

Review

Peter Mikuš, Milan Melník*, Andrea Forgácsová, Dominika Krajčiová and Emil Havránek

Gallium compounds in nuclear medicine and oncology

Abstract: An overview of the most important gallium compounds in nuclear medicine and oncology in the two last decades, with an emphasis on the last decade (especially the last 5 years), is given in this review. You can also find here some recent knowledge about $^{68}\text{Ge}/^{68}\text{Ga}$ radionuclide generator modern automated synthesis modules. Gallium has long been known to concentrate in skeletal tissue, particularly regions of bone deposition and remodeling. Elemental gallium is a potent inhibitor of bone resorption that acts to maintain or restore bone mass. There are several medically useful gallium radionuclides that have made extensive contribution in both the diagnosis and therapy of diseases. A huge variety of monofunctional and bifunctional chelators have been developed that allow the formation of stable $^{68}\text{Ga}(\text{III})$ complexes and convenient coupling to biomolecules such as amino acids, peptides, nanoparticles, or even whole cells. Gallium pharmaceuticals can be divided into two groups according to radioactivity, i.e., radiopharmaceuticals – using radioactive $\text{Ga}(\text{III})$ isotopes, and conventional pharmaceuticals – using non-radioactive $\text{Ga}(\text{III})$ ion. The pharmaceuticals can also be divided according to the target site of a drug, i.e., those having an impact on soft tissue (most of the drugs) and hard tissue (bones and bone metastasis). In oncology and nuclear medicine, gallium and its compounds have been applied for imaging as well as therapy, and their importance in this field is still growing.

Keywords: antineoplastics; complexes; gallium; nuclear medicine; oncology; radiopharmaceuticals.

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List of abbreviations

AE105-NH ₂	Asp-cyclohexylalanine-Phe-D-Ser-D-Arg-Tyr-Leu-Trp-Ser-NH ₂
AMBA	DO3A-CH(2)CO-G-4-aminobenzoyl-Q-W-A-V-G-H-L-M-NH(2)
ATP	adenosine triphosphate
Cyclo-MG1	cyclo[γ-D-Glu-Ala-Tyr-D-Lys]-Trp-Met-Asp-Phe-NH ₂
Cyclo-MG2	cyclo[γ-D-Glu-Ala-Tyr-D-Lys]-Trp-Nle-Asp-Phe-NH ₂
dNTP	intracellular deoxyribonucleoside
DOTA-TATE	DOTA-(Tyr3)-octreotate
DOTA	1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid
DOTANOC	DOTA-1-Nal3-octreotide
DOTATOC	DOTA-Tyr3-octreotide
DTPA	diethylenetriaminepentaacetic acid
EDTA	ethylenediamine- <i>N,N</i> -diacetic acid
EDTMP	ethylenediamine- <i>N,N,N',N'</i> -tetrakis(methylene phosphonate
FDG	18-fluoro-deoxyglucose
HER2	human epidermal growth factor receptor type 2
HMPAO	hexamethylpropyleneamine oxime
HYNIC	hydrazinonicotinamide
KP46	tris(8-quinolinolato)-gallium(III)
NODAGA	triazacyclononane,1-glutaric acid-4,7-acetic acid
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
PET	positron emission tomography
RADIGIS	radioisotope generator integrated system
sCCK8	cholecystokinin 8

*Corresponding author: Milan Melník, Faculty of Pharmacy, Department of Pharmaceutical Analysis and Nuclear Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, Slovak Republic, e-mail: qmelnik@stuba.sk

Peter Mikuš: Faculty of Pharmacy, Department of Pharmaceutical Analysis and Nuclear Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, Slovak Republic; and Faculty of Pharmacy, Toxicological and Antidoping Center, Comenius University in Bratislava, Bratislava, Slovak Republic

Andrea Forgácsová, Dominika Krajčiová and Emil Havránek: Faculty of Pharmacy, Department of Pharmaceutical Analysis and Nuclear Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, Slovak Republic

SPECT single photon emission computed
tomography
TF transferrin

Introduction

Gallium has shown efficacy in the treatment of several apparently diverse disorders. These disorders can be broadly categorized as (i) accelerated bone resorption, with or without elevated plasma calcium; (ii) autoimmune diseases and allograft rejection; (iii) certain cancers; and (iv) infectious diseases (Lessa et al., 2012).

Gallium has several medically useful radionuclides that have made extensive contribution in both the diagnosis and therapy of diseases. In the late 1940s, ^{72}Ga was localized in bones and was suggested to be potentially useful for treating bone tumors. After extending this theory, ^{67}Ga was concluded as a better radioisotope, as ^{72}Ga has a short half-life ($T_{1/2}$, 14 h) and was only available with a low specific activity. In contrast, ^{67}Ga with a longer $T_{1/2}$ (78 h) could easily be made with no carrier added (i.e., this isotope can be isolated from cyclotron without any carrier atom). However, the imaging equipment at that time were not sophisticated enough to produce a good-quality clinical study with this radioisotope; therefore, the development in this field was not so pronounced. The introduction of a $^{68}\text{Ge}/^{68}\text{Ga}$ generator, in the early 1960s, rekindled interest in radiogallium for bone tumor localization. In the early 1970s, ^{67}Ga reemerged, as its characteristics better matched with the newly developed instrument, the γ -camera, and the lower amount of carrier gallium that was added to obtain bone localization. The carrier-free nuclide was found, unexpectedly, to localize in certain soft tissue tumors, and a few years later in inflammatory processes. These two uses for ^{67}Ga continue to this day (Welch and Redvanly, 2003).

