Published in final edited form as:

Bioconjug Chem. 2009 May 20; 20(5): 825–841. doi:10.1021/bc800299f.

Radioimmunoimaging with longer-lived positron-emitting radionuclides: potentials and challenges

Tapan K. Nayak and Martin W. Brechbiel*

Radioimmune & Inorganic Chemistry Section, Radiation Oncology Branch, National Cancer Institute, National Institute of Health, Bethesda, MD-20892, USA

Abstract

Radioimmunoimaging and therapy has been an area of interest for several decades. Steady progress has been made towards clinical translation of radiolabeled monoclonal antibodies for diagnosis and treatment of diseases.

Tremendous advances have been made in imaging technologies such as positron emission tomography (PET). However, these advances have so far eluded routine translation into clinical radioimmunoimaging applications due to the mismatch between the short half-lives of routinely used positron-emitting radionuclides such as ^{18}F versus the pharmacokinetics of most intact monoclonal antibodies of interest. The lack of suitable positron-emitting radionuclides that match the pharmacokinetics of intact antibodies has generated interest in exploring the use of longer-lived positron emitters that are more suitable for radioimmunoimaging and dosimetry applications with intact monoclonal antibodies.

In this review, we examine the opportunities and challenges of radioimmunoimaging with select longer-lived positron-emitting radionuclides such as ^{124}I , ^{89}Zr and ^{86}Y with respect to radionuclide production, ease of radiolabeling intact antibodies, imaging characteristics, radiation dosimetry and clinical translation potential.

Keywords

PET imaging; radioimmunoimaging; monoclonal antibodies; longer-lived positron-emitters

Introduction and Background

Applications of radiation in medicine have been described now for over 100 years. The use of radiation in medicine branches from many scientific discoveries, most notably the discovery of x-rays in 1895 and its use in surgery (1). Since that time frame, over the next 60 years critical advances in nuclear medicine technology and instrumentation have resulted in methodologies and technologies for the visualization of many of the body's organs, including liver and spleen scanning, brain tumor localization, and studies of the gastrointestinal tract by the injection of radionuclides (2, 3). The greatest potential use of radiation in medicine is its utility to provide diagnostic information of pathological processes before the outset of structural changes in an organ. In these applications, very small amounts of radioactive material most often labeled or conjugated to "smart" targeting agents such as antibodies, peptides, and small molecules are introduced into the body. These

*Corresponding author: Dr. Martin W. Brechbiel, Building 10, Room 1B40, NCI-Bethesda, Bethesda, MD 20892, Fax: 301-402-192, martinwb@mail.nih.gov.

Authors declare no conflict of interests.

“smart” agents specifically target individual cells instead of just the general tissues or organs, and therefore provide more valuable information about actual pathology. One example of such “smart” targeting agents is the monoclonal antibody (mAb). Currently, 21 monoclonal antibodies (all intact) are approved by the U.S Food and Drugs Administration (U.S.F.D.A) for diagnosis and treatment of various illnesses.

Antibodies in Nuclear Medicine

Currently radiolabeled antibodies are clinically used for numerous applications such as oncology and cardiology (4, 5). Use of radiolabeled antibodies for targeting specific organs in animals have been explored almost half a century ago (6, 7). Radiolabeled antibodies have been used in the clinic for therapeutic and diagnostic purposes now for over 40 years (8, 9). In 1978, Goldenberg and colleagues successfully applied the principles of antibody-antigen binding by visualizing carcinoembryonic antigen (CEA) on tumors of patients with a history of cancer of diverse histopathology by injecting ^{131}I labeled goat IgG targeting CEA. However, the large scale application of radiolabeled antibodies in the clinic was hindered due to low production yields and concerns of toxic immune reactions after injecting antibodies of animal origin into humans. The introduction of hybridoma technology for monoclonal antibody (mAb) production as developed by Kohler and colleagues along with the evolution of recombinant DNA technology has addressed many of these problems (10). Chimeric and humanized monoclonal antibodies and even completely human antibodies are now the standard. Numerous clinical studies have since been reported describing the use of radiolabeled intact antibodies for diagnosis of cancer using γ -scintigraphy and single photon emission tomography (SPECT) imaging (4, 11–13).

In spite of great successes in pre-clinical animal models, the promise of radioimmunoimaging by γ -scintigraphy has not fully lived up to expectations, mostly due to differences in biodistribution and pharmacokinetic characteristics between animals and humans, and limitations of γ -scintigraphy in terms of intrinsic spatial resolution (14). Radionuclides that decay with γ -energies lower than 100 keV produce too much scatter, while γ -energies over 250 keV are difficult to collimate and therefore pose a challenge for quantitative γ -scintigraphy. To overcome the disadvantages posed by γ -scintigraphy attempts were made to exploit the superiorities of positron emission tomography (PET) for radioimmunoimaging. A single positron decay results in two 511 keV photons being emitted at 180 degrees. Most PET cameras contain a circular array of detectors with coincidence circuits designed to capture 511 keV photons emitted in opposite direction and therefore offer much better resolution and counting efficiency as compared to conventional γ -scintigraphy and SPECT cameras (15). However, a major limitation challenging PET radioimmunoimaging was that the half-life of most of the routinely used PET radionuclides such as ^{18}F ($t_{1/2} = 1.8$ h) and ^{11}C ($t_{1/2} = 0.3$ h). The half-lives of routinely used PET radionuclides simply did not match well with the biological half-lives (up to several days) and pharmacokinetic parameters of slowly localizing intact antibodies. To overcome the initial blood pool uptake and slow localization of the intact antibody, antibody fragments (Fab) were labeled with ^{18}F for PET imaging. However, ^{18}F labeled antibody fragments failed to demonstrate high tumor localization as demonstrated by parent intact antibodies (16, 17). ^{18}F labeled antibody fragments were cleared from the tumor relatively more quickly than intact antibodies and then rapidly excreted through the urinary route delivering high radiation doses to the kidneys (16, 17). Numerous attempts have been made to modify intact antibodies to increase tumor uptake and residence time, and concomitantly decrease kidney uptake for successful PET with ^{18}F (18, 19); however, to date results from these strategies have demonstrated low to moderate success. Another drawback of using ^{18}F as a choice of radionuclide for PET radioimmunoimaging is the cumbersome chemistry involved

which requires synthesis of ^{18}F labeled intermediate precursors for indirect labeling of antibodies with ^{18}F (17, 20)

As an alternative to the approach of modifying the antibody to match the physical characteristics of ^{18}F , the use of longer-lived positron emitters has been explored for radioimmunoimaging with intact antibodies (21–23). The half-lives of longer-lived positron emitters such as ^{124}I and ^{89}Zr (shown in Table 1) have a much closer match with the biological half-life of most intact antibodies. Antibodies labeled with longer-lived positron emitters can be imaged for 2–5 days after injection when the radioactivity in the blood pool has been cleared and target to background ratio is higher. In this report, we have examined the potentials and challenges of selected longer-lived positron-emitting radionuclides for radioimmunoimaging with intact antibodies. The criteria applied for selecting these longer-lived positron emitters (Table 1) included previously published reports with antibodies or clinical studies, a potentially suitable half-life (10 hr–140 h) versus biological half-life, an imageable positron emission, available or potentially available conjugation and radiolabeling chemistry, and reasonable or acceptable production logistics and radiation toxicity.

Selected longer-lived positron-emitting radionuclides for radioimmunoimaging

Numerous longer-lived positron-emitting radionuclides can be produced by cyclotrons, accelerators, and reactors. Selected longer-lived positron emitters ranging from halogens to metals are described below with respect to their production, feasibility of use in radiolabeling an intact antibody, biological applications and imaging characteristics.

Iodine-124

Iodine (atomic radius of 1.3 Å and electronegativity of 2.66) belongs to Group VII of the periodic table. ^{124}I is a positron-emitting radionuclide with a complex decay scheme associated with numerous high energy γ -emissions (0.6 MeV, 61 % abundance) and high-energy positron emissions (2.14 MeV, 24 % abundance) as shown in Table 1. ^{124}I ($t_{1/2}=100.2$ h) decays to a stable ^{124}Te daughter. The relatively longer half-life makes ^{124}I conducive for labeling intact antibodies for PET imaging over several days after administration. Additionally, the vast experience of radiolabeling proteins with the other Iodine radionuclides, i.e. ^{131}I , ^{125}I , and ^{123}I , promotes a significant level of confidence since existing chemistry and protocols are directly applicable for use with ^{124}I .

Production of ^{124}I

^{124}I can be produced using a conventional medical cyclotron using the $^{124}\text{Te}(\text{p},\text{n})^{124}\text{I}$ and $^{124}\text{Te}(\text{d},2\text{n})^{124}\text{I}$ reactions [Table 2]. Typically, highly enriched tellurium dioxide is used as the target and ^{124}I is isolated using dry distillation with minimal impurities (24–26). The yield ($<0.1\text{mCi}/\mu\text{Ah}$) from the $^{124}\text{Te}(\text{p},\text{n})^{124}\text{I}$ reaction is low at 15 MeV (27). Alternatively, ^{124}I can be produced through a $^{125}\text{Te}(\text{p},2\text{n})^{124}\text{I}$ reaction with about four times higher yields than the commonly used $^{124}\text{Te}(\text{p},\text{n})^{124}\text{I}$ and $^{124}\text{Te}(\text{d},2\text{n})^{124}\text{I}$ reactions (24, 27). However, the production of ^{124}I through the $^{125}\text{Te}(\text{p},2\text{n})^{124}\text{I}$ reaction requires a medium-high energy cyclotron and impurities are relatively higher than the $^{124}\text{Te}(\text{p},\text{n})^{124}\text{I}$ reaction.

Radiolabeling of antibodies with ^{124}I

Radio-iodinated antibodies have been used for *in vivo* applications for more than 60 years (6, 7, 28, 29). In the early 1960's, Hunter and co-workers described a method (Chloramine-T method) to prepare high specific-activity radio-iodinated antibodies using p-toluene

sulfonochloramide [Figure 1(a)] (30, 31). Briefly, the Chloramine-T method is an oxidative method which involves exposure of the substrate to Chloramine-T in the presence of NaI for a short time, producing high specific activity proteins labeled with carrier-free radioiodine. However, the major disadvantage of Chloramine-T method is the risk of oxidation of thiol groups and protein denaturation due to the presence of high concentrations of strong oxidizing agent which can compromise the intended biological use of the antibody. As an alternative, an enzymatic method using lactoperoxidase as a catalyst was developed for iodination of antibodies (32, 33). Lactoperoxidase catalyzes the oxidation of iodide using hydrogen peroxide as the enzyme substrate and is a milder oxidative agent than Chloramine-T. Due to the continuing concerns of protein denaturation and loss of biological activity by oxidizing agents, a newer technique (Bolton-Hunter method [Figure 1(b)]) was later developed using iodinated 3-(4-hydroxyphenyl)propionic acid *N*-hydroxysuccinimide ester, which reacts with free amino groups in the protein molecule to attach the radioiodine-labeled groups by amide bonds (34, 35). The Bolton-Hunter method is a non-oxidative technique which is less harsh on the proteins than alternative methods. Similarly, to avoid the interaction of oxidizing agent with the protein, an alternative solid-phase oxidation using 1,3,4,6-tetrachloro-3 α ,6 α -biphenyl glycoluril [Figure 1(c)] was developed and is commonly called as the Iodogen method (36). All of the above methods suffered from the drawback of *in vivo* deiodination in presence of enzymes. To overcome *in vivo* deiodination, another method was developed using *N*-succinimidyl 4-iodobenzoate (PIB) [Figure 1(d)] to specifically label antibodies and proteins (37). This method produced a more stable radio-iodinated antibody as compared to methods like Bolton-Hunter as PIB does not contain an *o*-phenol moiety which protects PIB derivatives from the deiodinases that can act to delabel *o*-iodophenols that are formed with the other radio-iodination methods. As a result of increased *in vivo* stability, the uptake in the thyroid was dramatically reduced when a comparison was made between the same antibody radioiodinated using the PIB and Chloramine-T methods (37). Many monoclonal antibodies are internalized via either clathrin dependent or independent pathways. Antibodies rapidly internalized (within 2–4 hours) via the clathrin-dependent endocytosis pathway are catabolized within lysosomes. Iodotyrosine is known to rapidly exit from the lysosome and the cell after catabolism and as result target to background ratios are poor (38–40). In order to overcome the issue of catabolism of conventional radio-iodinated antibodies, dilactitol-tyramine (DLT) and radioiodinated diethylenetriaminepentaacetic acid-appended peptides have successfully been used to residualize the radio-iodinated antibody within the cells (41–44). As a result of decreased catabolism, the tumor uptake of cell surface binding radio-iodinated antibodies was significantly higher than antibodies radiolabeled with Chloramine-T method (41, 42).

Detailed methodologies for radio-iodination of proteins can be found in a review article published in *Bioconjugate Chemistry* (45). Currently, most ^{124}I labeled antibodies are prepared using commercial iodination kits based on some of the above described techniques.

Biological studies with ^{124}I labeled antibodies

In the early 1990's, murine monoclonal antibody H17E2 recognizing placental alkaline phosphatase (PLAP) was radiolabeled with ^{124}I using the Iodogen method for targeting PLAP on HEp2 human tumor xenografts (21). The ^{124}I labeled H17E2 localized in the tumor for at least 7 days demonstrating the feasibility of using monoclonal antibodies labeled with ^{124}I for tumor localization studies (21). The utility of antibodies labeled with ^{124}I for PET imaging, imaging feasibility, and quantification studies were studied by Pentlow and co-workers (22). In this study, when compared to ^{18}F , the spatial resolution was only slightly degraded while the linearity was the same (22). This technique was translated to *in vivo* application by measurements of human neuroblastoma tumors in rats which had been injected with ^{124}I labeled 3F8 antibody demonstrating that quantitative PET

imaging of ^{124}I labeled antibodies was possible in biological systems. Use of ^{124}I in PET radioimmunomaging was further demonstrated by c-erb B2 quantification and visualization in tumor xenografts for up to 160 hours using ^{124}I labeled rat monoclonal antibody (ICR12) recognizing the external domain of the human c-erb B2 proto-oncogene product (23). With advances in imaging instrumentation and technology, whole-animal PET studies were performed for non-invasive measurements of tumor vascular endothelial growth factor (VEGF) in animal model using ^{124}I -SHPP-VG76e (46). Similarly, ^{124}I labeled engineered antibodies have been evaluated for *in vivo* imaging (47, 48).

Numerous applications of ^{124}I in humans for PET imaging have since been reported (49–51). PET with anti-VEGF ^{124}I -HuMV833 was conducted on twenty patients with progressive solid tumors with moderate success (52). A recently published clinical study successfully used a carbonic anhydrase-IX targeted ^{124}I -cG250 antibody to accurately identify clear-cell renal carcinoma and to predict the aggressiveness of the disease, information that could be valuable in treatment decision making (53).

Challenges

^{124}I is available from numerous academic centers and is also available commercially through sources such as IBA Molecular, but tends to be expensive. Current concerns remain high radiation dose and degraded image quality from high-energy γ -emissions, loss of spatial resolution as a result of high-energy positron [Table 1]. The image quality is further degraded due to decrease in tumor/background ratio as a result of intracellular catabolism of ^{124}I . Typically radioiodide localizes in the thyroid delivering high radiation doses and therefore potassium iodide solution has to be infused before protein labeled with ^{124}I infusion to block thyroid uptake.

Bromine-76

Bromine (atomic radius of 1.1 Å and electronegativity of 2.96) also belongs to the Group VII of the periodic table. ^{76}Br has a fairly high abundance (56%) of high-energy β^+ emission (3.9 MeV) and decays to a stable ^{76}Se daughter as shown in Table 1. The half-life of 16.2 h also makes ^{76}Br one of the most attractive radionuclides for PET imaging using intact antibodies that exhibit slow clearance. The positron abundance of ^{76}Br is almost double that of ^{124}I [Table 1] and the C-Br bond is stronger than the C-I bond in organic compounds therefore making it less susceptible to *in vivo* dehalogenation, thereby potentially minimizing concerns regarding background activity and associated toxicity (54). Due to the smaller atomic radius and greater electronegativity, ^{76}Br has greater polarity than ^{124}I . The increased polarity of ^{76}Br can alter biological properties and pharmacokinetics of the biomolecule which can either be unfavorable or favorable depending upon the objective and purpose of the study (55, 56).

