9 Antibodies as Vectors for Radiopharmaceutical Therapy

John E. Shively, Kirstin Zettlitz, Paul Yazaki, Anna Wu, and Jeffrey Wong

9.1 Fundamentals

Why Monoclonal Antibodies? Antibodies are an important arm of the immune system that can neutralize or eliminate toxins and pathogens that are recognized by the immune system as foreign. Since tumors arise from normal tissue, they do not usually elicit an antibody response. The advent of monoclonal antibodies that can be produced by immunizing a mouse with a human tumor raised the possibility that monoclonal antibodies could be used to directly treat tumors. In spite of this possibility, the majority of anti-tumor monoclonal antibodies is not sufficiently cytotoxic by themselves and must therefore be conjugated with drugs or radionuclides to elicit effective tumor killing. The choice of monoclonal antibodies as vehicles for targeted radiotherapy (RPT) is supported by an enormous knowledge base encompassing their formation, structure, function, and chemical or genetic modifications. First, it is informative to review the basic structure of an antibody from the viewpoint of a student or practitioner of radioimmunotherapy.

J. E. Shively (✉) · K. Zettlitz · P. Yazaki · A. Wu
Department of Immunology and Theranostics, Beckman Research Institute of City of Hope, Duarte, CA, USA
e-mail: jshively@coh.org

J. Wong
Department of Radiation Oncology, City of Hope National Medical Center, Duarte, CA, USA

Several different parts of an immunoglobulin's structure can be harnessed in the construction of a radioimmunoconjugate. In Fig. 9.1, we depict what many will recognize as a typical immunoglobulin G (IgG) composed of two heavy and two light chains. These chains are held together by both non-covalent interactions and covalent bonds. The latter are disulfides that can be reduced under mild conditions without destroying the overall structure of the immunoglobulin. Once reduced, the free thiols present convenient sites for the attachment of prosthetic groups or chelators for radionuclides (vide infra). Alternatively, radionuclides may be attached to surface residues such as lysines, of which there are up to 80 per biomolecule. When attaching radionuclides to an antibody, it is important to remember that the antigen binding sites are at the N-terminal distal ends of the molecule and that perturbing these regions can affect antigen binding. Since there are two antigen-binding sites, the overall affinity of the antibody for its antigen is increased via an effect called *avidity*. Thus, smaller, monovalent antibody fragments such as Fabs may exhibit reduced affinities for their target antigens compared to their full-length monoclonal antibody parent. Finally, the clearance of antibody from the circulation is controlled by many factors, including the molecular size of the antibody or its fragments and its ability to bind FcRn, a receptor on endothelial cells that promotes the transport of the immunoglobulin out of the vascular bed and into surrounding

L. Bodei et al. (eds.), *Radiopharmaceutical Therapy*, https://doi.org/10.1007/978-3-031-39005-0_9

tissues. These important topics will be discussed in more detail later.

Before we move on, it is important to ask a fundamental question: why use antibodies for RPT? First off, antibodies are natural targeting molecules that are easy to generate from many starting points using techniques that will be briefly mentioned later. In theory, one can generate antibodies to almost any antigen, whether a protein, carbohydrate, lipid, or small molecule. The theoretical number of possible different antibodies is estimated as high as a million trillion. In large molecules like proteins, antibodies may recognize an epitope composed of a linear sequence of amino acids or a three-dimensional structure that includes amino acids that are distant from one another in the protein's primary sequence. Knowledge of an antibody's target epitope is important since it may be masked by post-translational modifications or be disrupted by denaturation (the latter is especially germane in the case of three-dimensional epitopes).

Natural antibodies come in different isotypes such as IgG, IgM, IgA, and IgE, and each class has subclasses with different molecular sizes, half-lives, and functions. For example, the structure of the antibody shown in Fig. 9.1 is typical of a human IgG1 that has two disulfide bonds in the lower hinge connecting the two heavy chains and two additional disulfides in the upper hinge connecting the heavy and light chains. This arrangement of disulfide bridges varies between both isotypes and species. Since most clinically available antibodies are derived from natural antibodies from non-human sources, they can be engineered to more closely resemble human sequences (humanized) and tailor their blood clearance and effector functions (vide infra).

All of this structural and functional information must be considered when determining which type of antibody is most appropriate for RPT. For example, an antibody of the most appropriate isotype with a high affinity/avidity for its antigen is desirable. Care must also be taken to choose the suitable antigen, specifically one that is expressed at high levels in target tissues and at low levels in most (if not all) normal tissues.