A major advantage of a $^{68}\text{Ge}/^{68}\text{Ga}$ generator is its continuous source of ^{68}Ga , independently from an on-site cyclotron. The increase in knowledge of purification and concentration of the eluate and the complex ligand chemistry has led to ^{68}Ga -labeled pharmaceuticals with major clinical impact. ^{68}Ga -labeled pharmaceuticals have the potential to cover all of today's clinical options with $^{99\text{m}}\text{Tc}$, with the concordant higher resolution of positron emission tomography (PET) in comparison with single photon emission computed tomography (SPECT). ^{68}Ga -labeled analogues of octreotide (an octapeptide), such as DOTATOC, DOTANOC, and DOTA-TATE [where

DOTA is 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid, DOTANOC is DOTA-1-Nal3-octreotide, DOTATOC is DOTA-Tyr3-octreotide, and DOTA-TATE is DOTA-(Tyr3)-octreotate], are clinically applied in nuclear medicine. These analogues are now the most frequently applied of all of the ^{68}Ga -labeled pharmaceuticals (Breeman et al., 2011).

The ability of non-radioactive Ga(III) complexes to serve as therapeutic agents in oncology also has to be mentioned. For example, Ga-chloride or Ga-nitrate has inhibitory effects on cancer cell growth. Ga(III) ion is also used as a complexing metal for sonosensitizers such as porphyrins. They are used for sonodynamic therapy, the ultrasound-dependent enhancement of cytotoxic activities of certain compounds (sonosensitizers) (Rosenthal et al., 2004).

An aim of this work is to provide a brief view on and an evaluation of the most important gallium complexes applied in oncology in the period of the last two decades. Developments and new trends in this field are also briefly discussed.

Gallium and its isotopes

Gallium is the second metal ion, after platinum, to be used in cancer treatment. It is because of its ability to inhibit DNA and protein synthesis, and the activity of a variety of enzymes involving serum alkaline phosphatase and ribonucleotide reductase (Collery et al., 2002).

The solution and coordination chemistries of Ga(III) are somewhat similar to those of Al(III) and In(III); however, they are very similar to those of Fe(III). The biochemical similarities of these two ions, particularly concerning protein and chelate binding, are likely responsible for many of the physiological activities of gallium. Blood contains thousands of dissolved components, including many proteins, small-molecule ligands, anions, anionic groups, metal ions, and complexes of all of these components, in addition to colloidal and cellular components. *In vivo* studies using subnanomolar ^{67}Ga revealed that virtually whole gallium in blood is present in plasma, with traces in leukocytes (Bernstein, 1998).

Nearly all plasma gallium is tightly bound to the iron-transport protein, transferrin (TF). TF transports its metal load into cells through the TF receptor, a protein that can bind two TF molecules. This receptor binds most strongly to diferric TF, less strongly to monoferric TF, and weakly to apotransferrin (having no metal ions) at neutral pH levels.

The complex of metal-bearing TF and TF receptor is taken into the cell by endocytosis; the endosome is then acidified to release the metal. Malignant cells generally have a very high TF receptor expression. Gallium binds even more avidly to the related protein lactoferrin, which can remove Ga from TF. A third iron-binding protein to which gallium can bind is ferritin. Ferritin serves as iron storage, is present in most cells to varying degrees, and is particularly concentrated in the Kupffer cells of the liver; it is also concentrated in some other tissue macrophages (Lessa et al., 2012).

^{66}Ga has a half-life of 9.5 h and a high positron energy (4.15 MeV), and also has several γ -rays associated with the decay at energies >1 MeV. For this reason, it has been proposed as a radiodiagnostic agent. A ^{67}Ga - γ -emitter is one of the most widely used single photon markers for the presence of inflammation and malignancy. It has a 78 h half-life and decays by electron capture, emitting γ -rays at 93.3, 184.6, and 300.2 keV. The γ -ray at 184.6 keV is detected by γ -camera or SPECT imaging. The common way ^{68}Ge is used as the source of ^{68}Ga , which is employed very extensively in PET applications, is as a convenient source of positrons (positron emitter). It can also be produced directly, if that is desired (Welch and Redvanly, 2003). The short half-life of ^{68}Ga permits imaging applications with sufficient radioactivity while maintaining the patient's dose to an acceptable level. Furthermore, owing to superior resolution, ^{68}Ga -PET agents have the ability to replace the current SPECT agents in many applications (Smith et al., 2013). The ^{72}Ga isotope (14.3 h half-life) can be made either with fast or thermal neutrons. ^{72}Ga was used as the source of internal radiation because of the interest in this isotope as an irradiator of bone tumor (Brucer et al., 1953). The important nuclear characteristics of Ga isotopes are given in Table 1. Although the complexation properties of various isotopes of Ga(III) are the same, the characteristic radiation of particular isotopes is used for specific purposes, i.e., therapeutic (α or β radiation) and imaging (γ radiation).

$^{68}\text{Ge}/^{68}\text{Ga}$ generator and purification methods

Concerning gallium radioisotopes, this generator confers a huge advantage and makes a major impact on gallium radiopharmaceuticals. This fact is mentioned in almost all publications about ^{68}Ga . The invention of this generator and ^{68}Ga represent the very early applications of radionuclides to PET imaging at a time when even the wording of PET itself was not established (Rösch, 2013a).

In 1964, the first $^{68}\text{Ge}/^{68}\text{Ga}$ radionuclide generators were described. Although the generator design was by far not adequate in terms of today's level of chemical, radiopharmaceutical, and medical expectations, it perfectly met the needs of molecular imaging of this period (Rösch, 2013b). Today, it faces a renaissance in terms of new $^{68}\text{Ge}/^{68}\text{Ga}$ radionuclide generators, sophisticated ^{68}Ga radiopharmaceuticals, and state-of-the-art clinical diagnoses by means of PET/CT. Thanks to the pioneering achievement of radiochemists in Obninsk, Russia, a new type of $^{68}\text{Ge}/^{68}\text{Ga}$ generators became commercially available in the first years of the 21st century. Generator elutes based on hydrochloric acid provided 'cationic' ^{68}Ga instead of 'inert' ^{68}Ga -complexes, opening new pathways of Me(III)-based radiopharmaceutical chemistry (Rösch, 2013a). The increasing applications of generator-based ^{68}Ga radiopharmaceuticals (for diagnosis alone, but increasingly for treatment planning, thanks to the inherent option as expressed by theranostics) now ask for further developments – toward the optimization of $^{68}\text{Ge}/^{68}\text{Ga}$ generators both from the chemical and regulatory points of view. Dedicated chelators may be required to broaden the feasibility of ^{68}Ga labeling of more sensitive targeting vectors, and generator chemistry may be adopted to those chelators – or vice versa (Rösch, 2013b).