Production of ^{76}Br

^{76}Br can be prepared using a low-intermediate energy medical cyclotron. Typically, ^{76}Br is produced through the $^{76}\text{Se}(\text{p},\text{n})^{76}\text{Br}$ reaction in which a highly enriched ^{76}Se target is bombarded with 15–16 MeV protons (57). Alternatively, ^{76}Br can also be prepared through the $^{75}\text{As}({}^3\text{He},2\text{n})^{76}\text{Br}$ and $^{\text{nat}}\text{Br}(\text{p},\text{xn})^{76}\text{Kr} \rightarrow ^{76}\text{Br}$ reactions using high energy (over 50 MeV) protons (58, 59). Purification and recovery of ^{76}Br is performed by dry distillation methods. ^{76}Br produced through alternative routes contains other radionuclide impurities such as ^{77}Br and ^{75}Br which may result in lower yields due to separation and purification. The calculated yield for ^{76}Br produced through the $^{76}\text{Se}(\text{p},\text{n})^{76}\text{Br}$ reaction is almost 32 times greater than ^{76}Br produced through the $^{75}\text{As}({}^3\text{He},2\text{n})^{76}\text{Br}$ reaction [Table 2]. Another

disadvantage of ^{76}Br produced through the $^{75}\text{As}(^3\text{He},2n)^{76}\text{Br}$ reaction is the ^{77}Br ($t_{1/2}=57.03\text{ h}$) impurity.

Radiolabeling of antibodies with ^{76}Br

Numerous antibodies have been brominated using techniques and approaches similar to iodination such as Chloramine-T, enzymatic, *N*-succinimidyl *para*-(tri-methylstannyl)benzoate (60–62). Bromination of antibodies by oxidative methods can be more difficult than iodination due to the lower oxidation potential of bromide. Therefore, more powerful oxidizing agents are used for sufficient yield of bromination, potentially proving deleterious to proteins. Recently, a method was described for indirect radiobromination of antibodies to prevent protein denaturation in presence of strong oxidizing agents (63). In this method, indirect labeling was performed using the *p*-isothiocyanatobenzene derivative of the [^{76}Br]undecahydro-bromo-7,8-dicarba-nido-undecaborate(1–) (^{76}Br -NBI) as a precursor molecule. ^{76}Br -NBI was prepared using the Chloramine-T method and thereafter the coupling was performed without intermediate purification in an “one-pot” reaction (63). This technique resulted in improved intracellular retention of the radiolabeled antibody and decreased the loss of biological activity caused due to direct bromination with oxidizing agents. Similar indirect radio-bromination efforts have been reported using 4-hydroxyphenylethylmaleimide for site-specific bromination of a cysteine containing variant of the anti-HER2 affibody (64).

Biological studies with ^{76}Br labeled antibodies

Proteins have been brominated and evaluated in animals now for over 30 years (60). In the past decade, anti-CEA MAb 38S1 was radio-brominated and evaluated in animals (65, 66). A comparative study using ^{76}Br -38S1, ^{18}F FDG and ^{11}C -Met demonstrated the superiority of ^{76}Br -38S1 in successful identification and imaging of liver metastases in nude rats bearing human colon carcinoma (67). In recent years, more studies have been reported with ^{76}Br labeled intact antibodies and engineered antibodies for the purpose of PET imaging (56, 63, 64, 68). In a recent study, an ED-B fibronectin-binding human antibody derivative (L19-SIP) was labeled with ^{76}Br via an enzymatic approach using bromoperoxidase resulting in both radiolabeling yields and immunoreactivity of over 80% (56). This radio-brominated immunoconjugate (standardized uptake value of over 2.4) enabled detailed small-animal PET imaging of tumor neovasculature in F9 tumor-bearing animals (56). However, long retention of radioactivity in blood and very slow renal excretion were also observed, and as a result, the background activity in non-target organs was higher than that reported for the ^{125}I -labeled L19-SIP, illustrating the differences between iodinated and brominated antibody (56).

Clinically, small molecules labeled with ^{76}Br have been successfully used for *in vivo* quantitative PET neuroimaging (69, 70); however, few if any radio-brominated antibodies have been successfully used in the clinic.

Challenges

Besides the loss of intrinsic spatial resolution of about 5.3 mm due to high-energy positron (3.98 MeV) [Table 1], one of the major problems with ^{76}Br is the availability and large scale production. Enriched ^{76}Se is relatively expensive due to low natural abundance (1 mg/~US \$6) although target material recovery and recycling could obviate that aspect. This may be one of the contributing factors hindering commercialization of large quantities of ^{76}Br , although smaller quantities of ^{76}Br are available on request through MDS Nordion and Isotrace Technology. The β^+ energy of ^{76}Br is relatively higher than conventional PET radionuclides which may pose concerns of radiation doses for diagnostic purposes; however

the same could be viewed as a potential advantage if actually used for therapeutic applications.

Manganese-52

Manganese (atomic radius of 1.8 Å and electronegativity of 1.55) belongs to Group VIIB of the periodic table. ^{52}Mn ($t_{1/2}=134.2$ h) decays to a stable ^{52}Cr daughter. The half-life and suitable imaging characteristics as a result of optimum positron energy of 0.58 MeV [Table 1] makes ^{52}Mn one of the most attractive, under-utilized longer-lived positron emitters for PET imaging of intact antibodies.

Production

^{52}Mn can be produced using the $^{52}\text{Cr}(p,n)^{52}\text{Mn}$ reaction by irradiating a high purity Cr target with 16–17 MeV protons or by $^{51}\text{V}(^3\text{He},2n)^{52}\text{Mn}$ using a high-energy cyclotron (71–73).

Radiolabeling of antibodies with ^{52}Mn

Although ^{52}Mn seems to be a suitable candidate of radioimmunoimaging, relatively little effort has been made with it to label antibodies. Chelating agents such as dipyrroxyl diphosphate (74), macrocyclic Schiff-base ligands (73), cyclopentadienide (74) and porphyrin derivatives (75) have been suggested for Mn in different oxidation states. However, generation of stable $^{52}\text{Mn}(\text{II})$ labeled radioimmunoconjugates in aqueous conditions remains unclear due to complex redox chemistry.

To date, no peer-reviewed reports have been published describing the production or use of ^{52}Mn labeled antibodies.

Biological studies with ^{52}Mn

Radio-manganese has mostly been used as a cationic perfusion tracer and as a tracer to study the involvement of manganese in degenerative neurological diseases (71, 72, 75).

Challenges

^{52}Mn is one of the positron-emitters that have been understudied in nuclear medicine. Therefore, production, chelation and conjugation chemistry, and biological effects of ^{52}Mn all need to be extensively studied prior to embarking on radioimmunoimaging application studies.

Cobalt-55

Cobalt belongs to the Group VIIB of the periodic table. Cobalt has an atomic radius of 1.7 Å and electronegativity of 1.88. ^{55}Co decays to a longer-lived ^{55}Fe daughter ($t_{1/2} = 2.6$ y) mainly by positron emission of 1.5 MeV (76% abundant) with a half-life of 17.5 h [Table 1].

Production of ^{55}Co

The $^{54}\text{Fe}(d,n)^{55}\text{Co}$ reaction has been used to obtain ^{55}Co in good yields (32 MBq/μAh) and acceptable purity of over 99% using a medical cyclotron [Table 2] (76). Alternatively, ^{55}Co can be produced through $^{58}\text{Ni}(p,n)^{55}\text{Co}$ on medical cyclotron but yields are less than half that of $^{54}\text{Fe}(d,n)^{55}\text{Co}$ reaction [Table 2]. No-carrier-added ^{55}Co can be prepared using the $^{56}\text{Fe}(p,2n)^{55}\text{Co}$ reaction by bombardment with 28 MeV protons using a cyclotron or 40 MeV protons using a linear accelerator (77, 78). Better yields and decreased impurities of ^{55}Co are achieved when the reaction is carried out using 28 MeV protons and high-purity

target material. Typically, radiochemical separation is performed by solvent extraction techniques (78, 79).

Radiolabeling of antibodies with ^{55}Co

In studies performed at Brookhaven National Laboratory, an anti-CEA antibody fragment was labeled with ^{55}Co using cyclohexyl EDTA monoanhydride (CDTA-MA), 4-isothiocyanato-*trans*-1,2,-diaminocyclohexane *N,N,N',N'*-tetraacetic acid (4-ICE) and diethylenetriamine pentaacetic acid dianhydride (DTPA-DA) as bifunctional chelating agents (77). The radiolabeling was carried out at pH 5.5 at room temperature with an incubation time ranging from 2–4 hours. All three of the immunoconjugates were obtained in high labeling yields and demonstrated good serum stability after 4 days (77).

Biological studies with ^{55}Co labeled antibodies

^{55}Co labeled anti-CEA $\text{F(ab}')_2$ using DTPA-DA and 4-ICE as bifunctional chelating agents was evaluated in athymic mice bearing human colon carcinoma LS174T xenografts as potential PET imaging agent (77). In the same study, to evaluate the feasibility of the use of ^{55}Co as a PET tracer, a Jaszczak phantom study was performed on a whole body PET scanner, providing images of satisfactory resolution (77). *In vivo* biodistribution studies revealed the superiority of 4-ICE as a better ^{55}Co chelating agent as compared to DTPA-DA as the tumor uptake of 4-ICE conjugated antibody was 2.8 times with greater than that of DTPA-DA conjugated antibody.

In the past, ^{55}Co labeled bleomycin has been evaluated for imaging of tumors in pre-clinical and clinical settings (76, 78, 80). Other applications of ^{55}Co include evaluation of renal function (79). However, most applications of ^{55}Co in clinic are in the field of neuro-imaging to assess neuronal damage in stroke and traumatic brain injury (81, 82).

Challenges

Since the initial reports of successful use of ^{55}Co in radioimmunoimaging in early 1990's, no peer-reviewed reports appear to have been published in over 15 years demonstrating the use of ^{55}Co in radioimmunoimaging. Issues surrounding appropriate chelation chemistry should be addressable extrapolating from the prior reports and use of 1,4,7,10-tetraaminocyclododecane-*N,N,N',N'*-tetraacetic acid (DOTA) derivatives might also prove useful in this application. The dissociation kinetics of Co(II) -DOTA complexes have been suggested to be very slow at neutral and slightly acidic pH and therefore may offer better *in vivo* stability than DTPA complexes (83, 84). Cobalt has been used to determine the mean number of DOTA chelating groups conjugated per antibody using a metal-binding assay (85, 86). Size considerations might also direct one to investigate NOTA. This is an area of research that remains unsettled. Another potential challenge might be the radiation dose from the ^{56}Co impurity and the generated presence of the longer-lived ^{55}Fe daughter ($t_{1/2}=2.6$ y) (87). It is known that uncomplexed cobalt localizes in the bone and liver which could pose issues related to radiation toxicity in case the ^{55}Co complex is unstable *in vivo* (88).

Gallium-66

Gallium belongs to Group III of the periodic table. Gallium has an atomic radius of 1.8 Å and electronegativity of 1.81. ^{66}Ga has one of the highest energies of the candidates considered here for PET imaging ($E_{\text{max}}=4.15$ MeV, 56% abundant). Along with a complex spectrum of γ -rays, ^{66}Ga is one of the more difficult positron-emitters for quantitative PET imaging. However, due its favorable half-life of 9.4 h, although at the shorter end of this

criterion, use of ^{66}Ga has been explored for radioimmunoimaging in the past decade (89, 90).

Production of ^{66}Ga

^{66}Ga can be produced using a medical cyclotron employing the $^{66}\text{Zn}(p,n)^{66}\text{Ga}$ reaction [Table 2] (91). Alternatively, ^{66}Ga can be produced by ^4He bombardment of natural copper targets by means of the $^{63}\text{Cu}(^4\text{He}, n)^{66}\text{Ga}$ reaction (89, 92, 93). However, this method also yields an impurity in the form of ^{67}Ga ($t_{1/2} = 3.26$ d). Chemical purification of ^{66}Ga from the copper target using different techniques is described in detail in these published reports (89, 92, 93).

Radiolabeling of antibodies with ^{66}Ga

The co-ordination chemistry of Ga(III) is well established as it is known that Ga(III) readily binds to the harder oxygen donor chelating agents (94). Initially, GaCl_3 is complexed as an acetate or a citrate and this weak coordination complex is then used to prepare complexes of higher stability (94).

Monoclonal antimyosin antibody, Imciromab was labeled with ^{66}Ga in high yield (99%) by transcomplexation from an acetate buffer to the DTPA-conjugated protein (89, 90). One of the major concerns of using ^{66}Ga for labeling antibodies is the formation of the insoluble hydrolyzed $\text{Ga}(\text{OH})_3$ species above pH 4.5. The radiolabeling had to be carried out in a pH range between 3–4.5 conditions which may potentially degrade intact antibodies. Suitable chelation chemistry for Ga(III) radionuclides that has been evaluated includes EDTA, DTPA derivatives, and DOTA, the latter of which may be adequately stable *in vivo* within the context of the half-lives of the positron emitting gallium radionuclides (95). A critical parameter for *in vivo* stability of the Ga(III)-complex is its relatively stability to the Ga(III)-transferrin complex. The reported formation constant for Ga(III)-transferrin complex is $\log K_1 = 20.3$ (96) and the high plasma concentration of transferrin increases the risks of transchelation. The majority of the Ga(III) complexes used in nuclear medicine are thermodynamically stable and kinetically inert to exchange with transferrin *in vivo*. However, the complex formed with the chelator 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid (NOTA) is exceptionally stable and quite probably will emerge as the agent of choice for use with Ga(III) radionuclides (97, 98). Moreover, NOTA is a more suitable chelating agent for Ga(III) labeled antibodies as the Ga(III)-NOTA complex is formed at room temperature and therefore the protein is exposed to less harsh conditions as compared to heating as in the use of DOTA (99).

Biological studies with ^{66}Ga labeled antibodies

^{66}Ga -DTPA-antimyosin antibody was evaluated in dogs for imaging of myocardial necrosis by PET (89). Most biological applications of ^{66}Ga in molecular imaging have been with either peptides or small molecules as the radiolabeling in acidic conditions is more favorable as compared to use with antibodies (100, 101). The use of ^{66}Ga in humans dates back to 1954 (102), and in that study ^{66}Ga was used to investigate bone disease. As compared to ^{66}Ga , short-lived positron emitter ^{68}Ga ($t_{1/2} = 1.1$ h) obtained from a $^{68}\text{Ge}/^{68}\text{Ga}$ generator has gained immense popularity in the recent years due to availability of commercial $^{68}\text{Ge}/^{68}\text{Ga}$ generators and clinical successes with ^{68}Ga labeled somatostatin peptide (103, 104). In spite of such a short half-life, excellent results have been obtained from a ^{68}Ga labeled trastuzumab fragment in studying the tumor response to the heat shock protein 90 (Hsp90) inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) (105, 106). In these studies, a ^{68}Ga labeled Herceptin fragment was successfully used to image the pharmacodynamics of HER2 degradation in response to 17-AAG and was an earlier predictor of tumor response to 17-AAG therapy than ^{18}F -FDG PET (105, 106).

However, ^{68}Ga may not be a suitable candidate for imaging with intact antibodies due to their slower pharmacokinetics and tumor localization.

Challenges

Apart from unfavorable antibody radiolabeling procedures, the utility of ^{66}Ga in quantitative radioimmunoimaging is severely impaired by high positron energy (4.15 MeV) which results in loss of intrinsic spatial resolution of about 5.8 mm and additional high-energy γ -emission (1.03 MeV) resulting to poor signal to noise ratio [Table 1]. The half-life of 9.4 h may be relatively longer than existing radionuclides such ^{18}F ($t_{1/2}$ = 1.83 h), but the half-life is shorter with respect to the blood clearance and tumor localization of an intact antibody. Traditionally, gallium (^{67}Ga) is used for bone scans and therefore; ^{66}Ga lost from the chelate (if any) will localize in the bones delivering high radiation dose.

