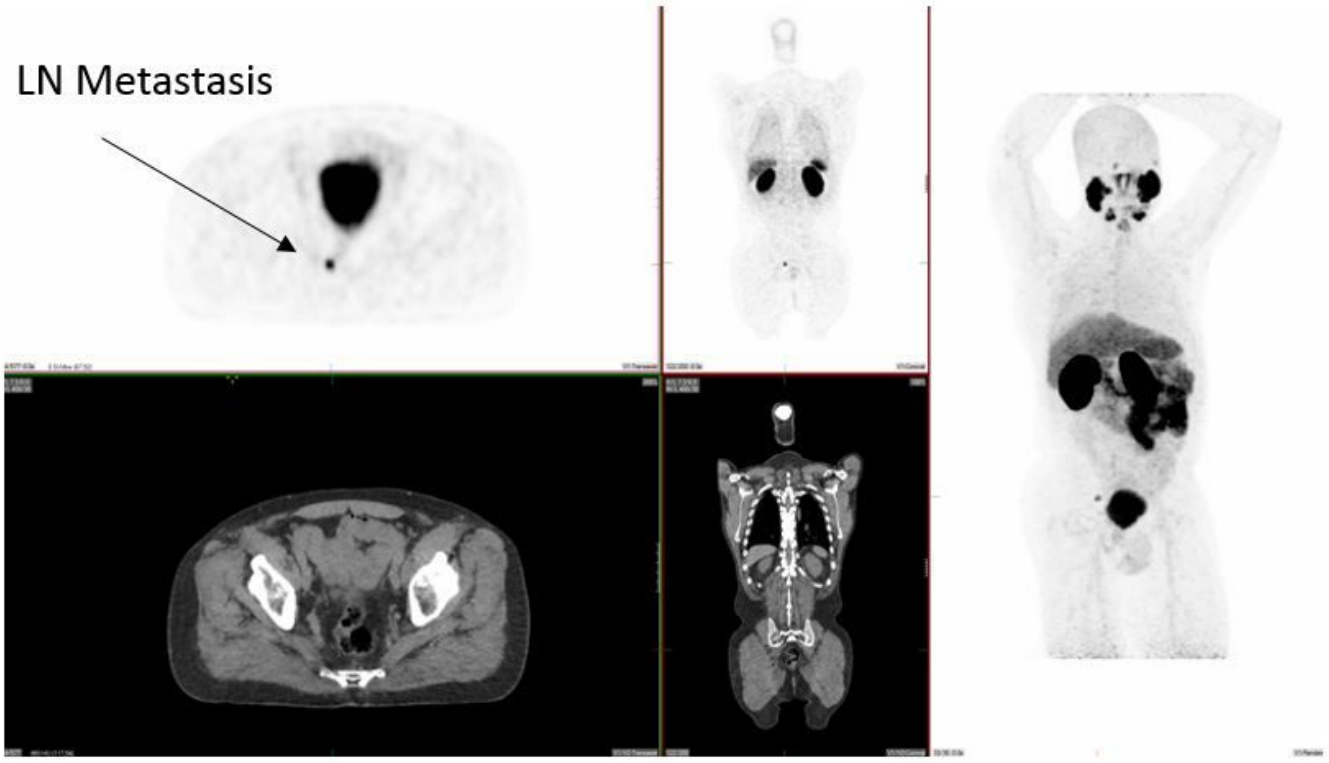

Figure 6

Images from the first patient scanned with [68Ga]Ga-PSMA-11 labeled with cyclotron produced 68Ga at the University of Michigan