Antibody Selection and Production It is unlikely that someone interested in developing a therapeutic radioimmunoconjugate would start a project with the goal of developing a new antibody by screening clones or using expression libraries, a time-consuming process that has been reviewed by others [1]. Therefore, we will start a discussion of the possible sources of antibodies for researchers seeking to create a novel radioimmunoconjugate.

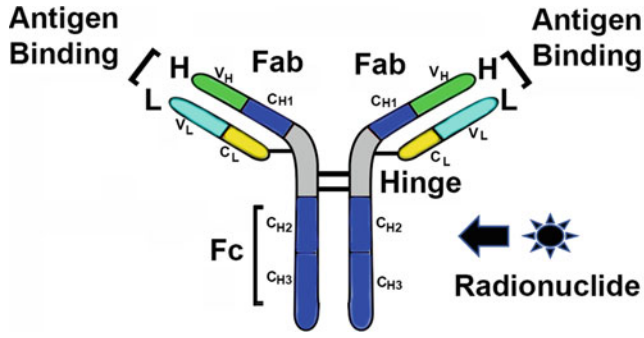

Fig. 9.1 Basic features of an antibody. An antibody has two heavy (H) and two light (L) chains with variable (V) and constant (C) domains. The two antigen binding sites comprise the N-terminal variable regions of the H and L chains, designated as V_H and V_L. Both sites contribute to antigen binding, thereby increasing binding in a process termed *avidity*. The two heavy chains are connected to each other and the light chains by disulfide bridges (thick lines) called the hinge region. The Fc region below the hinge region plays a role in the circulation half-life of the antibody and immune effector functions. The radionuclide can be appended almost anywhere on the antibody depending on the attachment chemistry

- Radiolabeled antibodies that have already been in the clinic but have not yet proven effective for RPT. These may be antibodies that were used for imaging only or ones that did not yield impressive results within a RPT trial but could be improved by choosing another radionuclide, dose, disease, or combination therapy.
- Antibodies approved for human use that are potential candidates for RPT but have not yet been used as radioimmunoconjugates in the clinic. These antibodies may include those that demonstrated impressive clinical results that justify their use as radioimmunoconjugates, or ones that failed to achieve their goals but can be improved with a therapeutic radionuclide payload. These antibodies can be rapidly radiolabeled, and their performance in imaging and/or therapy studies can be rapidly tested in preclinical studies.
- Antibodies that have shown promising results in preclinical studies that may or may not have included nuclear imaging trials.
- Antibodies that shown promising target specificity and efficacy in in vitro studies. These antibodies may not have entered animal studies and thus may require a great deal of in vivo development.

The list above is not comprehensive. It simply suggests several starting points—in order of increasing effort needed—from which radiolabeled antibodies can be developed for the clinic. The time, resources, and expertise available for a given project will also help guide the selection of an antibody. Since target specificity is of paramount importance for a radioimmunoconjugate, researchers should make tissue staining a critical early step in the selection and validation of a candidate antibody. This can be done by staining tissues blocks in a pathology core with expert opinions rendered by a seasoned pathologist. An example of the staining of the anti-CEA (carcinoembryonic antigen) antibody M5A [2] for normal vs. tumor tissue is shown in Fig. 9.2. In general, if an antibody stains a low percentage of cells in the target tissue or stains them with low intensity—or, conversely, stains normal tissues strongly—one can reasonably predict poor outcomes in human trials. It is important to remember than most animal models for growing human xenografts do not express the human variants of the antigen of interest. This can give false hope that an antibody is specific for the targeted tumor antigen and not the normal antigen also expressed in human organs. In addition, screening human cell lines can be misleading because they may or may not express the antigen of interest at the same level as normal or malignant tissue.