Arsenic-72

Arsenic belongs to Group III of the periodic table and has an atomic radius of 1.3 Å, and electronegativity of 2.18. ^{72}As ($t_{1/2}$ = 25.9 h) decays to a stable ^{72}Ge daughter with 88% high-energy positron emission [Table 1]. The longer half-life and positron abundance makes ^{72}As one of the more attractive potential candidates of PET radioimmunoimaging. ^{72}As can also serve as a potential imaging and dosimetry surrogate for the β^- -emitting ^{77}As ($t_{1/2}$ = 38.4 h) which can be used for radioimmunotherapy.

Production of ^{72}As

^{72}As can be produced by irradiating an enriched germanium target using the $^{72}\text{Ge}(\text{p},\text{n})^{72}\text{As}$ reaction, but the radionuclidic purity is low due to the formation of ^{71}As (10%) as shown in Table 2 (107). The target material can be recovered by dry distillation for reprocessing. Alternatively, ^{72}As can be produced from $^{72}\text{Se}/^{72}\text{As}$ radionuclide generators based on solid phase extraction and distillation in which ^{72}As can be eluted in aqueous solvents with over 40–70 % yield with less than 0.1 % contamination of ^{72}Se (108–110). However, for generator based production of ^{72}As , the production of ^{72}Se is tedious as it requires over 35 MeV bombardment of natural germanium target with helium ions. Another limitation of the $^{72}\text{Se}/^{72}\text{As}$ radionuclide generator is the relatively short half-life of ^{72}Se (8.40 d).

Radiolabeling of antibodies by ^{72}As

Radio-arsenic (^{74}As and ^{77}As) has been used to label the phosphatidylserine targeting chimeric monoclonal antibody Bavituximab (111). Bavituximab was first modified with *N*-succinimidyl S-acetylthioacetate (SATA) using a commercial kit. Direct labeling with radio-arsenic was performed after deprotection of the sulfhydryl groups using hydroxylamine [Figure 2]. The resultant radioimmunoconjugate demonstrated good stability over 72 hours during incubation in fetal bovine serum as determined by size-exclusion HPLC (111).

Biological studies with ^{72}As labeled antibodies

Bavituximab was labeled with ^{74}As ($t_{1/2}$ = 427 h, 30 % positron emission) as a surrogate for ^{72}As for a proof in principle study and evaluated for PET imaging in rats bearing Dunning prostate R3327-AT1 tumors (111). Tumors were clearly visualized after 48 hours with injection of 10 MBq of the radioimmunoconjugate with the highest tumor to background ratio being achieved at 72 hours. Localization of the radio-arsenic labeled immunoconjugate was confirmed by autoradiography. As a proof of principle, this study demonstrated successful use of radio-arsenic in PET radioimmunoimaging. However, one might argue that ^{72}As is a better candidate for radioimmunoimaging due to its more appropriate half-life and higher positron abundance. ^{72}As has been used in humans to study environmental toxicity caused by high doses of natural arsenic (112).

Challenges

Production of ^{72}As is limited to a few centers around the world and therefore the issue of a steady supply of clinical grade ^{72}As remains unclear. There have been efforts by the United States Department of Energy to assist in development of ^{72}As generators; however, the availability of a commercial generator seems unlikely in near future. Another drawback of ^{72}As is its association with a relatively high radiation dose which could restrict certain applications particularly when hematologic toxicity is a concern since the uncomplexed arsenic localizes in red blood cells (88).

Copper-64

Copper belongs to the Group IB of the periodic table and has an atomic radius of 1.6 Å, and an electronegativity of 1.90. ^{64}Cu has been a radionuclide of considerable interest for PET imaging due to its attractive physical and chemical properties. As compared to ^{18}F , ^{64}Cu has a longer half-life of 12.7 h and decays to stable ^{64}Ni and ^{64}Zn daughters. Besides the positron emission, ^{64}Cu also emits Auger electrons and has a β^- -emission, and therefore could potentially be used for radioimmunotherapy (113). Imaging characteristics as a result of optimum positron energy (0.66 MeV) and no high-energy γ -emission are one of the biggest factors that make ^{64}Cu an attractive candidate for PET imaging [Table 1]. The image quality and spatial resolution are equivalent to ^{18}F and require no major adjustments in data processing and analysis (114).

Production of ^{64}Cu

^{64}Cu can be produced in numerous ways. Initially, ^{64}Cu was prepared by use of the $^{64}\text{Zn}(\text{n,p})^{64}\text{Cu}$ reaction, but due to low yields and high contamination of ^{67}Cu , the $^{64}\text{Ni}(\text{p,n})^{64}\text{Cu}$ reaction was later employed to produce therapeutic quantities of ^{64}Cu (115–117). ^{64}Cu can also be produced through the $^{68}\text{Zn}(\text{p},\alpha\text{n})^{64}\text{Cu}$ reaction using a high-energy instrument (118–120). $^{68}\text{Zn}(\text{p},\alpha\text{n})^{64}\text{Cu}$ reaction gives a good yield, but the radionuclidic purity is as low as 75% and the final product contains around 25% of ^{67}Cu as shown in Table 2 (118, 120). High yield production of good quality ^{64}Cu has greatly improved over the past couple of years and this radionuclide is now commercially available from several sources such as Trace Life Science, Isotrace Technologies, MDS Nordion, and IBA Molecular.

Radiolabeling of antibodies with ^{64}Cu

Copper can exist in two primary oxidation states, Cu(I) and Cu(II). It has been demonstrated that kinetic inertness is more important than thermodynamic stability of Cu(II) complexes as Cu(II) complexes of EDTA and DTPA rapidly dissociated in human serum and a Cu(II)-albumin complex was formed (121)

To improve the *in vivo* stability, antibodies have been labeled with ^{64}Cu using various bifunctional chelating agents [Figure 3] such as derivatives of 1,4,8,11-tetraazacyclotetradecane (cyclam) (122), 1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid (TETA) (123, 124), and DOTA (125). However, all these bifunctional chelates and their derivatives were unsuccessful in preventing metabolic processing of the ^{64}Cu complex, presumably a factor of the redox chemistry of Cu(II), and as a result elevated liver uptake was observed. To overcome this *in vivo* instability of Cu(II) complexes, cross-bridged tetraamine chelating agents are currently being developed and evaluated (126). These cross-bridged chelating agents provided better geometric coordination and were inert in aqueous solutions (126). Most application of these chelating agents has been for labeling peptides (127). To our best knowledge, intact antibodies have not been labeled with ^{64}Cu using cross-bridged tetraamine chelating agents as yet due to required

harsh radiolabeling conditions utilizing high temperature and acidic pH that are not conducive for labeling heat and acid sensitive antibodies. To overcome these problems, a novel chelating agent SarAr: 1-*N*-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine conjugated to anti-GD2 monoclonal murine antibody 14.G2a and the corresponding chimeric monoclonal antibody ch14.8 were evaluated recently (128). SarAr is based on the macrobicyclic sarcophagine cage structure and was modified to contain the reactive aminobenzyl group for conjugation with the antibody. At pH 5.0 and room temperature conditions, the SarAr chelating agent forms a stable complex with Cu(II) within the multiple macrocyclic rings comprising the sarcophagine cage structure. Therefore, radiolabeling of SarAr-mAb was performed at less harsh conditions (128). The resulting radioimmunoconjugate was shown to be stable in presence of liver proteins known to transchelate with ^{64}Cu (128). Alternately, attempts have been made to radiolabel antibodies with ^{64}Cu using porphyrin based chelating agents (129).

Biological studies with ^{64}Cu labeled antibodies

Numerous studies using ^{64}Cu labeled antibodies have been reported for PET radioimmunoimaging and radioimmunotherapy. Clinically approved antibodies such as Cetuximab and Abegrin have been labeled with ^{64}Cu for PET imaging in pre-clinical models (130–132). ^{64}Cu -DOTA-cetuximab was evaluated in mice bearing EGFR positive and EGFR negative tumors for quantitative PET imaging (132). In this study, ^{64}Cu -DOTA-cetuximab showed prominent uptake in EGFR-expressing tumors, but low accumulation in EGFR-negative tumors. A good linear correlation between the calculated %ID/g values as measured by PET imaging and the EGFR expression level as measured by western blot was exhibited (132). Subcutaneous xenografts and peritoneal metastases were clearly visualized by PET imaging with an anti-L1-CAM antibody chCE7 labeled with ^{64}Cu in mice bearing L1-CAM tumors (133, 134). However, this PET study also revealed that radio-metabolites of the copper immunoconjugate non-specifically localized in the lymph nodes through the reticuloendothelial system which could be critical in imaging lymph node metastasis (134). Recently, novel approaches in terms of chelating agents and linkers have been used to decrease the liver uptake and increase the target/background ratio (128, 135). The evaluation of ^{64}Cu -SarAr-14.G2a demonstrated that the use of a better chelating agent could greatly improve the tumor/background ratio as MicroPET imaging confirmed significant uptake of the radioimmunoconjugate in GD-2-positive tumors, with minimal uptake in GD-2-negative tumors and non-target tissues of interest such as the liver (128). Another use of ^{64}Cu labeled antibodies was to serve as a PET surrogate for ^{67}Cu labeled antibodies used for radioimmunotherapy (13). On comparison with ^{67}Cu , it was found that the ^{64}Cu labeled antibody alone reduced tumor growth, however, a much higher dose (5 times higher) of ^{64}Cu was required to produce effects comparable to ^{67}Cu and the therapy with ^{64}Cu was largely ineffective in treating larger tumors (136). To date, there has been one clinical study reported that utilized ^{64}Cu -TETA-1A3 to evaluate 36 patients with suspected primary or advanced colorectal cancer (137). In this study, all patients had CT scans and PET scans 4 to 36 hours after being injected with 370 MBq of ^{64}Cu -TETA-1A3 directed towards lipid antigens present in human colon carcinoma. The positive predictive value of ^{64}Cu -TETA-1A3 PET radioimmunoimaging was excellent, ranging from 89% (40/45) to 96% (43/45) and detected 11 new occult tumor sites including 9 small abdominopelvic foci less than 2.0 cm in diameter that were previously not detected by CT or MRI. This study demonstrated that PET radioimmunoimaging with ^{64}Cu may have important applications in clinical oncology, particularly for detecting smaller lesions undetected by CT or MRI.

Challenges

Excellent results have been obtained while imaging intact antibodies with ^{64}Cu , but ^{64}Cu seems to be a more suitable candidate for imaging when applied to use antibody fragments,

diabodies, and other engineered targeting vectors which have faster localization and clearance (124, 132). Copper is a constituent of many metalloenzymes related to cellular respiratory oxidation process (88) and therefore, the release and accumulation of ^{64}Cu from the most traditional radioimmunoconjugates in the organs such as liver remains a concern in spite of efforts to reduce the non-specific uptake by developing better chelating agents and linkers.

Yttrium-86

Yttrium belongs to Group IIIB of the periodic table. Yttrium has an atomic radius of 2.3 Å and electronegativity of 1.22. The primary purpose for exploring the viability of ^{86}Y for PET imaging was to use it as an imaging and dosimetry surrogate for the therapeutic radionuclide ^{90}Y which has no imageable emission. ^{86}Y is an attractive candidate for studying ^{90}Y due its half-life (14. 2 h) which allows imaging over several days.

Production of ^{86}Y

^{86}Y can be produced on a medical cyclotron by irradiating SrCO_3 or SrO with 2–6 microA of beam current for <4 hours applying the $^{86}\text{Sr}(p,n)^{86}\text{Y}$ reaction. As compared to SrCO_3 , SrO is a superior target as a SrO target can withstand at least a 6 microA of beam current, a significant improvement over a maximum of 2 microA on the SrCO_3 target resulting in yields that are almost doubled with minimum contaminants (138). At our own institution, we have developed a simple and efficient semi-automated method to purify ^{86}Y which does not compromise the biological properties of the ^{86}Y labeled antibody (139, 140).

Radiolabeling of antibodies with ^{86}Y

In physiological conditions, the primary oxidation state of yttrium is Y(III). Chelation chemistry is well established for Y(III) radionuclides and several bifunctional derivatives of both DOTA and DTPA [Figure 4] have demonstrated their utility in this arena (95). The majority of the chelation chemistry for yttrium labeled antibodies was carried out with ^{90}Y and ^{88}Y . In 1976, ^{90}Y -DTPA was used for radionuclide therapy of brain tumors in patients (141), and since then cyclic anhydride-DTPA was used to conjugate to antibodies for radiolabeling with ^{90}Y (142). However, when ^{90}Y -DTPA antibodies were evaluated *in vivo*, ^{90}Y was released from that DTPA and deposited in the bones causing radiation toxicity (143, 144). In order to improve the *in vivo* stability of the radioimmunoconjugate, backbone substituted DTPA derivatives such as *p*-isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (SCN-Bz-DTPA) and a methyl derivative of *p*-isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (SCN-Bz-Mx-DTPA also known as 1B4M-DTPA) (143–145) were evaluated. The 4-isothiocyanatobenzyl group (SCN-Bz) substituted onto the carbon backbone of DTPA for use in linkage to the antibody and the methyl groups were incorporated onto the backbone to sterically hinder the release of radiometal from the chelate. Comparative biological studies in mice demonstrated the superiority of backbone substituted DTPA radioimmunoconjugates over cyclic anhydride DTPA radioimmunoconjugates (143, 144). With the better understanding of the coordination chemistry and geometry of DTPA derivatives, a cyclohexyl derivative of DTPA was developed which demonstrated excellent stability *in vitro* and *in vivo* (146, 147). Interestingly, stereochemistry of CHX-DTPA played a major role in *in vivo* stability of $^{90}\text{Y}/^{88}\text{Y}$ radioimmunoconjugate and subsequent bone uptake (146, 148). The single enantiomeric [(*R*)-2-amino-3-(4-isothiocyanatophenyl)propyl]-*trans*-(*S,S*)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A''-DTPA) radioimmunoconjugate was found to be more stable as compared to the corresponding diastereomeric pair of CHX-B-DTPA radioimmunoconjugates (148). After 7 days post injection, bone uptake of the ^{88}Y labeled CHX-B'-DTPA or CHX-B''-DTPA radioimmunoconjugates was 5 times higher than that of

the ^{88}Y labeled CHX-A''-DTPA radioimmunoconjugate (148). In order to further improve the stability, DOTA derivatives such as SCN-Bz-DOTA were explored for radiolabeling antibodies with ^{90}Y (149, 150). In a comparative study, it was found that DOTA conjugated antibody was better than DTPA conjugated antibody in terms of stability (150). Since then numerous pre-clinical and clinical studies have been published reporting DTPA and DOTA based chelating agents for ^{90}Y radioimmunotherapy and thus extrapolating to ^{86}Y for PET imaging (151–153).

Biological studies with ^{86}Y labeled antibodies

^{86}Y labeled antibodies were most recently evaluated to perform dosimetry for ^{90}Y labeled antibodies used for radioimmunotherapy as they were expected to be superior to ^{111}In labeled antibodies in terms of similarities in both chemical and biological behavior (153). Numerous studies have been reported with ^{86}Y labeled antibodies including clinically approved trastuzumab (Herceptin) (152, 154) and cetuximab (Erbix) (154). In a recent study, ^{86}Y -CHX-A''-DTPA-trastuzumab was evaluated in mice bearing intraperitoneal SKOV3 ovarian tumors (152). In this study, PET and MRI studies were performed to assess the therapeutic potential of ^{90}Y -CHX-A''-DTPA-trastuzumab using ^{86}Y -CHX-A''-DTPA-trastuzumab as an imaging surrogate for dosimetry. Intraperitoneal tumors were visualized by PET imaging 2 days after injecting ^{86}Y -CHX-A''-DTPA-trastuzumab and the tumor localization was confirmed by MRI studies. ^{86}Y -CHX-A''-DTPA-trastuzumab localized to sites of disease with minimal normal organ uptake (152). In the study performed at our institution, we could clearly visualize LS-174T colon carcinoma xenografts in mice after 2 days of injecting ^{86}Y -CHX-A''-DTPA-trastuzumab (tumor uptake was greater than 30% ID/g) (154). In the same study, the HER1-targeted ^{86}Y -CHX-A''-DTPA cetuximab was evaluated in mice bearing LS-174T colon carcinoma, SKOV3 ovarian carcinoma and DU-145 prostate carcinoma (154). All the tumors were clearly visualized at 1 day post-injection with the tumor uptake ranging from 20–30% ID/g.