Generator-produced ^{68}Ga is typically of suboptimal purity, mainly due to the breakthrough of the parent radionuclide ^{68}Ge , and Fe(III) impurity (Belosi et al., 2013).

Table 1 Nuclear characteristics of Ga isotopes (Brucer et al., 1953; Welch and Redvanly, 2003).

Characteristic	^{67}Ga	^{68}Ga	^{66}Ga	^{72}Ga
Beam energy (MeV)	12–22	12–22	8–12	
γ Photon energy (keV)	93, 184, 300	–	1039, 2750	835
% Photons/disintegration	38, 24, 22	178	114, 37, 23	96
Electrons (keV)	84, 92	1900 (β^+)	4153 (β^+)	β^-
Half-life	78 h	68 min	9.5 h	14.3 h
Production method	$^{68}\text{Zn}(p,2n)^{67}\text{Ga}$	^{68}Ge daughter, $^{66}\text{Zn}(\alpha,2n)^{68}\text{Ge}$	$^{63}\text{Cu}(\alpha,n)^{66}\text{Ga}$	^{72}Ge
Contaminant	^{66}Ga , ^{65}Zn	^{68}Ge	^{67}Ga	
Way of detection	γ -Camera, SPECT	PET	γ -Camera, PET	γ -Camera

Anionic, cationic, and fractional elution methods are well known (Mueller et al., 2013). Modern automated synthesis modules adopt both fractionation methods and purification methods to get rid of ^{68}Ge breakthrough (Costa et al., 2013; Schultz et al., 2013).

There is also a good example of a $^{68}\text{Ge}/^{68}\text{Ga}$ generator combined with an automated ^{68}Ga elute purification-concentration unit [radioisotope generator integrated system (RADIGIS)], specially designed for ^{68}Ga processing (RADIGIS- ^{68}Ga) (Le, 2013). Another of the many examples of automated purification systems is described where [^{68}Ga] Ga-AMBA [AMBA=DO3A-CH(2)CO-G-(4-aminobenzoyl)-Q-W-A-V-G-H-L-M-NH(2), one of bombesin analogues (Schroeder et al., 2011)] was HPLC purified and Sep-Pak purified, using the built-in radiodetector of the Tracerlab FX F-N synthesizer to monitor fractionated $^{68}\text{Ge}/^{68}\text{Ga}$ generator elution and purification (Cagnolini et al., 2010).

There is a number of fully automated systems to produce ^{68}Ga precursors with various chelators already available and ready to use with an appropriate ligand.

Gallium pharmaceuticals

As was mentioned, Ga(III) and Fe(III) have some familiar similarities in biochemical behavior, and thus the free isotope ion binds to proteins in blood and tends to concentrate in areas of rapid cell division, which is typical for cancer cells, and in areas of higher concentration of leukocytes (such as inflammation sites). Ga(III) binds to TF, leukocyte lactoferrin, bacterial siderophores, inflammatory proteins, and cell membranes in neutrophils, both living and dead (Campain et al., 2010). These binding properties of Ga(III) are used in the diagnosis and therapy of both soft tissue and hard tissue diseases. The specificity of Ga(III) binding in the body can be properly modified through appropriate ligands and/or chelators. Although all the isotopes of Ga (^{66}Ga , ^{67}Ga , ^{68}Ga) can be used for imaging using different imaging equipment (PET, SPECT, and γ -camera), ^{66}Ga and ^{67}Ga , thanks to being sources of γ -rays with high energy, are appropriate for imaging as well. Their complexation with a suitable ligand (and, in case of a need, with a chelator as well) can provide a more specific targeting of tumor cells and enhance the cytotoxicity by synergic effects (e.g., by complexing with an already known cytotoxic drug or by a sonosensitizer) (Maecke et al., 2005; Maecke and Andre, 2007; Fani et al., 2008; Roesch and Riss, 2010; Wadas et al., 2010). ^{67}Ga imaging has proved useful in high-grade lymphomas, either to evaluate lymphoma at the initial staging or to assess the response to therapy.

The sensitivity of ^{67}Ga imaging, however, is highly dependent on the cell type and the size and location of the lesion (Kostakoglu and Goldsmith, 2000). ^{67}Ga imaging of lymphoma provides information about the presence or absence of an active lymphoma. Most important, the positive predictive value for the presence of active disease has consistently been shown to be greater than that for morphologic imaging techniques (such as CT and MRI), primarily owing to the presence of residual mass in treated lymphoma (Tumeh et al., 1987; Front et al., 1990; Gasparini et al., 1993; Front and Israel, 1995). However, ^{68}Ga , used only for the diagnosis of diseases, can be modified as well into a pharmacologically active molecular structure in the same way.