Clinical use of ^{86}Y labeled agent used for dosimetry ^{90}Y labeled has been illustrated by the use of ^{86}Y -DOTA-Phe¹-Tyr³-octreotide (SMT-487) for dosimetry of ^{90}Y -SMT-487 in patients with metastatic carcinoid tumors (155). In this study, the maximum dose that could be delivered to the tumor without exceeding the 23-Gy cut-off dose to kidneys was successfully calculated using PET imaging, and therefore presumably reduced the incidence of potential renal toxicity caused by ^{90}Y -SMT-487 therapy.

Challenges

^{86}Y is commercially available (Isotrace Technology) in a limited capacity and is also only available at centers capable of producing ^{86}Y on a regular basis. The high positron energy of 3.14 MeV (34% abundance) and addition γ -emission of 1.08 MeV (83 % abundance) significantly affects the spatial resolution and image quality respectively [Table 1]. Uncomplexed ^{86}Y localizes in the bone and therefore can cause radiation toxicity in the events of “leakage” from the immunoconjugate and thus may be not a suitable candidate to image bone metastasis.

Zirconium-89

Zirconium belongs to Group IVB of the periodic table, has an atomic radius of 2.2 Å, and an electronegativity of 1.33. ^{89}Zr ($t_{1/2}$ = 78.4 h) decays to an ultra short-lived daughter $^{89\text{m}}\text{Y}$ ($t_{1/2}$ = 16 s) with a 23% β^+ emission and 77% electron capture [Table 1]. Due to its appropriate half-life and imaging characteristics, ^{89}Zr labeled antibodies are currently being evaluated in the clinic as PET surrogates for both ^{90}Y and ^{177}Lu therapy studies despite the

possibility of using the elementally matched ^{86}Y for the former and that the latter possesses an imagable γ -emission (156).

Production of ^{89}Zr

^{89}Zr can be produced through the $^{89}\text{Y}(\text{p},\text{n})^{89}\text{Zr}$ reaction in which inexpensive natural yttrium foil is irradiated with a 14–14.5 MeV proton beam and the target is separated by ion-exchange chromatography or solvent extraction (26, 157, 158). Alternatively, ^{89}Zr can also be produced through the $^{89}\text{Y}(\text{d},2\text{n})^{89}\text{Zr}$ reaction (159). A relatively high yield of 43 MBq/ μAh is obtained with the $^{89}\text{Y}(\text{p},\text{n})^{89}\text{Zr}$ reaction with less than 0.2% impurity of ^{88}Zr ($t_{1/2}=85$ d and daughter ^{88}Y ; $t_{1/2}=105$ d) [Table 2].

Radiolabeling of antibodies with ^{89}Zr

Over the past 20 years attempts have been made to label proteins and antibodies with ^{89}Zr using DTPA derivatives and porphyrins as chelating agents (160, 161). Proteins and antibodies were labeled with ^{89}Zr using desferal (Df) conjugates (162, 163). Briefly, incorporation of Df-groups onto monoclonal antibodies was performed in a two-step procedure. In the first step, maleimide groups were incorporated into the protein with the assistance of succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC). In the second step, the thioester of Df formed by conjugation to SATA was further converted to a free thiol with hydroxylamine to facilitate reaction with the maleimide groups of the antibody with subsequent radiolabeling with ^{89}Zr . The final purified product was obtained through gel filtration (162, 163). This protocol was further modified by formation of *N*-succinyl-desferrioxamine B of Df (N-SucDf) as an intermediate (164). In this method, the hydroxamate groups of N-sucDf were temporarily blocked with formation of the Fe(III) complex. The N-sucDf-Fe complex was then esterified and coupled to antibodies. Thereafter, Fe(III) was removed by reduction to Fe(II) and transchelation to EDTA, mAb-N-sucDf is then radiolabeled with ^{89}Zr at pH 7.2–7.4 with a 30 minute incubation at room temperature (164).

Biological studies with ^{89}Zr labeled antibodies

Considering the success of ^{90}Y -labeled mAbs in clinical radioimmunotherapy (12, 151, 165), ^{86}Y -labeled mAbs are currently being developed in pre-clinical models for clinical dosimetry purposes (139, 152, 153). However, the half-life of ^{90}Y is nearly 4 times greater than that of ^{86}Y and therefore predicting ^{90}Y dosimetry of slowly clearing antibody may not be feasible or even appropriate. Due to these reasons, ^{89}Zr was explored as one of the possible radionuclides that could be used for dosimetry analysis of ^{90}Y -labeled mAbs (166). Clinically used antibodies such as HER1-targeted cetuximab (156), VEGF-targeted bevacizumab (167) and CD-20-targeted ibritumomab tiuxetan have been labeled with ^{89}Zr -Df conjugate (168). All the above studies demonstrated the use of ^{89}Zr labeled antibodies in quantitative PET imaging; additionally, the tumor localization of the ^{89}Zr -mAb was similar to that of the $^{111}\text{In}/^{90}\text{Y}$ -mAb.

Due to the feasibility in production and purification of ^{89}Zr labeled mAb, clinical studies were performed with CD44V6-specific chimeric mAb, U36 labeled with ^{89}Zr (169). In this study, ^{89}Zr -U36 was evaluated in patients with squamous cell carcinoma of the head and neck (HNSCC), who were at high risk of having neck lymph node metastases. All primary tumors ($n = 17$) as well as lymph node metastases in 18 of 25 positive levels (sensitivity 72%) and in 11 of 15 positive sides (sensitivity 73%) were detected using PET radioimmunoinaging (169). Interpretation of PET was correct in 112 of 121 operated levels (accuracy 93%) and in 19 of 25 operated sides (accuracy 76%). For CT/MRI, sensitivities of 60% and 73% and accuracies of 90% and 80% were found per level and side, respectively. ^{89}Zr -immunoPET demonstrated a sensitivity of 85% and accuracy of 95% as

compared to sensitivity of 62 % and accuracy of 88% for ^{18}F -FDG-PET (169). Thus, this study demonstrated the utility and advantages of ^{89}Zr PET radioimmunoimaging in clinical setting in comparison with CT/MRI and ^{18}F -FDG (169). Similarly, all known tumor lesions previously identified by ^{18}F -FDG were successfully imaged by ^{89}Zr -Df-Zevalin in a pilot PET imaging study in a patient with Non-Hodgkin's Lymphoma (168)

Challenges

Uncomplexed ^{89}Zr localizes in the bone and therefore can deliver high radiation dose to the bone marrow, therefore the need for stable chelation chemistry remains an important consideration with this element (88). In spite of the recent success of ^{89}Zr PET imaging, clinical grade ^{89}Zr is not commercially available yet (although, ^{89}Zr can be obtained from IBA Molecular on request). Another major disadvantage is the currently used ^{89}Zr -mAb production scheme. The currently used conjugation and radiolabeling schemes are often tedious, time-consuming and involve numerous steps that increase the margin for error for the cGMP (current Good Manufacturing Practice) production. In the currently used procedure, the Fe(III) complex is first formed with desferal which is then activated for conjugation through extension of the terminal amine with succinic anhydride followed by the conversion of the formed carboxylate into an active ester. After conjugation of the antibody, the Fe(III) is then reduced and displaced with ^{89}Zr (164). Alternate, simpler conjugation and radiolabeling schemes that can be successfully carried out at local radiopharmacies are warranted to bolster the increased appeal of ^{89}Zr PET radioimmunoimaging. One of the suggested applications of ^{89}Zr has been to use it as a surrogate for ^{90}Y and ^{131}I labeled antibodies, however, ^{89}Zr labeled antibodies, while close to, also do not exactly match the biodistribution and pharmacokinetics of ^{90}Y and ^{131}I labeled antibodies (168, 170).

Opportunities and Challenges

Production

The reliable large-scale availability of the above desired longer-lived positron emitting radionuclides in suitable quality for clinical applications is critical for potential clinical translation. Many of the above described radionuclides such as ^{64}Cu , ^{76}Br , ^{86}Y and ^{72}As can be produced in good yields at low-energy medical cyclotrons (26). Most of the longer-lived positron-emitting radionuclides are currently produced by employing low-energy nuclear reactions such as (p, n) and (d, n) which may require isotopically enriched targets which are usually expensive. Alternatively, a higher-energy dual-particle cyclotron or a multi-particle intermediate-energy cyclotron could be used for production of some these radionuclides employing nuclear reactions such as (p, xn) and (^3He , xn). Two of the most important criteria in medical application of positron-emitters are quality and yield, and therefore the target material is critical. It is absolutely essential to produce target material that is economical, may be efficiently recovered for reuse, can withstand high-energy beam fluxes of charged particles, and most importantly, also be easily isolated from the product to give high-purity final product. One such example that meets these criteria has been the production of ^{64}Cu . Originally, ^{64}Cu was produced through the $^{64}\text{Zn}(n,p)^{64}\text{Cu}$ reaction with resulting in low yields and a high contamination of ^{67}Cu , unless highly enriched pure ^{64}Zn was used. The low yields compounded with contamination of the β^- -emitting therapeutic radionuclide ^{67}Cu were impediments to routine large-scale clinical applications of ^{64}Cu for PET imaging. As an alternative, the $^{64}\text{Ni}(p,n)^{64}\text{Cu}$ reaction was employed to obtain higher yields and better quality ^{64}Cu (115, 116). However, the biggest drawback of this method remains the cost of the highly enriched ^{64}Ni target material which can be a hindrance in large-scale production of ^{64}Cu at multiple centers across the world. With better understanding and characterization of cross-section data, integral yields and chemical

processing methods, it is possible to obtain good quality positron-emitters in large yields using a medical cyclotron that can be potentially used for radioimmunoimaging. Most commonly used production routes of selected longer-lived positron emitters for radioimmunoimaging with their corresponding yields and radionuclidic impurities are listed in Table 2.

Imaging characteristics

One of the major factors impacting image quality is the additional γ -emissions and the energy of the positron which can contribute to the background noise and the loss of intrinsic spatial resolution respectively. Some of the longer-lived positron-emitting radionuclides such as ^{76}Br , ^{72}As , ^{124}I and ^{66}Ga have high-energy positron emissions which result in loss of intrinsic spatial resolution and poor signal to noise ratios [Table 1]. In clinical settings, both radiation dosimetry and chemistry as a result of inadequate specific activity and stability may limit the amount of radioactivity injected, thereby further degrading image quality. Loss of spatial resolution could also limit the use of radioimmunoconjugates labeled with some of the described longer-lived positron-emitting radionuclides for the detection of metastasis and smaller lesions which could be critical for their use in assessing or predicting clinical outcomes. Successful attempts have been made to improve the image characteristics of high-energy positron emitters by developing methods to correct for scatter fractions and spurious coincidences (171–173). Image reconstruction algorithms have been developed which include models of the statistical nature of nuclear decays and tomography responses which improve the spatial resolution, and at the same time reduce the image noise (114, 171–173). Further advances in instrumentation and image processing may additionally improve spatial resolution and the ability to perform more accurate quantitative analysis. However, implementing these improvements in the clinic may be complex as new instruments and imaging software will require FDA approval.

Potential therapeutic applications and radiation toxicity

Tumor therapy using antibodies carrying radioactivity has been reported now for over 50 years (174, 175). In 1966, McCradle and co-workers reported the use of ^{131}I labeled antibody to human fibrinogen as a clinical diagnostic and therapeutic agent (8). In this study, 50 patients with reported neoplasm were imaged and a high percentage of patients showed positive scans for metastases to the brain which was a remarkable achievement during that period when targeted non-invasive radioimmunoimaging was still in its infancy. This study demonstrated the value of molecular imaging with radiolabeled antibodies for the assessment of primary and metastatic lesions (8). In this same study, two previously imaged patients were treated with higher doses of ^{131}I labeled antibody to human fibrinogen resulting in regression of their lesions (8). Since that time numerous studies have been reported demonstrating the utility of antibodies labeled with radionuclides such as ^{111}In , ^{177}Lu and ^{64}Cu which could be used for diagnosis as well as therapy (4, 176). For better clinical acceptance, cost-benefit effectiveness and numerous other reasons including the need of surrogate radionuclide, a radiolabeled antibody that could be used as a diagnostic as well as therapeutic becomes a desirable combination.

One of the major concerns in radioimmunotherapy is the non-uniform absorbed dose distribution in the tumor resulting in less effective therapy in spite of dose escalation (177). One possible solution might be use of longer-lived radionuclides with longer emission ranges which may increase mean absorbed dose to the tumor as they are more likely to penetrate into the tumor before decaying in the body than shorter-lived radionuclides (178). Longer-lived positron-emitting radionuclides can deliver high absorbed doses to the target site as the doses delivered by positrons are as high as negatrons of the same energy. Considering the success of negatron-emitting radioimmunotherapy in pre-clinical and

clinical studies, longer-lived positron-emitting radioimmunoconjugates may potentially be used for therapeutic applications. The longer half-life may minimize the effects of non-uniformity up to an extent. However, the choice of radionuclide will be based upon the function of biological and pharmacokinetic properties of the targeting antibody (normal organs uptake and clearance), disease state, physical half-life of the radionuclide, abundance and energy of the positron emission and additional γ -rays. For example, assuming a biological half-life of 100 hours and whole-body uniform distribution in an adult human, based on the values given in the International Commission on Radiological Protection (ICRP) publications, the effective dose from ^{52}Mn ($t_{1/2}$ = 134.2 h) will be approximately 0.9–1.1 mSv/MBq as compared to 0.02–0.04 mSv/MBq from ^{64}Cu ($t_{1/2}$ = 12.7 h) and 0.2–0.3 mSv/MBq from ^{76}Br ($t_{1/2}$ = 16.2 h) (159). Another important parameter in terms of therapeutic applications of longer-lived positron emitters is the positron abundance and the dose rate; a dose rate of 2 – 3 cGy/hr has been suggested as being required to overcome the proliferation of most types of tumor cells (179). Therefore, the portion of the total dose that is delivered below this rate is essentially unnecessary and can potentially pose a hazard. Theoretically, high specific activity longer-lived positron-emitting radioimmunoconjugates could be used for therapy; however toxicity and dose to the normal organs may pose a few challenges and will depend on whether the radioimmunoconjugate is delivered systemically or locally.

Economics, regulatory affairs and clinical acceptance

Currently, the use of PET in the clinical setting revolves around ^{18}F -FDG, which has great clinical acceptance as it can be used to image most metabolically active tumors and lesions irrespective of any expression of cell surface molecular targets such as EGFR and HER2/ μ . This characteristics of ^{18}F -FDG can also be disadvantageous too as it does not provide information regarding the status of cell surface targets which could be essential for patients about to undergo immunotherapy or radioimmunotherapy. Under those circumstances, PET radioimmunoimaging could play a critical role in risk stratification. In the past year, sales of Herceptin (trastuzumab), Avastin (Bevacizumab), Rituxan (Rituximab) and Erbitux (Cetuximab) each all exceeded one billion U.S. dollars (180). Based on previous results, not all patients may respond to immunotherapy due to low-density of target expression or changes in the target levels. In all likelihood, not all patients undergo some form of diagnostic scan to evaluate the status of target in the progression of illness. PET radioimmunoimaging could serve as one of the potential tools for screening patients that may or may not respond to therapy, thereby acting as a cost containment tool. Contrary to that positive aspect, a sizeable financial investment and commitment is required to develop a clinically FDA approved radiopharmaceutical. In order to recover the cost spent in developing the PET radioimmunoconjugate, sales and use volumes have to be significantly high which will be directly related to the clinical acceptance of PET radioimmunoimaging.

The number of radiopharmaceuticals approved by the US FDA in the past decade has been limited due to more scrutiny and a tedious approval process. Radiation toxicity and required pre-approval studies could potentially be a major obstacle for the approval of radioimmunoconjugates labeled with longer-lived positron emitters. Thus, one can question the rationale behind the objective of developing a novel PET radioimmunoconjugate considering the level of required investment and resources versus failure to acquire approval of the agent by the FDA.

Other regulatory barriers include waste disposal of large quantities of longer-lived high energy positron emitters, decontamination, occupational hazards, and the radiation exposure to normal population by out-patients undergoing high-dose longer-lived positron-emitting radionuclide protocols.

Lack of standards for calibration, decay characteristics

A recent study has highlighted the differences in published values of decay data and energies of some of the described radionuclides (119). These differences in values could impact the overall biological and dosimetry results as the same amount of radioactivity quantified from dose calibrator and PET image could vary from institution to institution depending of the values used. Therefore, there is a need to establish standards for calibration of instruments for these longer-lived positron emitters to avoid variances which could result in errors.

Future perspective

It is clear from the selected examples cited in this report that efforts are continuing to being made to develop radioimmunoconjugates labeled with longer-lived positron-emitting radionuclides for PET imaging. The superiority of PET in radioimmunoimaging was demonstrated by a recent clinical study in which a comparison was made between ^{131}I -G250, ^{124}I -cG250 and ^{18}F -FDG for detection of metastatic renal-cell carcinoma (53, 181). ^{131}I -cG250 was able to detect 30% of metastatic lesions as compared to 69% for ^{18}F -FDG (181); however, the results were far better when ^{124}I -cG250 was used as the sensitivity of detection of tumor was 94 % (53, 181). When compared to ^{124}I -cG250, ^{131}I -cG250 did not provide the same resolution, contrast, or the potential for quantification as ^{124}I -cG250 (53). Considering the advantages of PET radioimmunoimaging, further efforts are warranted to improve PET instrumentation and image processing, increase production of clinical grade high-yield longer-lived positron emitters, and continue to create efficient radiolabeling methods to produce high-yield biologically active stable radioimmunoconjugates in the least amount of time and variability. And, lastly, but perhaps the most critical criterion for successful clinical translation, continued efforts have to be applied towards making wise selections of the most appropriate antibody matched with the most appropriate radionuclide for the desired application within an appropriate patient population. However, a few questions still remain unanswered: how many positron-emitting radionuclides do we really need to evaluate to “fit the bill” and to be able to ask and answer questions related to radioimmunoimaging? What are the alternatives in case of unexpected unreliable availability of the radionuclide? Is it worth the capital investments related to PET imaging if techniques such as biopsies and CT imaging are going to remain the mainstay of cancer diagnosis? Can future generation SPECT systems be as good as currently used PET (182, 183)? If so, then why develop newer positron-emitting products if ^{111}In based SPECT can offer precise quantitation in future? And finally, should be there be a common set of criteria and guidelines for choosing the radionuclide?

In conclusion, we believe that radioimmunoimaging with longer-lived positron-emitting radionuclides holds significant promise, but several scientific and non-scientific challenges have to be conquered before realizing the true potentials of PET radioimmunoimaging with intact antibodies.

Acknowledgments

This report was supported by the Intramural Research Program of the NIH, NCI, Center for Cancer Research and the United States Department of Health and Human Services. Gratitude is expressed to Mike Slackenry for constant encouragement and support.

Literature Cited

1. Cattell HW. Application of the X-Rays to Surgery. Science. 1896; 3:344–346.
2. Wagner HN Jr, Bardfeld PA. Evaluation of structure and function of spleen with radioactive tracers. JAMA. 1967; 199:202–6. [PubMed: 5333945]

3. Wagner HN Jr. The current status of nuclear medicine. *JAMA*. 1963; 183:500–3. [PubMed: 13998247]
4. Boswell CA, Brechbiel MW. Development of radioimmunotherapeutic and diagnostic antibodies: an inside-out view. *Nucl Med Biol*. 2007; 34:757–78. [PubMed: 17921028]
5. Khaw BA. Antibodies for molecular imaging in the cardiovascular system. *J Nucl Cardiol*. 2005; 12:591–604. [PubMed: 16171720]
6. Melcher LR, Masouredis SP. The in vivo stability of the I131 protein label of rabbit antibody in guinea pigs as determined by the quantitative precipitin reaction. *J Immunol*. 1951; 67:393–402. [PubMed: 14898075]
7. Pressman D. The zone of localization of antibodies; the specific localization of antibodies to rat kidney. *Cancer*. 1949; 2:697–700. [PubMed: 18149956]
8. McCardle RJ, Harper PV, Spar IL, Bale WF, Andros G, Jiminez F. Studies with iodine-131-labeled antibody to human fibrinogen for diagnosis and therapy of tumors. *J Nucl Med*. 1966; 7:837–47. [PubMed: 5955363]
9. Goldenberg DM, DeLand F, Kim E, Bennett S, Primus FJ, van Nagell JR Jr, Estes N, DeSimone P, Rayburn P. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med*. 1978; 298:1384–6. [PubMed: 349387]
10. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975; 256:495–7. [PubMed: 1172191]
11. Goldenberg DM, Kim EE, Deland F, Spremulli E, Nelson MO, Gockerman JP, Primus FJ, Corgan RL, Alpert E. Clinical studies on the radioimmunodetection of tumors containing alpha-fetoprotein. *Cancer*. 1980; 45:2500–5. [PubMed: 6155193]
12. O'Donnell RT, DeNardo SJ, Yuan A, Shen S, Richman CM, Lara PN, Griffith IJ, Goldstein DS, Kukis DL, Martinez GS, Mirick GR, DeNardo GL, Meyers FJ. Radioimmunotherapy with (111)In(90)Y-2IT-BAD-m170 for metastatic prostate cancer. *Clin Cancer Res*. 2001; 7:1561–8. [PubMed: 11410491]
13. DeNardo GL, Kukis DL, Shen S, DeNardo DA, Meares CF, DeNardo SJ. 67Cu-versus 131I-labeled Lym-1 antibody: comparative pharmacokinetics and dosimetry in patients with non-Hodgkin's lymphoma. *Clin Cancer Res*. 1999; 5:533–41. [PubMed: 10100704]
14. Mach JP, Carrel S, Forni M, Ritschard J, Donath A, Alberto P. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma: a critical evaluation. *N Engl J Med*. 1980; 303:5–10. [PubMed: 7189578]
15. Phelps ME. PET: the merging of biology and imaging into molecular imaging. *J Nucl Med*. 2000; 41:661–81. [PubMed: 10768568]
16. Otsuka FL, Welch MJ, Kilbourn MR, Dence CS, Dilley WG, Wells SA Jr. Antibody fragments labeled with fluorine-18 and gallium-68: in vivo comparison with indium-111 and iodine-125-labeled fragments. *Int J Rad Appl Instrum B*. 1991; 18:813–6. [PubMed: 1787093]
17. Garg PK, Garg S, Bigner DD, Zalutsky MR. Localization of fluorine-18-labeled Mel-14 monoclonal antibody F(ab')₂ fragment in a subcutaneous xenograft model. *Cancer Res*. 1992; 52:5054–60. [PubMed: 1516061]
18. Cai W, Olafsen T, Zhang X, Cao Q, Gambhir SS, Williams LE, Wu AM, Chen X. PET imaging of colorectal cancer in xenograft-bearing mice by use of an 18F-labeled T84.66 anti-carcinoembryonic antigen diabody. *J Nucl Med*. 2007; 48:304–10. [PubMed: 17268029]
19. Kramer-Marek G, Kiesewetter DO, Martiniova L, Jagoda E, Lee SB, Capala J. [(18)F]FBEM-Z(HER2:342)-Affibody molecule-a new molecular tracer for in vivo monitoring of HER2 expression by positron emission tomography. *Eur J Nucl Med Mol Imaging*. 2008; 35:1008–18. [PubMed: 18157531]
20. Vaidyanathan G, Bigner DD, Zalutsky MR. Fluorine-18-labeled monoclonal antibody fragments: a potential approach for combining radioimmunoscinigraphy and positron emission tomography. *J Nucl Med*. 1992; 33:1535–41. [PubMed: 1634947]
21. Snook DE, Rowlinson-Busza G, Sharma HL, Epenetos AA. Preparation and in vivo study of 124I-labelled monoclonal antibody H17E2 in a human tumour xenograft model. A prelude to positron emission tomography (PET). *Br J Cancer Suppl*. 1990; 10:89–91. [PubMed: 2383487]

22. Pentlow KS, Graham MC, Lambrecht RM, Cheung NK, Larson SM. Quantitative imaging of I-124 using positron emission tomography with applications to radioimmunodiagnosis and radioimmunotherapy. *Med Phys.* 1991; 18:357–66. [PubMed: 1870476]
23. Bakir MA, Eccles S, Babich JW, Aftab N, Styles J, Dean CJ, Lambrecht RM, Ott RJ. c-erbB2 protein overexpression in breast cancer as a target for PET using iodine-124-labeled monoclonal antibodies. *J Nucl Med.* 1992; 33:2154–60. [PubMed: 1460508]
24. Hohn A, Nortier FM, Scholten B, van der Walt TN, Coenen HH, Qaim SM. Excitation functions of $^{125}\text{Te}(\text{p}, \text{xn})$ -reactions from their respective thresholds up to 100 MeV with special reference to the production of ^{124}I . *Appl Radiat Isot.* 2001; 55:149–56. [PubMed: 11393753]
25. Sajjad M, Bars E, Nabi HA. Optimization of ^{124}I production via $^{124}\text{Te}(\text{p}, \text{n})^{124}\text{I}$ reaction. *Appl Radiat Isot.* 2006; 64:965–70. [PubMed: 16725332]
26. Tang L. Radionuclide production and yields at Washington University School of Medicine. *Q J Nucl Med Mol Imaging.* 2007; 52:122–33.
27. Kondo K, Lambrecht RM, Wolf AP. Iodine-123 production for radiopharmaceuticals--XX excitation functions of the $^{124}\text{Te}(\text{p}, 2\text{n})^{123}\text{I}$ and $^{124}\text{Te}(\text{p}, \text{n})^{124}\text{I}$ reactions and the effect of target enrichment on radionuclidic purity. *Int J Appl Radiat Isot.* 1977; 28:395–401. [PubMed: 863535]
28. Pressman D, Hill RF, Foote FW. The Zone of Localization of Anti-Mouse-Kidney Serum as Determined by Radioautographs. *Science.* 1949; 109:65–66. [PubMed: 17777137]
29. Yalow RS, Berson SA. Assay of plasma insulin in human subjects by immunological methods. *Nature.* 1959; 184(Suppl 21):1648–9. [PubMed: 13846363]
30. Hunter WM, Greenwood FC. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature.* 1962; 194:495–6. [PubMed: 14450081]
31. Greenwood FC, Hunter WM, Glover JS. The Preparation of I-131-Labelled Human Growth Hormone of High Specific Radioactivity. *Biochem J.* 1963; 89:114–23. [PubMed: 14097352]
32. Marchalonis JJ. An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochem J.* 1969; 113:299–305. [PubMed: 4185494]
33. David GS. Solid state lactoperoxidase: a highly stable enzyme for simple, gentle iodination of proteins. *Biochem Biophys Res Commun.* 1972; 48:464–71. [PubMed: 5065068]
34. Rudinger J, Ruegg U. Appendix: Preparation of N-succinimidyl 3-(4-hydroxyphenyl)propionate. *Biochem J.* 1973; 133:538–539. [PubMed: 16742792]
35. Bolton AE, Hunter WM. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I -containing acylating agent. *Biochem J.* 1973; 133:529–39. [PubMed: 4733239]
36. Salacinski PR, McLean C, Sykes JE, Clement-Jones VV, Lowry PJ. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3 α ,6 α -diphenyl glycoluril (Iodogen). *Anal Biochem.* 1981; 117:136–46. [PubMed: 7316186]
37. Wilbur DS, Hadley SW, Hylarides MD, Abrams PG, Beaumier PA, Morgan AC, Reno JM, Fritzberg AR. Development of a stable radioiodinating reagent to label monoclonal antibodies for radiotherapy of cancer. *J Nucl Med.* 1989; 30:216–26. [PubMed: 2738650]
38. Press OW, DeSantes K, Anderson SK, Geissler F. Inhibition of catabolism of radiolabeled antibodies by tumor cells using lysosomotropic amines and carboxylic ionophores. *Cancer Res.* 1990; 50:1243–50. [PubMed: 2297772]
39. Geissler F, Anderson SK, Press O. Intracellular catabolism of radiolabeled anti-CD3 antibodies by leukemic T cells. *Cell Immunol.* 1991; 137:96–110. [PubMed: 1832089]
40. Geissler F, Anderson SK, Venkatesan P, Press O. Intracellular catabolism of radiolabeled anti- μ antibodies by malignant B-cells. *Cancer Res.* 1992; 52:2907–15. [PubMed: 1581908]
41. Sharkey RM, Behr TM, Mattes MJ, Stein R, Griffiths GL, Shih LB, Hansen HJ, Blumenthal RD, Dunn RM, Juweid ME, Goldenberg DM. Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22. *Cancer Immunol Immunother.* 1997; 44:179–88. [PubMed: 9191878]
42. Mattes MJ, Shih LB, Govindan SV, Sharkey RM, Ong GL, Xuan H, Goldenberg DM. The advantage of residualizing radiolabels for targeting B-cell lymphomas with a radiolabeled anti-CD22 monoclonal antibody. *Int J Cancer.* 1997; 71:429–35. [PubMed: 9139880]