Renewed interest in ^{68}Ga has recently arisen from several reasons. First, PET has developed during the last decade from a research tool into a powerful diagnostic and imaging tool for routine clinical applications. Second, $^{68}\text{Ge}/^{68}\text{Ga}$ generators have been developed that produce suitable elutes for labeling that can be converted into ^{68}Ga -labeled pharmaceuticals for PET studies. Third, there are many DOTA peptides that can be labeled with ^{68}Ga . Fourth, a variety of monofunctional and bifunctional chelators have been developed that allow the formation of stable $^{68}\text{Ga}(\text{III})$ complexes and convenient coupling to biomolecules. Fifth, the availability of PET radiolabeled pharmaceuticals by the introduction of ^{68}Ga in radiopharmacy, independent of an on-site cyclotron, has opened new applications and possibilities. Coupling of ^{68}Ga to small peptides and biomolecules has recently been reviewed (Maecke et al., 2005; Maecke and Andre, 2007; Fani et al., 2008; Roesch and Riss, 2010; Wadas et al., 2010), and ^{68}Ga is potentially an alternative to ^{18}F - and ^{11}C -based radiopharmacy. Last but not least, equipment, including generators, purification and concentration of elute, techniques of radiolabeling, robotics, and PET cameras, have improved during the last decade.

Gallium pharmaceuticals can be divided into two groups according to their radioactivity. These are the radiopharmaceuticals employing radioactive $^{67,68}\text{Ga}(\text{III})$ isotopes, e.g., Ga(imidazole)₃ and its derivatives, as epidermal growth factor receptors (Garcia et al., 2009); GaCl₃, for imaging of lymphoma, inflammatory processes, transmitting nerve impulses, and regulatory fluid in and out of cells (Collery et al., 2000; Silvola et al., 2011); Ga-folate, as diagnostic agent for receptor-positive tumor (Melpomeni et al., 2011); Ga-citrate, suitable for scintigraphy for the detection of a wide variety of diseases: Hodgkin's, lung cancer, malignant lymphoma, and pancreatic cancer (Kunn et al., 1997; Liu et al., 2003; Lin et al., 2007); Ga-nitroimidazole and its derivative, for PET imaging of tumor hypoxia (Goldman, 1982; Juchau, 1989; Kunn et al.,

1997; Fernández et al., 2013); and Ga- biphosphonate, for PET tracing of bone lesions (Fellner et al., 2012). There are also some conventional pharmaceuticals employing non-radioactive Ga(III) pharmaceuticals used in oncology, e.g., Ga-EDTMP, for therapy of bone metastasis (Su et al., 2005); Ga(8-quinolinate)₃, for treating renal cell cancer (Collery et al., 2000; Jalilian et al., 2005; Rudnev et al., 2006); Ga-semicarbazones, with a wide spectrum of antitumor effects in the ovary, breast, and colon (Rudnev et al., 2006; Kalinowski et al., 2009; Gambino et al., 2011); Ga(maltol)₃, for the treatment of several types of cancer, including liver cancer and lymphoma (Rudnev et al., 2006); Ga(nitrate)₃, for the treatment of lymphomas, bone metastasis, and bladder cancer (Collery et al., 2002; Jakupec and Keppler, 2004); Ga-porphyrins, a sonosensitizer in photodynamic therapy (Rosenthal et al., 2004; Jalilian et al., 2005); Gapyrazole and its derivatives, as antitumor agents for the therapy of ovarian adenocarcinoma and human lung carcinoma (Tajiri et al., 1994; Balbi et al., 2011); and Gathiolate ligands, for their dose-dependent antiproliferative effect toward cancer cells (Gallego et al., 2011). Besides low molecular weight ligands (Gallego et al., 2011; Collery et al., 2002; Rosenthal et al., 2004; Garcia et al., 2009), protein biopolymers and even whole cells have been successfully employed in Ga(III) pharmaceuticals, as illustrated in Table 2. The structure and properties of such protein ligands are further illustrated and mentioned in Table 3. According to the target site of a drug, we can divide them into pharmaceuticals with an impact on hard tissue (bones and bone metastasis) and soft tissue (mainly the liver, spleen, ovaries, prostate, breasts, intestine and mucous tissue, various receptors in tumor cells, or any tissue undergoing inflammatory processes; i.e., most of the drugs), as will be discussed in the next two subsections, respectively. Gallium complexes in oncology can be used for imaging and/or therapy, which is discussed therein as well. The main advantage of radiopharmaceuticals over other drugs is that they are able to visualize metabolic pathways and the functions of cells and tissues.

Hard tissue gallium pharmaceuticals

Gallium has long been known to concentrate in skeletal tissue, particularly regions of bone deposition and remodeling. In growing bone, gallium is concentrated in the metaphysis, particularly in the hypertrophic cartilage zone (growth plate); it is also concentrated in regions of fracture healing. To a less extent, gallium accumulates on

the endosteal and periosteal surfaces of diaphyseal bone (Bernstein, 1998). A possible way of Ga getting into bone is through calcium channels or by a simple diffusion. Only a minimum amount goes through the TF receptor (Bernstein, 1998; Liu et al., 2003).

Elemental gallium is a potent inhibitor of bone resorption that acts to maintain or restore bone mass. By virtue of these biological effects, gallium compounds are potentially useful treatments for a variety of diseases that are characterized by accelerated bone loss, including cancer-related hypercalcemia, bone metastases, Paget's disease, and postmenopausal osteoporosis (Warrell, 1995).

Gallium nitrate was originally developed as an anti-neoplastic agent; however, further studies have revealed that this drug has extremely potent effects on the turnover of bone, and that low doses can be used to reduce bone resorption. The results of randomized double-blind studies have suggested that this drug has superior clinical efficacy relative to etidronate, calcitonin, and pamidronate for the acute control of cancer-related hypercalcemia (Warrell, 1997). Gallium nitrate injection (Ganite) has been approved by the US Food and Drug Administration as an intravenous treatment for patients with cancer-related hypercalcemia (Su et al., 2005).

⁶⁷Ga whole-body scan is commonly used in nuclear medicine, and it is useful for finding bone tumors. ⁶⁷Ga-nitrate or citrate is used as a carrier molecule. ⁶⁷Ga is imaged with a γ-camera, a SPECT camera, or SPECT/CT hybrid machines.