43. Stein R, Goldenberg DM, Thorpe SR, Basu A, Mattes MJ. Effects of radiolabeling monoclonal antibodies with a residualizing iodine radiolabel on the accretion of radioisotope in tumors. *Cancer Res.* 1995; 55:3132–9. [PubMed: 7606734]
44. Stein R, Govindan SV, Mattes MJ, Chen S, Reed L, Newsome G, McBride BJ, Griffiths GL, Hansen HJ, Goldenberg DM. Improved iodine radiolabels for monoclonal antibody therapy. *Cancer Res.* 2003; 63:111–8. [PubMed: 12517786]
45. Wilbur DS. Radiohalogenation of proteins: an overview of radionuclides, labeling methods, and reagents for conjugate labeling. *Bioconjug Chem.* 1992; 3:433–70. [PubMed: 1463775]
46. Collingridge DR, Carroll VA, Glaser M, Aboagye EO, Osman S, Hutchinson OC, Barthel H, Luthra SK, Brady F, Bicknell R, Price P, Harris AL. The development of [(124)I]iodinated-VG76: a novel tracer for imaging vascular endothelial growth factor in vivo using positron emission tomography. *Cancer Res.* 2002; 62:5912–9. [PubMed: 12384557]
47. Sundaresan G, Yazaki PJ, Shively JE, Finn RD, Larson SM, Raubitschek AA, Williams LE, Chatziioannou AF, Gambhir SS, Wu AM. 124I-labeled engineered anti-CEA minibodies and diabodies allow high-contrast, antigen-specific small-animal PET imaging of xenografts in athymic mice. *J Nucl Med.* 2003; 44:1962–9. [PubMed: 14660722]
48. Fortin MA, Salnikov AV, Nestor M, Heldin NE, Rubin K, Lundqvist H. Immuno-PET of undifferentiated thyroid carcinoma with radioiodine-labelled antibody cMAb U36: application to antibody tumour uptake studies. *Eur J Nucl Med Mol Imaging.* 2007; 34:1376–87. [PubMed: 17277931]
49. Phan HT, Jager PL, Paans AM, Plukker JT, Sturkenboom MG, Sluiter WJ, Wolffenbuttel BH, Dierckx RA, Links TP. The diagnostic value of (124)I-PET in patients with differentiated thyroid cancer. *Eur J Nucl Med Mol Imaging.* 2008
50. Freudenberg LS, Antoch G, Frilling A, Jentzen W, Rosenbaum SJ, Kuhl H, Bockisch A, Gorges R. Combined metabolic and morphologic imaging in thyroid carcinoma patients with elevated serum thyroglobulin and negative cervical ultrasonography: role of (124)I-PET/CT and FDG-PET. *Eur J Nucl Med Mol Imaging.* 2008
51. Kolbert KS, Pentlow KS, Pearson JR, Sheikh A, Finn RD, Humm JL, Larson SM. Prediction of absorbed dose to normal organs in thyroid cancer patients treated with 131I by use of 124I PET and 3-dimensional internal dosimetry software. *J Nucl Med.* 2007; 48:143–9. [PubMed: 17204711]
52. Jayson GC, Zweit J, Jackson A, Mulatero C, Julyan P, Ranson M, Broughton L, Wagstaff J, Hakansson L, Groenewegen G, Bailey J, Smith N, Hastings D, Lawrance J, Haroon H, Ward T, McGown AT, Tang M, Levitt D, Marreud S, Lehmann FF, Herold M, Zwierzina H. Molecular imaging and biological evaluation of HuMV833 anti-VEGF antibody: implications for trial design of antiangiogenic antibodies. *J Natl Cancer Inst.* 2002; 94:1484–93. [PubMed: 12359857]
53. Divgi CR, Pandit-Taskar N, Jungbluth AA, Reuter VE, Gonen M, Ruan S, Pierre C, Nagel A, Pryma DA, Humm J, Larson SM, Old LJ, Russo P. Preoperative characterisation of clear-cell renal carcinoma using iodine-124-labelled antibody chimeric G250 (124I-cG250) and PET in patients with renal masses: a phase I trial. *Lancet Oncol.* 2007; 8:304–10. [PubMed: 17395103]
54. Vedenyev, VIGLV.; Kondrat'Yev, VN.; Medvedev, VA. Bond Energies, ionization Potentials and electron Affinities. Edward Arnold; London: 1966.
55. Orlova A, Hoglund J, Lubberink M, Lebeda O, Gedda L, Lundqvist H, Tolmachev V, Sundin A. Comparative biodistribution of the radiohalogenated (Br, I and At) antibody A33. Implications for in vivo dosimetry. *Cancer Biother Radiopharm.* 2002; 17:385–96. [PubMed: 12396703]
56. Rossin R, Berndorff D, Friebe M, Dinkelborg LM, Welch MJ. Small-animal PET of tumor angiogenesis using a (76)Br-labeled human recombinant antibody fragment to the ED-B domain of fibronectin. *J Nucl Med.* 2007; 48:1172–9. [PubMed: 17574989]
57. Bergstrom M, Lu L, Fasth KJ, Wu F, Bergstrom-Pettermann E, Tolmachev V, Hedberg E, Cheng A, Langstrom B. In vitro and animal validation of bromine-76-bromodeoxyuridine as a proliferation marker. *J Nucl Med.* 1998; 39:1273–9. [PubMed: 9669410]
58. Loc'h C, Mardon K, Valette H, Brutesco C, Merlet P, Syrota A, Maziere B. Preparation and pharmacological characterization of [76Br]-meta-bromobenzylguanidine ([76Br]MBBG). *Nucl Med Biol.* 1994; 21:49–55. [PubMed: 9234263]

59. Scott-Robson S, Capala J, Malmberg P, Lundqvist H. Production of ⁷⁶Br and its use in labeling proteins. *Acta Radiol Suppl.* 1991; 376:64. [PubMed: 1666784]
60. Knight LC, Harwig SL, Welch MJ. In vitro stability and in vivo clearance of fibrinogen or serum albumin labeled with ⁷⁷Br, ¹³¹I, or ¹²⁵I by direct or indirect synthetic methods. *J Nucl Med.* 1977; 18:282–8. [PubMed: 839278]
61. Wilbur DS, Hyllarides MD. Radiolabeling of a monoclonal antibody with N-succinimidyl para-[⁷⁷Br]bromobenzoate. *Int J Rad Appl Instrum B.* 1991; 18:363–5. [PubMed: 2071449]
62. Lovqvist A, Sundin A, Ahlstrom H, Carlsson J, Lundqvist H. ⁷⁶Br-labeled monoclonal anti-CEA antibodies for radioimmuno positron emission tomography. *Nucl Med Biol.* 1995; 22:125–31. [PubMed: 7735162]
63. Winberg KJ, Persson M, Malmstrom PU, Sjoberg S, Tolmachev V. Radiobromination of anti-HER2/neu/ErbB-2 monoclonal antibody using the p-isothiocyanatobenzene derivative of the [⁷⁶Br]undecahydro-bromo-7,8-dicarba-nido-undecaborate(1–) ion. *Nucl Med Biol.* 2004; 31:425–33. [PubMed: 15093812]
64. Mume E, Orlova A, Larsson B, Nilsson AS, Nilsson FY, Sjoberg S, Tolmachev V. Evaluation of ((4-hydroxyphenyl)ethyl)maleimide for site-specific radiobromination of anti-HER2 antibody. *Bioconjug Chem.* 2005; 16:1547–55. [PubMed: 16287254]
65. Lovqvist A, Sundin A, Ahlstrom H, Carlsson J, Lundqvist H. Pharmacokinetics and experimental PET imaging of a bromine-76-labeled monoclonal anti-CEA antibody. *J Nucl Med.* 1997; 38:395–401. [PubMed: 9074527]
66. Lovqvist A, Lundqvist H, Lubberink M, Tolmachev V, Carlsson J, Sundin A. Kinetics of ⁷⁶Br-labeled anti-CEA antibodies in pigs; aspects of dosimetry and PET imaging properties. *Med Phys.* 1999; 26:249–58. [PubMed: 10076983]
67. Lovqvist A, Sundin A, Roberto A, Ahlstrom H, Carlsson J, Lundqvist H. Comparative PET imaging of experimental tumors with bromine-76-labeled antibodies, fluorine-18-fluorodeoxyglucose and carbon-11-methionine. *J Nucl Med.* 1997; 38:1029–35. [PubMed: 9225786]
68. Hoglund J, Tolmachev V, Orlova A, Lundqvist H, Sundin A. Optimized indirect (⁷⁶Br)-bromination of antibodies using N-succinimidyl para-[⁷⁶Br]bromobenzoate for radioimmuno PET. *Nucl Med Biol.* 2000; 27:837–43. [PubMed: 11150718]
69. Maziere B, Loc'h C, Baron JC, Sgouropoulos P, Duquesnoy N, D'Antona R, Cambon H. In vivo quantitative imaging of dopamine receptors in human brain using positron emission tomography and [⁷⁶Br]bromospiperone. *Eur J Pharmacol.* 1985; 114:267–72. [PubMed: 3877639]
70. Martinot JL, Peron-Magnan P, Huret JD, Mazoyer B, Baron JC, Boulenger JP, Loc'h C, Maziere B, Caillard V, Loo H, et al. Striatal D2 dopaminergic receptors assessed with positron emission tomography and [⁷⁶Br]bromospiperone in untreated schizophrenic patients. *Am J Psychiatry.* 1990; 147:44–50. [PubMed: 2293788]
71. Davidsson L, Cederblad A, Hagebo E, Lonnerdal B, Sandstrom B. Intrinsic and extrinsic labeling for studies of manganese absorption in humans. *J Nutr.* 1988; 118:1517–21. [PubMed: 3145332]
72. Sastri CS, Petri H, Kueppers G, Erdtmann G. Production of manganese-52 of high isotopic purity by ³He-activation of vanadium. *Int J Applied Radiation and Isotopes.* 1981; 32:246–247.
73. Lambrecht RM, Wolf AP. The cyclotron production of ³⁸K, ⁵¹Mn, ⁵²Mn, and ⁷⁷Kr for positron emission tomography. *Journal of Labelled Compounds and Radiopharmaceuticals.* 1979; 16:129–130.
74. Kong G, Jackson C, Koh DM, Lewington V, Sharma B, Brown G, Cunningham D, Cook GJR. The use of ¹⁸F-FDG PET/CT in colorectal liver metastases-comparison with CT and liver MRI. *European Journal of Nuclear Medicine and Molecular Imaging.* 2008;1–7. [PubMed: 17828536]
75. Cikrt M. Biliary excretion of ²⁰³Hg, ⁶⁴Cu, ⁵²Mn, and ²¹⁰Pb in the rat. *Br J Ind Med.* 1972; 29:74–80. [PubMed: 5060249]
76. Sharma HZJ, Smith AM, Downey S. Production of cobalt-55, a short-lived, positron emitting radiolabel for bleomycin. *International Journal of Radiation and Instrumentation [A].* 1986; 37:105–109.

77. Srivastava SCMLF, Kolsky KL, Mease RC, Joshi V, Meinken GE, Pyatt B, Wolf AP, Schlyder DJ, Levy AV, Fowler JS. Production and use of Cobalt-55 as an antibody label for PET imaging. *Journal of Labelled Compounds and Radiopharmaceuticals*. 1994; 35:389–391.
78. Lagunas-Solar MC, Jungerman JA. Cyclotron production of carrier-free cobalt-55, a new positron-emitting label for bleomycin. *International Journal of Applied Radiation and Isotopes*. 1979; 30:25–32.
79. Goethals P, Volkaert A, Vandewielle C, Dierckx R, Lameire N. ⁵⁵Co-EDTA for renal imaging using positron emission tomography (PET): a feasibility study. *Nucl Med Biol*. 2000; 27:77–81. [PubMed: 10755649]
80. Nieweg OE, Beekhuis H, Paans AM, Piers DA, Vaalburg W, Welleweerd J, Wiegman T, Woldring MG. Detection of lung cancer with ⁵⁵Co-bleomycin using a positron camera. A comparison with ⁵⁷Co-bleomycin and ⁵⁵Co-bleomycin single photon scintigraphy. *Eur J Nucl Med*. 1982; 7:104–7. [PubMed: 6175521]
81. Stevens H, Jansen HM, De Reuck J, Lemmerling M, Strijckmans K, Goethals P, Lemahieu I, de Jong BM, Willemsen AT, Korf J. ⁵⁵Cobalt (Co) as a PET-tracer in stroke, compared with blood flow, oxygen metabolism, blood volume and gadolinium-MRI. *J Neurol Sci*. 1999; 171:11–8. [PubMed: 10567044]
82. Jansen HM, Paans AM, vd Vliet AM, Veenma-van der Duin L, Bolwijn-Meijer CJ, Pruim J, Willemsen AT, Franssen EJ, Minderhoud JM, Korf J. Cobalt-55 positron emission tomography in ischemic stroke. *Clin Neurol Neurosurg*. 1997; 99:6–10. [PubMed: 9107460]
83. Byegård JSG, Skålberg M. The stability of some metal EDTA, DTPA and DOTA complexes: Application as tracers in groundwater studies. *Journal of Radioanalytical and Nuclear Chemistry*. 1999; 241:281–290.
84. Heppeler A, Andre JP, Buschmann I, Wang X, Reubi JC, Hennig M, Kaden TA, Maecke HR. Metal-ion-dependent biological properties of a chelator-derived somatostatin analogue for tumour targeting. *Chemistry*. 2008; 14:3026–34. [PubMed: 18246556]
85. Kukis DL, DeNardo SJ, DeNardo GL, O'Donnell RT, Meares CF. Optimized conditions for chelation of yttrium-90-DOTA immunoconjugates. *J Nucl Med*. 1998; 39:2105–10. [PubMed: 9867151]
86. Kukis DL, DeNardo GL, DeNardo SJ, Mirick GR, Miers LA, Greiner DP, Meares CF. Effect of the extent of chelate substitution on the immunoreactivity and biodistribution of 2IT-BAT-Lym-1 immunoconjugates. *Cancer Res*. 1995; 55:878–84. [PubMed: 7850803]
87. Beekhuis H, Nieweg OE. Radiation absorbed doses from Co-57- and Co-55 bleomycin. *J Nucl Med*. 1984; 25:478–85. [PubMed: 6085803]
88. Bowen, H. Trace Elements in Biochemistry. Academic Press; London: 1966. The Biogeochemistry of the Elements; p. 173-239.
89. Goethals P, Coene M, Slegers G, Vogelaers D, Everaert J, Lemahieu I, Colardyn F, Heyndrickx GR. Production of carrier-free ⁶⁶Ga and labeling of antimyosin antibody for positron imaging of acute myocardial infarction. *Eur J Nucl Med*. 1990; 16:237–40. [PubMed: 2351172]
90. Graham MC, Pentlow KS, Mawlawi O, Finn RD, Daghighian F, Larson SM. An investigation of the physical characteristics of ⁶⁶Ga as an isotope for PET imaging and quantification. *Med Phys*. 1997; 24:317–26. [PubMed: 9048374]
91. Szelecsenyi F, Tarkanyi F, Kovacs Z, Bergman J, Heselius SJ, Solin O. Production of ⁶⁶Ga and ⁶⁷Ga at a compact cyclotron. *Acta Radiol Suppl*. 1991; 376:62–3. [PubMed: 1666783]
92. Goethals P, Coene M, Slegers G, Agon P, Deman J, Schelstraete K. Cyclotron production of carrier-free ⁶⁶Ga as a positron emitting label of albumin colloids for clinical use. *Eur J Nucl Med*. 1988; 14:152–4. [PubMed: 3261243]
93. Zweit J, Sharma H, Downey S. Production of gallium-66, a short-lived, positron emitting radionuclide. *Int J Rad Appl Instrum [A]*. 1987; 38:499–501.
94. Green MA, Welch MJ. Gallium radiopharmaceutical chemistry. *Int J Rad Appl Instrum B*. 1989; 16:435–48. [PubMed: 2681083]
95. Brechbiel MW. Bifunctional chelates for metal nuclides. *Q J Nucl Med Mol Imaging*. 2008; 52:166–73. [PubMed: 18043537]