The specificity of Ga pharmaceuticals toward the target is given by the ligand in Ga compound. For example, EDTMP (ethylenediamine-*N,N,N',N'*-tetrakis(methylene phosphonate)) is a ligand with a high affinity for skeletal tissue, and its Ga(III) complex is used to treat bone metastasis (Su et al., 2005). Bone metastases are a serious aggravation for patients with cancer. Therefore, early recognition of bone metastases is of great interest for the further treatment of patients. Bisphosphonates are widely used for scintigraphy of bone lesions with ^{99m}Tc. Using the ⁶⁸Ge/⁶⁸Ga generator together with a macrocyclic bisphosphonate, a comparable PET tracer comes into focus. The bisphosphonate DOTA-conjugated ligand reveals great potential for the diagnosis of bone metastases by means of PET/CT (Fellner et al., 2012).

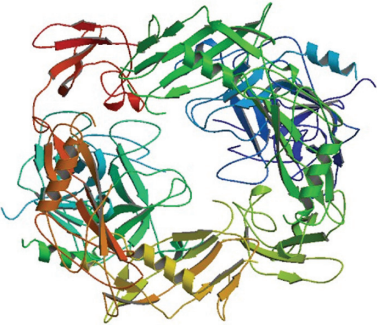
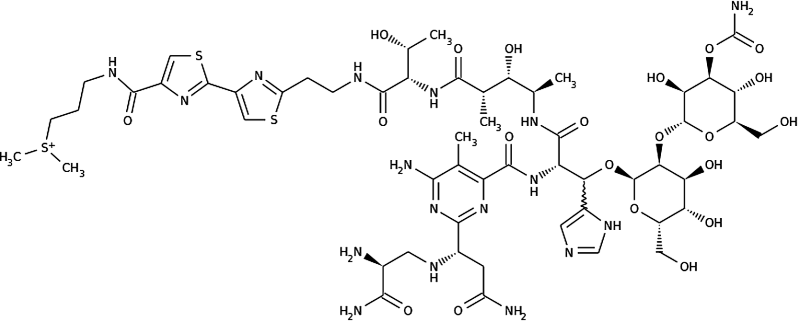
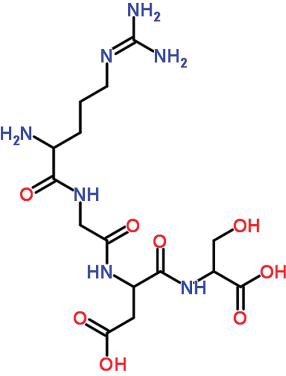
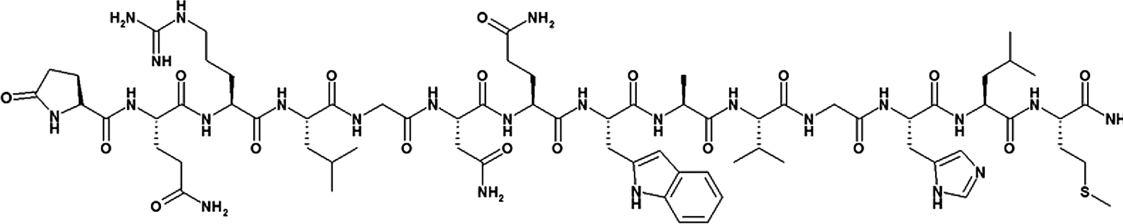
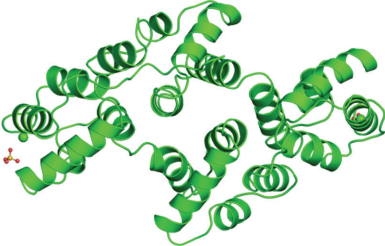
Soft tissue gallium pharmaceuticals

Unlike in hard tissues, gallium penetrates soft tissues mainly through TF receptors. Isotopes such as ⁶⁸Ga have

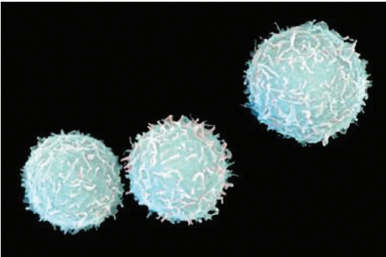
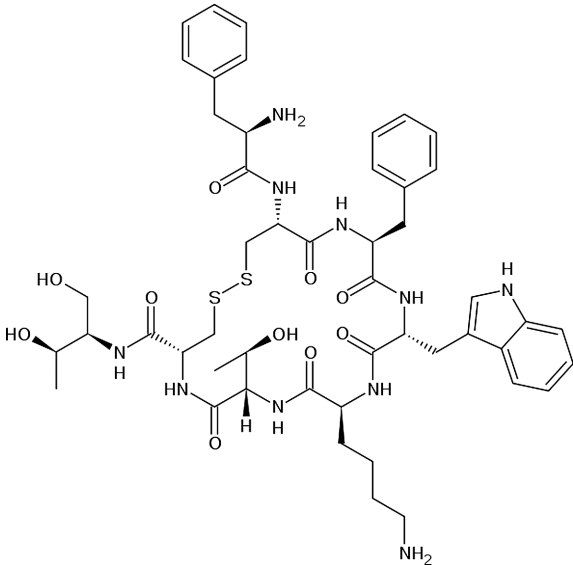
Table 2 Examples of protein ligands for Ga pharmaceuticals.