96. Harris WR, Chen Y. Electron paramagnetic resonance and difference ultraviolet studies of Mn²⁺ binding to serum transferrin. *J Inorg Biochem.* 1994; 54:1–19. [PubMed: 8151309]
97. Eisenwiener KP, Prata MI, Buschmann I, Zhang HW, Santos AC, Wenger S, Reubi JC, Macke HR. NODAGATOC, a new chelator-coupled somatostatin analogue labeled with [67/68Ga] and [111In] for SPECT, PET, and targeted therapeutic applications of somatostatin receptor (hsst2) expressing tumors. *Bioconjug Chem.* 2002; 13:530–41. [PubMed: 12009943]
98. Lee J, Garmestani K, Wu C, Brechbiel MW, Chang HK, Choi CW, Gansow OA, Carrasquillo JA, Paik CH. In vitro and in vivo evaluation of structure-stability relationship of 111In- and 67Ga-labeled antibody via 1B4M or C-NOTA chelates. *Nucl Med Biol.* 1997; 24:225–30. [PubMed: 9228656]
99. Velikyan I, Maecke H, Langstrom B. Convenient preparation of 68Ga-based PET-radiopharmaceuticals at room temperature. *Bioconjug Chem.* 2008; 19:569–73. [PubMed: 18205327]
100. Mathias CJ, Lewis MR, Reichert DE, Laforest R, Sharp TL, Lewis JS, Yang ZF, Waters DJ, Snyder PW, Low PS, Welch MJ, Green MA. Preparation of 66Ga- and 68Ga-labeled Ga(III)-deferoxamine-folate as potential folate-receptor-targeted PET radiopharmaceuticals. *Nucl Med Biol.* 2003; 30:725–31. [PubMed: 14499330]
101. Ugur O, Kothari PJ, Finn RD, Zanzonico P, Ruan S, Guenther I, Maecke HR, Larson SM. Ga-66 labeled somatostatin analogue DOTA-DPhe1-Tyr3-octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. *Nucl Med Biol.* 2002; 29:147–57. [PubMed: 11823119]
102. Van Der Werff JT. Clinical investigations on the use of radioactive gallium (Ga66 and Ga67) in bone diseases. *Acta radiol.* 1954; 41:343–7. [PubMed: 13158135]
103. Henze M, Dimitrakopoulou-Strauss A, Milker-Zabel S, Schuhmacher J, Strauss LG, Doll J, Macke HR, Eisenhut M, Debus J, Haberkorn U. Characterization of 68Ga-DOTA-D-Phe1-Tyr3-octreotide kinetics in patients with meningiomas. *J Nucl Med.* 2005; 46:763–9. [PubMed: 15872348]
104. Koukouraki S, Strauss LG, Georgoulas V, Eisenhut M, Haberkorn U, Dimitrakopoulou-Strauss A. Comparison of the pharmacokinetics of 68Ga-DOTATOC and [18F]FDG in patients with metastatic neuroendocrine tumours scheduled for 90Y-DOTATOC therapy. *Eur J Nucl Med Mol Imaging.* 2006; 33:1115–22. [PubMed: 16763820]
105. Smith-Jones PM, Solit D, Afroze F, Rosen N, Larson SM. Early tumor response to Hsp90 therapy using HER2 PET: comparison with 18F-FDG PET. *J Nucl Med.* 2006; 47:793–6. [PubMed: 16644749]
106. Smith-Jones PM, Solit DB, Akhurst T, Afroze F, Rosen N, Larson SM. Imaging the pharmacodynamics of HER2 degradation in response to Hsp90 inhibitors. *Nat Biotechnol.* 2004; 22:701–6. [PubMed: 15133471]
107. Jennewein M, Qaim SM, Hermanne A, Jahn M, Tsyganov E, Slavine N, Seliounine S, Antich PA, Kulkarni PV, Thorpe PE, Mason RP, Rösch F. A new method for radiochemical separation of arsenic from irradiated germanium oxide. *Applied Radiation and Isotopes.* 2005; 63:343–351. [PubMed: 15955705]
108. Jennewein M, Qaim SM, Kulkarni PV, Mason RP, Hermanne A, Rösch F. A no-carrier-added 72Se/72As radionuclide generator based on solid phase extraction. *Radiochimica Acta.* 2005; 93:579–583.
109. Jennewein M, Schmidt A, Novgorodov AF, Qaim SM, Rösch F. A no-carrier-added 72Se/72As radionuclide generator based on distillation. *Radiochimica Acta.* 2004; 92:245–249.
110. Al-Kouraishi SH, Boswell GGJ. An isotope generator for 72As. *International Journal of Applied Radiation and Isotopes.* 1978; 29:607–609.
111. Jennewein M, Lewis MA, Zhao D, Tsyganov E, Slavine N, He J, Watkins L, Kodibagkar VD, O'Kelly S, Kulkarni P, Antich PP, Hermanne A, Rosch F, Mason RP, Thorpe PE. Vascular Imaging of Solid Tumors in Rats with a Radioactive Arsenic-Labeled Antibody that Binds Exposed Phosphatidylserine. *Clin Cancer Res.* 2008; 14:1377–85. [PubMed: 18316558]
112. Turner PC. The calculation of absorbed tissue doses from arsenic-72. *Phys Med Biol.* 1962; 7:329–34. [PubMed: 13994909]

113. Connett JM, Buettner TL, Anderson CJ. Maximum tolerated dose and large tumor radioimmunotherapy studies of ^{64}Cu -labeled monoclonal antibody 1A3 in a colon cancer model. *Clin Cancer Res.* 1999; 5:3207s–3212s. [PubMed: 10541365]
114. Ruangma A, Bai B, Lewis JS, Sun X, Welch MJ, Leahy R, Laforest R. Three-dimensional maximum a posteriori (MAP) imaging with radiopharmaceuticals labeled with three Cu radionuclides. *Nucl Med Biol.* 2006; 33:217–26. [PubMed: 16546676]
115. Obata A, Kasamatsu S, McCarthy DW, Welch MJ, Saji H, Yonekura Y, Fujibayashi Y. Production of therapeutic quantities of (^{64}Cu) using a 12 MeV cyclotron. *Nucl Med Biol.* 2003; 30:535–9. [PubMed: 12831992]
116. McCarthy DW, Shefer RE, Klinkowstein RE, Bass LA, Margeneau WH, Cutler CS, Anderson CJ, Welch MJ. Efficient production of high specific activity ^{64}Cu using a biomedical cyclotron. *Nucl Med Biol.* 1997; 24:35–43. [PubMed: 9080473]
117. Zinn KR, Chaudhuri TR, Cheng TP, Morris JS, Meyer WA Jr. Production of no-carrier-added ^{64}Cu from zinc metal irradiated under boron shielding. *Cancer.* 1994; 73:774–8. [PubMed: 8306259]
118. Hilgers K, Stoll T, Skakun Y, Coenen HH, Qaim SM. Cross-section measurements of the nuclear reactions $\text{natZn(d,x)}^{64}\text{Cu}$, $^{66}\text{Zn(d,}\alpha)^{64}\text{Cu}$ and $^{68}\text{Zn(p,}\alpha)^{64}\text{Cu}$ for production of ^{64}Cu and technical developments for small-scale production of ^{67}Cu via the $^{70}\text{Zn(p,}\alpha)^{67}\text{Cu}$ process. *Appl Radiat Isot.* 2003; 59:343–51. [PubMed: 14622933]
119. Qaim SM. Decay data and production yields of some non-standard positron emitters used in PET. *Q J Nucl Med Mol Imaging.* 2008; 52:111–6. [PubMed: 18174879]
120. Szelecsényi F, Steyn GF, Kovács Z, Vermeulen C, Van Der Meulen NP, Dolley SG, Van Der Walt TN, Suzuki K, Mukai K. Investigation of the $^{66}\text{Zn(p,2p)}^{64}\text{Cu}$ and $^{68}\text{Zn(p,x)}^{64}\text{Cu}$ nuclear processes up to 100 MeV: Production of ^{64}Cu . *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms.* 2005; 240:625–637.
121. Cole WC, DeNardo SJ, Meares CF, McCall MJ, DeNardo GL, Epstein AL, O'Brien HA, Moi MK. Serum stability of ^{67}Cu chelates: comparison with ^{111}In and ^{57}Co . *Int J Rad Appl Instrum B.* 1986; 13:363–8. [PubMed: 3539884]
122. Franz J, Freeman GM, Barefield EK, Volkert WA, Ehrhardt GJ, Holmes RA. Labeling of antibodies with ^{64}Cu using a conjugate containing a macrocyclic amine chelating agent. *International Journal of Radiation Applications and Instrumentation. Part B Nuclear Medicine and Biology.* 1987; 14:479–484.
123. Rogers BE, Anderson CJ, Connett JM, Guo LW, Edwards WB, Sherman EL, Zinn KR, Welch MJ. Comparison of four bifunctional chelates for radiolabeling monoclonal antibodies with copper radioisotopes: biodistribution and metabolism. *Bioconjug Chem.* 1996; 7:511–22. [PubMed: 8853465]
124. Anderson CJ, Connett JM, Schwarz SW, Rocque PA, Guo LW, Philpott GW, Zinn KR, Meares CF, Welch MJ. Copper-64-labeled antibodies for PET imaging. *J Nucl Med.* 1992; 33:1685–91. [PubMed: 1517844]
125. Ping Li W, Meyer LA, Capretto DA, Sherman CD, Anderson CJ. Receptor-binding, biodistribution, and metabolism studies of ^{64}Cu -DOTA-cetuximab, a PET-imaging agent for epidermal growth-factor receptor-positive tumors. *Cancer Biother Radiopharm.* 2008; 23:158–71. [PubMed: 18454685]
126. Anderson CJ, Wadas TJ, Wong EH, Weisman GR. Cross-bridged macrocyclic chelators for stable complexation of copper radionuclides for PET imaging. *Q J Nucl Med Mol Imaging.* 2008; 52:185–92. [PubMed: 18043536]
127. Sprague JE, Peng Y, Sun X, Weisman GR, Wong EH, Achilefu S, Anderson CJ. Preparation and biological evaluation of copper-64-labeled tyr3-octreotate using a cross-bridged macrocyclic chelator. *Clin Cancer Res.* 2004; 10:8674–82. [PubMed: 15623652]
128. Voss SD, Smith SV, DiBartolo N, McIntosh LJ, Cyr EM, Bonab AA, Dearling JL, Carter EA, Fischman AJ, Treves ST, Gillies SD, Sargeson AM, Huston JS, Packard AB. Positron emission tomography (PET) imaging of neuroblastoma and melanoma with ^{64}Cu -SarAr immunoconjugates. *Proc Natl Acad Sci U S A.* 2007; 104:17489–93. [PubMed: 17954911]

129. Bhalgat MK, Roberts JC, Mercer-Smith JA, Knotts BD, Vessella RL, Lavalley DK. Preparation and biodistribution of copper-67-labeled porphyrins and porphyrin-A6H immunoconjugates. *Nucl Med Biol.* 1997; 24:179–85. [PubMed: 9089710]
130. Ping Li W, Meyer LA, Capretto DA, Sherman CD, Anderson CJ. Receptor-Binding, Biodistribution, and Metabolism Studies of (64)Cu-DOTA-Cetuximab, a PET-Imaging Agent for Epidermal Growth-Factor Receptor-Positive Tumors. *Cancer Biother Radiopharm.* 2008; 23:158–71. [PubMed: 18454685]
131. Cai W, Wu Y, Chen K, Cao Q, Tice DA, Chen X. In vitro and in vivo characterization of 64Cu-labeled Abegrin, a humanized monoclonal antibody against integrin alpha v beta 3. *Cancer Res.* 2006; 66:9673–81. [PubMed: 17018625]
132. Cai W, Chen K, He L, Cao Q, Koong A, Chen X. Quantitative PET of EGFR expression in xenograft-bearing mice using 64Cu-labeled cetuximab, a chimeric anti-EGFR monoclonal antibody. *Eur J Nucl Med Mol Imaging.* 2007; 34:850–8. [PubMed: 17262214]
133. Grunberg J, Novak-Hofer I, Honer M, Zimmermann K, Knogler K, Blauenstein P, Ametamey S, Maecke HR, Schubiger PA. In vivo evaluation of 177Lu- and 67/64Cu-labeled recombinant fragments of antibody chCE7 for radioimmunotherapy and PET imaging of L1-CAM-positive tumors. *Clin Cancer Res.* 2005; 11:5112–20. [PubMed: 16033825]
134. Novak-Hofer I, Honer M, Ametamey S, Grunberg J, Missimer J, Zimmermann K, Schubiger PA. Imaging of renal carcinoma xenografts with 64Cu-labelled anti-L1-CAM antibody chCE7. *Eur J Nucl Med Mol Imaging.* 2003; 30:1066. [PubMed: 12761598]
135. Li L, Bading J, Yazaki PJ, Ahuja AH, Crow D, Colcher D, Williams LE, Wong JY, Raubitschek A, Shively JE. A versatile bifunctional chelate for radiolabeling humanized anti-CEA antibody with In-111 and Cu-64 at either thiol or amino groups: PET imaging of CEA-positive tumors with whole antibodies. *Bioconjug Chem.* 2008; 19:89–96. [PubMed: 17988078]
136. Connett JM, Anderson CJ, Guo LW, Schwarz SW, Zinn KR, Rogers BE, Siegel BA, Philpott GW, Welch MJ. Radioimmunotherapy with a 64Cu-labeled monoclonal antibody: a comparison with 67Cu. *Proc Natl Acad Sci U S A.* 1996; 93:6814–8. [PubMed: 8692901]
137. Philpott GW, Schwarz SW, Anderson CJ, Dehdashti F, Connett JM, Zinn KR, Meares CF, Cutler PD, Welch MJ, Siegel BA. RadioimmunoPET: detection of colorectal carcinoma with positron-emitting copper-64-labeled monoclonal antibody. *J Nucl Med.* 1995; 36:1818–24. [PubMed: 7562049]
138. Yoo J, Tang L, Perkins TA, Rowland DJ, Laforest R, Lewis JS, Welch MJ. Preparation of high specific activity (86)Y using a small biomedical cyclotron. *Nucl Med Biol.* 2005; 32:891–7. [PubMed: 16253815]
139. Garmestani K, Milenic DE, Plascjak PS, Brechbiel MW. A new and convenient method for purification of 86Y using a Sr(II) selective resin and comparison of biodistribution of 86Y and 111In labeled Herceptin. *Nucl Med Biol.* 2002; 29:599–606. [PubMed: 12088731]
140. Park LS, Szajek LP, Wong KJ, Plascjak PS, Garmestani K, Googins S, Eckelman WC, Carrasquillo JA, Paik CH. Semi-automated 86Y purification using a three-column system. *Nucl Med Biol.* 2004; 31:297–301. [PubMed: 15013497]
141. Smith S, Thomas RM, Steere HA, Beatty HE, Dawson KB, Peckham MJ. Therapeutic irradiation of the central nervous system using intrathecal 90Y--DTA. *Br J Radiol.* 1976; 49:141–7. [PubMed: 1065444]
142. Hnatowich DJ, Virzi F, Doherty PW. DTPA-coupled antibodies labeled with yttrium-90. *J Nucl Med.* 1985; 26:503–9. [PubMed: 3989606]
143. Sharkey RM, Motta-Hennessy C, Gansow OA, Brechbiel MW, Fand I, Griffiths GL, Jones AL, Goldenberg DM. Selection of a DTPA chelate conjugate for monoclonal antibody targeting to a human colonic tumor in nude mice. *Int J Cancer.* 1990; 46:79–85. [PubMed: 2114375]
144. Washburn LC, Sun TT, Lee YC, Byrd BL, Holloway EC, Crook JE, Stubbs JB, Stabin MG, Brechbiel MW, Gansow OA, et al. Comparison of five bifunctional chelate techniques for 90Y-labeled monoclonal antibody CO17-1A. *Int J Rad Appl Instrum B.* 1991; 18:313–21. [PubMed: 2071444]
145. Brechbiel MW, Gansow OA. Backbone-substituted DTPA ligands for 90Y radioimmunotherapy. *Bioconjug Chem.* 1991; 2:187–94. [PubMed: 1932218]