Protein ligand	Usage	Gallium type	Other ions used	Chelators used	References
Streptokinase	SPECT imaging of thrombi in many cardiovascular diseases	$^{67,68}\text{Ga}$	$^{99\text{m}}\text{Tc}$, ^{131}I	DTPA-dianhydride	(Jalilian et al., 2009)
Bleomycin	SPECT imaging and/or therapy of neoplastic tissues	^{68}Ga	^{111}In , ^{57}Co , $^{99\text{m}}\text{Tc}$, radioferric salts, and ^{105}Rh	–	(Jalilian et al., 2005)
Arg-Gly-Asp (RGD) and bombesin (BBN) analogues	Arg-Gly-Asp (RGD) peptide images integrin $\alpha_v\beta_3$ expression through PET; bombesin (BBN) analogues target the gastrin-releasing peptide receptor and visualize/treat prostate cancer	$^{67,68}\text{Ga}$	^{111}In , ^{177}Lu , ^{18}F , ^{64}Cu	DOTA, NOTA	(Liu et al., 2009a,b; Schroeder et al., 2011; Fournier et al., 2012; Inkster et al., 2013; Sturzu et al., 2014; Varasteh et al., 2013)
Annexin V	Apoptosis-detecting radioligand, SPECT/PET imaging	$^{67,68}\text{Ga}$	$^{99\text{m}}\text{Tc}$, ^{123}I , ^{124}I , ^{125}I , ^{111}In , ^{18}F , ^{64}Cu	DOTA	(Lahorte et al., 2004)
White blood cells	SPECT imaging of acute inflammation and infection	^{67}Ga	^{111}In , $^{99\text{m}}\text{Tc}$	DOTA, HMPAO, oxine	(Roivainen et al., 2012)
Octreotide derivatives such as DOTANOC, DOTATOC	PET imaging methods in the diagnosis of thoracic and gastroenteropancreatic (GEP) neuroendocrine tumors (NETs)	^{68}Ga	^{111}In , $^{99\text{m}}\text{Tc}$, ^{90}Y , ^{177}Lu	DOTA	(Breeman et al., 2011; Treglia et al., 2012)
AE105- NH_2	PET imaging of the urokinase-type plasminogen activator receptor (u-PAR) expression in tumor cells	^{68}Ga	^{64}Cu	DOTA, NODAGA	(Leung, 2004–2013; Persson et al., 2012)
Anti-HER2 nanobodies (2Rs15d)	PET tracer of high-specific-contrast imaging of HER2-positive tumors and breast cancer	^{68}Ga	$^{99\text{m}}\text{Tc}$	NOTA	(Vaneycken et al., 2011; Xavier et al., 2013)
Cyclo-MG1 and cyclo-MG2	A minigastrin analogue for PET imaging of gastrin receptor overexpressed in most tumor tissues, such as medullary thyroid carcinomas (>90%), astrocytomas (>65%), and stromal ovarian cancers (100%)	^{68}Ga	^{111}In , $^{99\text{m}}\text{Tc}$	DOTA, EDDA, HYNIC	(Chopra, 2004–2013a,b)
sCCK8 derivatives	PET imaging of gastrin receptor overexpressed in most tumor tissues, such as medullary thyroid carcinomas (>90%), astrocytomas (>65%), and stromal ovarian cancers (100%)	^{68}Ga	^{111}In , $^{99\text{m}}\text{Tc}$	DOTA, EDDA, HYNIC	(Chopra, 2004–2013a,b)

Table 3 Structure and properties of some protein ligands for Ga pharmaceuticals.

Ligand	Structure	Properties of ligand
Streptokinase	$C_{2100}H_{3278}N_{566}O_{669}S_4$ 	A protein binding and activating human plasminogen; causes lysis of venous thrombi
Bleomycin	$C_{555}H_{84}N_{17}O_{21}S_3$ 	A glycopeptide antibiotic causing breaks in DNA
Arg-Gly-Asp	$C_{15}H_{27}N_7O_8$ 	A short amino acid sequence; plays a role in many recognition systems involved in cell-to-cell and cell-to-matrix adhesion
Bombesin	$C_{71}H_{110}N_{24}O_{18}S$ 	A peptide that stimulates gastrin release from G cells
Annexin V		A member of the calcium and phospholipid binding superfamily of Annexin proteins

(Table 3 Continued)

Ligand	Structure	Properties of ligand
White blood cells		Cells of the immune system involved in defending the body against infectious disease and foreign materials
Octreotide	$C_{49}H_{66}N_{10}O_{10}S_2$ 	An octapeptide that mimics natural somatostatin pharmacologically, although it is a more potent inhibitor of growth hormone, glucagon, and insulin than the natural hormone

suitable physical properties with a high positron yield reaching 89% of all disintegrations. Its half-life of 68 min matches the pharmacokinetics of many peptides and other small molecules owing to a fast blood clearance, quick diffusion, and target localization (Al-Nahhas et al., 2007). The peptide-binding properties and proper half-life of ^{68}Ga indicates its suitability for soft tissues.

^{67}Ga seems to behave as an analogue of the ferric ion, accumulating within the tumor cell by simple diffusion and possible penetration through the calcium channels. Many studies have demonstrated the usefulness of ^{67}Ga scan in detecting Hodgkin's disease (cancer of lymph tissue found in the lymph nodes, spleen, liver, and bone marrow) (Liu et al., 2003).

Whole-body scintigraphy with ^{67}Ga -citrate (^{67}Ga -citrate scan) has been one of the most widely used tumor-seeking radiopharmaceutical, with great value in detecting lung cancer and malignant lymphoma. Meanwhile, pancreatic cancer is detectable by ^{67}Ga -citrate scan at low sensitivity (Kunn et al., 1997). ^{67}Ga and labeled leukocytes are

useful in the detection of an unknown infectious source. However, the delay in the diagnosis of a ^{67}Ga -citrate scan (72 h for a whole-body scan) and the complicated labeling technique of a ^{67}Ga -leukocyte scan are major drawbacks to their clinical use (e.g., higher time/preparation demands are not acceptable in case of acute inflammations). ^{67}Ga -citrate scan was the first radionuclide method to be widely used in the imaging of inflammation. The mechanism of gallium uptake in infectious sites is not completely understood. Multiple factors are thought to contribute to the accumulation and retention of ^{67}Ga in inflammatory lesions, including increased capillary permeability at the inflammatory sites, binding of gallium to tissue proteins such as lactoferrin, as well as direct leukocyte and bacterial uptake (Lin et al., 2007). Nowadays, ^{67}Ga -citrate scan is supplanted by FDG scan in developed countries.