146. Camera L, Kinuya S, Garmestani K, Wu C, Brechbiel MW, Pai LH, McMurty TJ, Gansow OA, Pastan I, Paik CH, et al. Evaluation of the serum stability and in vivo biodistribution of CHX-DTPA and other ligands for yttrium labeling of monoclonal antibodies. *J Nucl Med.* 1994; 35:882–9. [PubMed: 8176477]
147. Camera L, Kinuya S, Garmestani K, Pai LH, Brechbiel MW, Gansow OA, Paik CH, Pastan I, Carrasquillo JA. Evaluation of a new DTPA-derivative chelator: comparative biodistribution and imaging studies of ¹¹¹In-labeled B3 monoclonal antibody in athymic mice bearing human epidermoid carcinoma xenografts. *Nucl Med Biol.* 1993; 20:955–62. [PubMed: 8298575]
148. Kobayashi H, Wu C, Yoo TM, Sun BF, Drumm D, Pastan I, Paik CH, Gansow OA, Carrasquillo JA, Brechbiel MW. Evaluation of the in vivo biodistribution of yttrium-labeled isomers of CHX-DTPA-conjugated monoclonal antibodies. *J Nucl Med.* 1998; 39:829–36. [PubMed: 9591585]
149. Deshpande SV, DeNardo SJ, Kukis DL, Moi MK, McCall MJ, DeNardo GL, Meares CF. Yttrium-90-labeled monoclonal antibody for therapy: labeling by a new macrocyclic bifunctional chelating agent. *J Nucl Med.* 1990; 31:473–9. [PubMed: 2324823]
150. Harrison A, Walker CA, Parker D, Jankowski KJ, Cox JP, Craig AS, Sansom JM, Beeley NR, Boyce RA, Chaplin L, et al. The in vivo release of ⁹⁰Y from cyclic and acyclic ligand-antibody conjugates. *Int J Rad Appl Instrum B.* 1991; 18:469–76. [PubMed: 1917516]
151. Wong JY, Chu DZ, Williams LE, Liu A, Zhan J, Yamauchi DM, Wilczynski S, Wu AM, Yazaki PJ, Shively JE, Leong L, Raubitschek AA. A phase I trial of (90)Y-DOTA-anti-CEA chimeric T84.66 (cT84.66) radioimmunotherapy in patients with metastatic CEA-producing malignancies. *Cancer Biother Radiopharm.* 2006; 21:88–100. [PubMed: 16706629]
152. Palm S, Enmon RM Jr, Matei C, Kolbert KS, Xu S, Zanzonico PB, Finn RL, Koutcher JA, Larson SM, Sgouros G. Pharmacokinetics and Biodistribution of (86)Y-Trastuzumab for (90)Y dosimetry in an ovarian carcinoma model: correlative MicroPET and MRI. *J Nucl Med.* 2003; 44:1148–55. [PubMed: 12843231]
153. Lovqvist A, Humm JL, Sheikh A, Finn RD, Koziorowski J, Ruan S, Pentlow KS, Jungbluth A, Welt S, Lee FT, Brechbiel MW, Larson SM. PET imaging of (86)Y-labeled anti-Lewis Y monoclonal antibodies in a nude mouse model: comparison between (86)Y and (111)In radiolabels. *J Nucl Med.* 2001; 42:1281–7. [PubMed: 11483692]
154. Regino, CM.; Wong, D.; Garmestani, K.; Baidoo, K.; Williams, K.; Seidel, M.; Green, J.; Choyke, M.; Brechbiel, PM. 99th Annual Meeting of the American Association for Cancer Research. American Association for Cancer Research; San Diego: 2008. p. Abstract # 3159
155. Jamar F, Barone R, Mathieu I, Walrand S, Labar D, Carlier P, de Camps J, Schran H, Chen T, Smith MC, Bouterfa H, Valkema R, Krenning EP, Kvols LK, Pauwels S. ⁸⁶Y-DOTA0)-D-Phe1-Tyr3-octreotide (SMT487)—a phase 1 clinical study: pharmacokinetics, biodistribution and renal protective effect of different regimens of amino acid co-infusion. *Eur J Nucl Med Mol Imaging.* 2003; 30:510–8. [PubMed: 12582815]
156. Perk LR, Visser GW, Vosjan MJ, Stigter-van Walsum M, Tijink BM, Leemans CR, van Dongen GA. (89)Zr as a PET surrogate radioisotope for scouting biodistribution of the therapeutic radiometals (90)Y and (177)Lu in tumor-bearing nude mice after coupling to the internalizing antibody cetuximab. *J Nucl Med.* 2005; 46:1898–906. [PubMed: 16269605]
157. Hohn A, Zimmermann K, Schaub E, Hirzel W, Schubiger PA, Schibli R. Production and separation of “non-standard” positron emission tomography nuclides at a large cyclotron facility: the experiences at the Paul Scherrer Institute Switzerland. *Q J Nucl Med Mol Imaging.* 2008
158. Meijs WE, Herscheid JD, Haisma HJ, Pinedo HM. Evaluation of desferal as a bifunctional chelating agent for labeling antibodies with Zr-89. *Int J Rad Appl Instrum [A].* 1992; 43:1443–7.
159. Pagani M, Stone-Elander S, Larsson SA. Alternative positron emission tomography with non-conventional positron emitters: effects of their physical properties on image quality and potential clinical applications. *Eur J Nucl Med.* 1997; 24:1301–27. [PubMed: 9323273]
160. Link JM, Krohn KA, Eary JF. ⁸⁹Zr for antibody labeling and positron emission tomography. *Journal of Labelled Compounds and Radiopharmaceuticals.* 1986; 23:1297–1298.
161. Ali SA, Shankland EG. Radiochemical synthesis of ⁸⁹Zr-porphyrin for positron label of monoclonal antibodies. *Journal of Labelled Compounds and Radiopharmaceuticals.* 1991; 30:326.

162. Meijs WE, Haisma HJ, Klok RP, van Gog FB, Kievit E, Pinedo HM, Herscheid JD. Zirconium-labeled monoclonal antibodies and their distribution in tumor-bearing nude mice. *J Nucl Med.* 1997; 38:112–8. [PubMed: 8998164]
163. Meijs WE, Haisma HJ, Van der Schors R, Wijbrandts R, Van den Oever K, Klok RP, Pinedo HM, Herscheid JD. A facile method for the labeling of proteins with zirconium isotopes. *Nucl Med Biol.* 1996; 23:439–48. [PubMed: 8832698]
164. Verel I, Visser GW, Boellaard R, Stigter-van Walsum M, Snow GB, van Dongen GA. ⁸⁹Zr immuno-PET: comprehensive procedures for the production of ⁸⁹Zr-labeled monoclonal antibodies. *J Nucl Med.* 2003; 44:1271–81. [PubMed: 12902418]
165. Witzig TE, Gordon LI, Cabanillas F, Czuczman MS, Emmanouilides C, Joyce R, Pohlman BL, Bartlett NL, Wiseman GA, Padre N, Grillo-Lopez AJ, Multani P, White CA. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J Clin Oncol.* 2002; 20:2453–63. [PubMed: 12011122]
166. Verel I, Visser GW, van Dongen GA. The promise of immuno-PET in radioimmunotherapy. *J Nucl Med.* 2005; 46(Suppl 1):164S–71S. [PubMed: 15653665]
167. Nagengast WB, de Vries EG, Hospers GA, Mulder NH, de Jong JR, Hollema H, Brouwers AH, van Dongen GA, Perk LR, Lub-de Hooge MN. In vivo VEGF imaging with radiolabeled bevacizumab in a human ovarian tumor xenograft. *J Nucl Med.* 2007; 48:1313–9. [PubMed: 17631557]
168. Perk LR, Visser OJ, Stigter-van Walsum M, Vosjan MJ, Visser GW, Zijlstra JM, Huijgens PC, van Dongen GA. Preparation and evaluation of (⁸⁹)Zr-Zevalin for monitoring of (⁹⁰)Y-Zevalin biodistribution with positron emission tomography. *Eur J Nucl Med Mol Imaging.* 2006; 33:1337–45. [PubMed: 16832633]
169. Borjesson PK, Jauw YW, Boellaard R, de Bree R, Comans EF, Roos JC, Castelijns JA, Vosjan MJ, Kummer JA, Leemans CR, Lammertsma AA, van Dongen GA. Performance of immuno-positron emission tomography with zirconium-89-labeled chimeric monoclonal antibody U36 in the detection of lymph node metastases in head and neck cancer patients. *Clin Cancer Res.* 2006; 12:2133–40. [PubMed: 16609026]
170. Perk LR, Stigter-van Walsum M, Visser GW, Kloet RW, Vosjan MJ, Leemans CR, Giaccone G, Albano R, Comoglio PM, van Dongen GA. Quantitative PET imaging of Met-expressing human cancer xenografts with (⁸⁹)Zr-labelled monoclonal antibody DN30. *Eur J Nucl Med Mol Imaging.* 2008
171. Laforest R, Liu X. Image quality with non-standard nuclides in positron emission tomography. *Q J Nucl Med Mol Imaging.* 2007
172. Beattie BJ, Finn RD, Rowland DJ, Pentlow KS. Quantitative imaging of bromine-76 and yttrium-86 with PET: a method for the removal of spurious activity introduced by cascade gamma rays. *Med Phys.* 2003; 30:2410–23. [PubMed: 14528963]
173. Vandenberghe S. Three-dimensional positron emission tomography imaging with ¹²⁴I and ⁸⁶Y. *Nucl Med Commun.* 2006; 27:237–45. [PubMed: 16479243]
174. Bale WF, Spar IL. Studies directed toward the use of antibodies as carriers of radioactivity for therapy. *Adv Biol Med Phys.* 1957; 5:285–356. [PubMed: 13520434]
175. Bale WF, Spar IL, Goodland RL. Experimental radiation therapy of tumors with I-131-carrying antibodies to fibrin. *Cancer Res.* 1960; 20:1488–94. [PubMed: 13686301]
176. Milenic DE, Brady ED, Brechbiel MW. Antibody-targeted radiation cancer therapy. *Nat Rev Drug Discov.* 2004; 3:488–99. [PubMed: 15173838]
177. O'Donoghue JA. Implications of nonuniform tumor doses for radioimmunotherapy. *J Nucl Med.* 1999; 40:1337–41. [PubMed: 10450686]
178. Howell RW, Goddu SM, Rao DV. Application of the linear-quadratic model to radioimmunotherapy: further support for the advantage of longer-lived radionuclides. *J Nucl Med.* 1994; 35:1861–9. [PubMed: 7965170]
179. Fowler JF. Radiobiological aspects of low dose rates in radioimmunotherapy. *Int J Radiat Oncol Biol Phys.* 1990; 18:1261–9. [PubMed: 2347734]

180. Nunn AD. Molecular imaging and personalized medicine: an uncertain future. *Cancer Biother Radiopharm.* 2007; 22:722–39. [PubMed: 18158763]
181. Brouwers AH, Dorr U, Lang O, Boerman OC, Oyen WJ, Steffens MG, Oosterwijk E, Mergenthaler HG, Bihl H, Corstens FH. 131 I-cG250 monoclonal antibody immunoscintigraphy versus [18 F]FDG-PET imaging in patients with metastatic renal cell carcinoma: a comparative study. *Nucl Med Commun.* 2002; 23:229–36. [PubMed: 11891480]
182. Rahmim A, Zaidi H. PET versus SPECT: strengths, limitations and challenges. *Nucl Med Commun.* 2008; 29:193–207. [PubMed: 18349789]
183. Beekman F, van der Have F. The pinhole: gateway to ultra-high-resolution three-dimensional radionuclide imaging. *Eur J Nucl Med Mol Imaging.* 2007; 34:151–61. [PubMed: 17143647]

Figure 1. General scheme and reagents of radio-halogenation of intact antibodies using commonly used methods (a) Chloramine-T method, (b) Bolton-hunter method, (c) Iodogen method and (d) *N*-succinimidyl 4-iodobenzoate based method.

Figure 2.
Representative scheme of radiolabeling intact antibodies with radio-arsenic using SATA approach.

Figure 3. Selected chelating agents used for radiolabeling intact antibodies with ^{64}Cu .

Figure 4. Selected chelating agents used for radiolabeling intact antibodies with $^{86}\text{Y}/^{90}\text{Y}$.

Characteristics of selected longer-lived positron-emitting radionuclides for radioimmunoinaging. Some of the data presented in the table were obtained from references (4, 159) and <http://www.nndc.bnl.gov/nudat2/> (Nuclear Structure and Decay Data Searchable Database, National Nuclear Data Center, Brookhaven National Laboratory, USA) accessed on 03/07/2008.

Table 1

Radionuclide	Half-life	Production	Daughter (half-life)	β^+ max in MeV (β^+ yields)	γ -energies in MeV (γ yields)	Mean range (mm)	Intrinsic spatial resolution loss (mm)	Human studies
^{18}F	1.83 h	$^{20}\text{Ne}(\text{d}, \alpha)^{18}\text{F}$ $^{18}\text{O}(\text{p}, \text{n})^{18}\text{F}$	^{18}O (stable)	0.63 (97 %)	0.14 (41 %)	0.69	0.7	Yes
^{124}I	100.2 h	$^{124}\text{Te}(\text{p}, \text{n})^{124}\text{I}$ $^{124}\text{Te}(\text{d}, 2\text{n})^{124}\text{I}$ $^{125}\text{Te}(\text{p}, 2\text{n})^{124}\text{I}$	^{124}Te (stable)	2.14 (24 %)	0.60 (61 %)	3.25	2.3	Yes
^{76}Br	16.0 h	$^{76}\text{Se}(\text{p}, \text{n})^{76}\text{Br}$ $^{75}\text{As}(\alpha, \text{He}, 2\text{n})^{76}\text{Br}$	^{76}Se (stable)	3.98 (56 %)	0.55 (74 %)	5.07	5.3	Yes
^{52}Mn	134.2 h	$^{52}\text{Cr}(\text{p}, \text{n})^{52}\text{Mn}$ $^{51}\text{V}(\alpha, \text{He}, 2\text{n})^{52}\text{Mn}$ $^{52}\text{Cr}(\alpha, \text{He}, \text{t})^{52}\text{Mn}$	^{52}Cr (stable)	0.58 (29 %)	0.74 (90 %)	5.00	0.6	Yes
^{55}Co	17.5 h	$^{54}\text{Fe}(\text{d}, \text{n})^{55}\text{Co}$ $^{56}\text{Fe}(\text{p}, 2\text{n})^{55}\text{Co}$	^{55}Fe (2.6 y)	1.50 (76 %)	0.48 (20 %)	5.74	1.6	Yes
^{66}Ga	9.4 h	$^{66}\text{Zn}(\text{p}, \text{n})^{66}\text{Ga}$ $^{63}\text{Cu}(4\text{He}, \text{n})^{66}\text{Ga}$	^{66}Zn (stable)	4.15 (56 %)	1.03 (36.9 %)	8.06	5.8	Yes
^{72}As	25.9 h	$^{72}\text{Ge}(\text{p}, \text{n})^{72}\text{As}$	^{72}Ge (stable)	3.32 (88 %)	0.83 (80 %)	5.01	3.6	Yes
^{64}Cu	12.7 h	$^{64}\text{Ni}(\text{p}, \text{n})^{64}\text{Cu}$ $^{68}\text{Zn}(\text{p}, \text{n})^{64}\text{Cu}$ $^{64}\text{Ni}(\text{d}, 2\text{n})^{64}\text{Cu}$	^{64}Ni , ^{64}Zn (stable)	0.66 (18 %)	-	0.70	0.7	Yes
^{86}Y	14.7 h	$^{86}\text{Sr}(\text{p}, \text{n})^{86}\text{Y}$ $^{86}\text{Rb}(\alpha, \text{He}, 2\text{n})^{86}\text{Y}$	^{86}Sr (stable)	3.15 (34 %)	1.08 (83 %)	2.46	1.8	Yes
^{89}Zr	78.4 h	$^{89}\text{Y}(\text{p}, \text{n})^{89}\text{Zr}$ $^{89}\text{Y}(\text{d}, 2\text{n})^{89}\text{Zr}$	$^{89\text{m}}\text{Y}$ (16 s)	0.90 (23 %)	-	1.18	1.0	Yes

Table 2

Commonly used production routes of selected long-lived positron-emitting radioimmunoimaging and corresponding yields and radionuclidic impurities (adapted from reference (119)).

Radionuclide	Commonly used production route	Energy range (MeV)	Calculated yield (MBq/μAh)	Radionuclidic impurity
¹²⁴ I	¹²⁴ Te(p, n) ¹²⁴ I ¹²⁴ Te(d, 2n) ¹²⁴ I	12 → 8 14 → 10	16 17.5	¹²⁵ I (0.1 %) ¹²⁵ I (1.7 %)
⁷⁶ Br	⁷⁶ Se(p, n) ⁷⁶ Br ⁷⁵ As(³ He, 2n) ⁷⁶ Br	15 → 8 18 → 10	360 11	- ⁷⁷ Br (1.6 %)
⁵² Mn	⁵² Cr(p, n) ⁵² Mn ⁵² Cr(³ He, t) ⁵² Mn	20 → 10 36 → 10	0.4 5.6	⁵⁴ Mn (0.5 %) ⁵⁴ Mn (0.8 %)
⁵⁵ Co	⁵⁴ Fe(d, n) ⁵⁵ Co ⁵⁸ Ni(p, n) ⁵⁵ Co	10 → 5 15 → 7	32 14	⁵⁶ Co, ⁵⁷ Co (0.1 %) ⁵⁷ Co (0.5 %)
⁶⁶ Ga	⁶⁶ Zn(p, n) ⁶⁶ Ga	13 → 8	433	-
⁷² As	⁷² Ge(p, n) ⁷² As	18 → 8	93	⁷¹ As (10 %)
⁶⁴ Cu	⁶⁴ Ni(p, n) ⁶⁴ Cu ⁶⁸ Zn(p, n) ⁶⁴ Cu	12 → 9 35 → 20	240 100	⁶¹ Cu (0.4 %) ⁶⁷ Cu (25 %)
⁸⁶ Y	⁸⁶ Sr(p, n) ⁸⁶ Y	14 → 10	400	^{87m} Y (3 %)
⁸⁹ Zr	⁸⁹ Y(p, n) ⁸⁹ Zr	12 → 6	43	⁸⁸ Zr (0.1 %)