Chelating agents are molecules that have the ability to form more than one bond to a metal ion, thereby increasing the stability of the ion complex. Compounds such as DOTA, DTPA, DOTANOC, DOTATATE, chloride, or citrate

acid are chelating agents of different strengths. Several studies showed that somatostatin receptor PET and PET/CT, using different radiopharmaceuticals (e.g., [^{68}Ga]DOTANOC, [^{68}Ga]DOTATOC, and [^{68}Ga]DOTATATE), are accurate imaging methods in the diagnosis of thoracic (mainly pulmonary and thymic) and gastroenteropancreatic neuroendocrine tumors (Breeman et al., 2011).

Radiolabeled peptides are of increasing interest in nuclear oncology. Special emphasis has been given to the development of peptides labeled with positron emitters. Several bifunctional chelators based on 1,4,7-triazacyclononane- N,N',N'' -triacetic acid and 1,4,7,10-tetraazacyclododecane- N,N',N'',N''' -tetraacetic acid macrocycles are available for coupling to peptides and other biomolecules. In addition to these hydrophilic chelators, a lipophilic tetradentate S(3)N legend was developed. The recent clinical experience with ^{68}Ga -peptides includes imaging neuroendocrine tumors, particularly carcinoid [^{68}Ga]DOTA,Tyr(3)-octreotide, localizing neuroendocrine tumors with higher sensitivity than [^{111}In]diethylenetriaminepentaacetic acid-octreotide, as well as neuroectodermal tumors such as pheochromocytoma and paraganglioma or prostate cancer. *In vitro* and animal testing are still progressing alongside clinical studies, with promising results in the use of [^{68}Ga]DOTA-rhenium-cyclized α -melanocyte stimulating hormone and [^{68}Ga]DOTA-napamide in melanoma, [^{68}Ga]DOTA-PEG(4)-BN(7-14) for the imaging of bombesin receptor-positive tumors, and [^{68}Ga]ethylene dicysteine-metronidazole for imaging tumor hypoxia. In addition to tumors, the [^{68}Ga]DOTA peptide inhibitor of vascular peptide protein 1 is being assessed for imaging inflammatory reaction. An additional value following a positive scan is the use of β -emitters labeled to the same peptides for radionuclide treatment (Pagou et al., 2009). The use of peptides for PET molecular imaging has undeniable advantages: the possibility of targeting through peptide-receptor interaction, small size and low-molecular weight conferring good penetration in the tissue or at cellular level, low toxicity, no antigenicity, and the possibility of a wide choice for radiolabeling (for the representative examples, see Table 2) (Morgat et al., 2013). In conclusion, the recent introduction of ^{68}Ga -peptides could greatly contribute to the management of a wide range of clinical conditions, including tumors and inflammation (Pagou et al., 2009).

Complexation of gallium with organic ligands has been recognized as a promising strategy for creating tumor-inhibiting therapeutics with a number of advantages over gallium salts concerning oral bioavailability. Indeed, their evolved hydrolytic stability and membrane

penetration ability render gallium complexes improved intestinal absorption functions compared with nitrate or chloride salt, which leads to increased plasma concentrations of gallium. Owing to such benefits, as well as better antiproliferative properties, two oral compounds, tris(8-quinolinolato)-gallium(III) (KP46) and tris(3-hydroxy-2-methyl-4*H*-pyran-4-onato)gallium(III) (gallium maltolate), have been selected from a series of gallium complexes for clinical development. KP46 has finished phase I trials, with the outcome of promising tolerability and evidence of clinical activity in renal cell carcinoma; however, it also reduces the viability of A549 human malignant lung adenocarcinoma cells. Gallium maltolate is a promising chemotherapeutic agent for the treatment of hepatocellular carcinoma. Likewise, bis(2-acetylpyridine-4,4-dimethyl-3-thiosemicarbazonato- N,N,S)gallium(III) tetrachlorogallate(III) (KP1089) was the first representative of the class of α -*N*-heterocyclic thiosemicarbazone complexes that has been assayed for antineoplastic activity in human tumor cell lines – ovary, breast, and colon (Collery et al., 2000; Arion et al., 2002; Chua et al., 2006; Rudnev et al., 2006; Chitambar et al., 2007; Timerbaev, 2009).

Another promising bioactive organic ligand is the nanobodies. Nanobodies are the smallest fully functional antigen-binding antibody fragments possessing ideal properties as probes for molecular imaging. The anti-human epidermal growth factor receptor type 2 (HER2) nanobody was labeled with ^{68}Ga through a 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) derivative, and its use for HER2 iPET imaging was assessed. Preclinical validation showed high-specific-contrast imaging HER 2-positive tumors with no observed toxicity. [^{68}Ga]NOTA-2Rs15d is ready for the first-in-human clinical trials (Xavier et al., 2013).

Over the years, increasing attention has been paid to pyrazoles in drug and related research owing to the ability of metal complexes to be anticancer (e.g., human colorectal cell lines, gastric cell lines, leukemia cells, and many others), antibacterial/parasitic, and antiviral agents (Tajiri et al., 1994).

The trivalent gallium cation is capable of inhibiting tumor growth, mainly because of its resemblance to ferric iron. It affects cellular acquisition of iron by binding to TF, and it interacts with the iron-dependent enzyme ribonucleotide reductase, resulting in reduced intracellular deoxyribonucleoside triphosphate (dNTP) pools and inhibition of DNA synthesis. The abundance of TF receptors and the upregulation of ribonucleotide reductase render tumor cells susceptible to the cytotoxicity of gallium. A remarkable clinical activity in lymphomas and bladder cancer has

been documented in clinical studies employing intravenous gallium nitrate (Jakupec and Keppler, 2004).

Another way to treat cancer is through photodynamic therapy. Tetrapyrroles are multisided natural products that are of relevance in clinical medicine. Owing to their specific accumulation in tumor tissue, porphyrins, metalloporphyrins, and chlorins have been used in photodynamic therapy and optical imaging in connection with radionuclides (Zoller et al., 2013). Ga-porphyrins are used for sonodynamic therapy, the ultrasound-dependent enhancement of cytotoxic activities of certain compounds (sonosensitizers). The possible mechanisms of sonodynamic therapy include generation of sonosensitizer-derived radicals that initiate chain peroxidation of membrane lipids through peroxy and/or alkoxy radicals; the physical destabilization of the cell membrane by the sonosensitizer, thereby rendering the cell more susceptible to shear forces; or ultrasound-enhanced drug transport across the cell membrane (sonoporation) (Rosenthal et al., 2004). For example, photodynamic therapy using the new photosensitizer Ga-porphyrin complex could be applied for the treatment of pancreatic cancer (Tajiri et al., 1994). Tetrapyrroles are multisided natural products that are of relevance in clinical medicine. Owing to their specific accumulation in tumor tissue, porphyrins, metalloporphyrins, and chlorines have been used in photodynamic therapy and optical imaging, for example, labeled using ^{68}Ga . A proof-of-concept PET study of ^{68}Ga -labeled tetrapyrrole complexes suggests the suitability of these novel tracer candidates for PET imaging (Zoller et al., 2013). Other examples are metal nanoparticles that have become of great interest in research as photosensitizers in photodynamic therapy of cancer, drug delivery, and photodynamic antimicrobial therapy (Managa et al., 2014).

Conclusions

Early and specific tumor detection, and also therapy selection and response evaluation, are some challenges of personalized medicine (Morgat et al., 2013). The development of new radiopharmaceuticals and their availability are crucial factors influencing the expansion of clinical nuclear medicine. The contribution of Ga in this way is undeniable. It has earned its place in the treatment of bone tumors and bone metastases (e.g., Ga-nitrate), as well as in whole-body scan γ -scintigraphy (^{67}Ga -citrate). The number of new ^{68}Ga -based imaging agents for PET has increased greatly in the last decade. ^{68}Ga has been used for labeling of a broad range of molecules (small

organic molecules, peptides, short amino acid sequences, proteins, and oligonucleotides) as well as particles (nanoparticles, body cells, monoclonal antibodies), thus demonstrating its potential to become a PET analogue of the legendary generator-produced γ -emitting $^{99\text{m}}\text{Tc}$ but with the added value of higher sensitivity and resolution, as well as quantitation and dynamic scanning (Velikyan, 2013). This is exactly where Ga pharmaceuticals are heading today, focusing mainly on the ^{68}Ga isotope while it is available right on site, in hospitals, thanks to the generator mentioned above; high purity and radiochemical yields (important factors for the development of an automated processing system); lower toxicity compared with $^{66,67}\text{Ga}$ isotopes; dynamic scanning; and a number of chelators available for complexing with biomolecules. In a review on the continuing role of radionuclide generator systems for nuclear medicine, Knapp and Mirzadeh (1994) stated that ‘despite the availability of the $^{68}\text{Ge}/^{68}\text{Ga}$ generator application of ^{68}Ga , radiopharmaceuticals may suffer from the complex ligand chemistry required for Ga(III) complexation to useful tissue-specific radiopharmaceuticals’. Indeed, at that time (1994), no ^{68}Ga -labeled pharmaceuticals were in clinical studies. However, in 2011, two ^{68}Ga -labeled pharmaceuticals – [^{68}Ga]N-[(4,7,10-tricarboxymethyl-1,4,7,10-tetraazacyclododec-1-yl)acetyl]-D-phenylalanyl-L-cysteinyl-L-tyrosyl-D-tryptophanyl-L-lysyl-L-threoninyl-L-cysteinyl-L-threonine-cyclic(27) disulfide and [^{68}Ga]pasireotide tetraxetan – for the diagnosis of gastroenteropancreatic endocrine tumors were found. However, these drugs are still not authorized by the European Medicines Agency but are in a pipeline. There is an urgent need to reduce the cost (i.e., time and money) of developing imaging agents for routine clinical use (Breeman et al., 2011). Generator-produced ^{68}Ga and the development of small chelator-coupled peptides (and other small biomolecules) may open a new generation of freeze-dried, good manufacturing practice-produced, kit-formulated PET radiopharmaceuticals similar to $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ -generator-based, $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals (Maecke et al., 2005).

From this review, it is obvious that Ga has a wide spectrum of use in cancer therapy, mainly as an imaging agent for the early detection of cancer cells or malignant changes in the body, as a therapeutic agent for treating various cancers, and even as a sonosensitizer. Another example of the importance of radionuclide medicine lies in the early diagnosis of abscesses. Most reports agree that CT is the imaging method of choice for the diagnosis of intra-abdominal abscesses. However, in patients with a distortion of normal anatomy because of recent trauma or surgery, an early infection before the development of

discrete fluid collections is more difficult to detect with CT. In these clinical situations, radionuclide scanning may be of great value (Lin et al., 2007). Ga(III) complexes with pharmaceutically active organic ligands may show additive or synergistic effects of both metal and ligands in one same compound. If the metal acts by a mechanism that is distinct from that of the ligands, different targets would be reached by the administration of the complex. In addition, organic ligands may be useful carriers of gallium into the cells. Although the pharmacological properties of gallium have been extensively investigated, there is not a great number of non-radioactive Ga(III) complexes that have been examined for their therapeutic potential (Lessa et al., 2012).

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