The amount of antibody required for a therapeutic study is an important consideration. Often

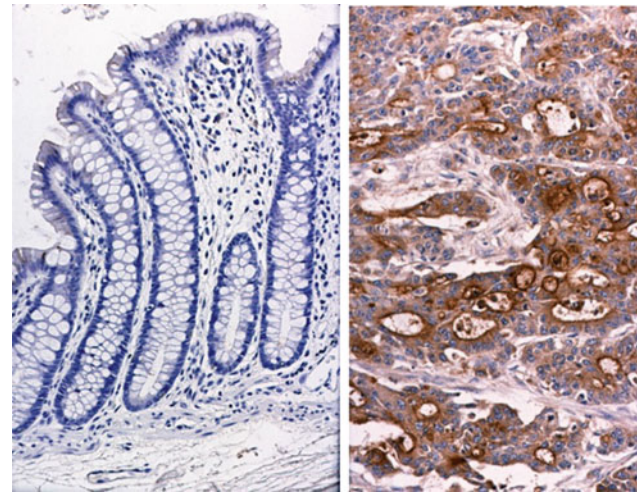

Fig. 9.2 Immunostaining of normal vs. malignant colon with anti-CEA antibody. Normal colon (left) and malignant colon (right) stained with anti-CEA antibody conjugated to HRP and counterstained with hematoxylin. The results are representative of over 10 normal specimens and 50 colon tumor specimens

as little as 5 mg of the radioimmunoconjugate will be needed per dose assuming a sufficient specific activity can be achieved. However, some antibodies are rapidly cleared in humans, necessitating the addition of a so-called "cold" dose. Here, it is important to remember that only a small fraction of the antibody molecules is actually radiolabeled. Thus, each injected dose contains mostly unlabeled antibody. If the systemic concentration of antibody is too low for it to bind its target based on its affinity and clearance properties, then more cold (unlabeled) antibody may need to be added to the radiolabeled dose. This is usually done via an initial infusion of the unlabeled antibody followed soon thereafter by the administration of the radioimmunoconjugate. If this is the case, then the amount of antibody required for a clinical trial may be greatly increased. The good news is that the cold antibody need not be chemically modified.

The production of clinical grade antibodies requires a trained staff operating under SOPs in a GMP facility. If the antibody is already a commercially approved agent, an IND is still required for the radiolabeled version, since it is considered a new agent. A summary of the details for the GMP production of an antibody and its journey through the IND process are shown below:

- Selection of a stable, high-expressing mammalian cell culture clone
- Generation of a master and working cell bank
- Production in a bioreactor (grams to kilo scale)
- Purification, including viral inactivation/removal steps
- Bioconjugation in the case of radiometal-labeled immunoconjugates
- Formulation
- Vialing the final product
- Viral validation studies
- Lot release testing
- Toxicity studies
- Preclinical studies
- IND submission

Antibody Fragments and Engineered Antibodies Since intact antibodies clear rather slowly from circulation due to their molecular size (150 kDa) and the presence of their Fc domain that binds FcRn, there has been significant interest in building radioimmunoconjugates out of antibody fragments that retain the antigen-binding portion of mAbs but have a lower mass and lack an Fc domain (Fig. 9.3). The clearance rates of antibody fragments depend on their molecular size and the molecular weight cutoff of kidney filtration, about 60 kDa. Indeed, experimental measurements have shown that that diabodies—with a molecular weight of 55 kDa—are still cleared by the kidney. Figure 9.3 illustrates 6 antibody fragments in decreasing order of molecular size. The clearance of radioimmunoconjugates through the kidneys is a potential concern, because the retention of the radiolabel within the kidneys can lead to the kidneys becoming a dose-limiting organ due to their intrinsic radiosensitivity [3]. For this reason, radiolabeled antibody fragments that clear to the kidney are not favored for RPT. A second consideration surrounding the pharmacokinetics of radiolabeled antibody fragments is that their rapid clearance from circulation may mean substantially less absolute uptake in the target tissue which can translate into lower radiation doses. For these reasons, it is important to optimize the pharmacokinetic profile of a radioimmunoconjugate, whether it is a full-length antibody or a fragment.

The examples of antibody fragments shown in Fig. 9.3 were taken from our work on an anti-CEA antibody that began with the screening of murine hybridomas [4, 5], followed by engineering a half-murine, half-human chimeric version [6], and finally generating a fully humanized antibody [2]. The enzymatic production of divalent F $(ab')_2$ and monovalent Fab fragments was later described [7], as well as the engineering of scFv and diabody constructs [8–11]. Thus, starting from an intact murine monoclonal antibody, it is possible to generate antibody fragments that are entirely murine or—by genetic engineering—to convert monoclonal antibodies to chimeric or fully human mAbs and fragments. Since monoclonal antibodies arise from two genes, the heavy and light chains (Fig. 9.1), genetic engineering,

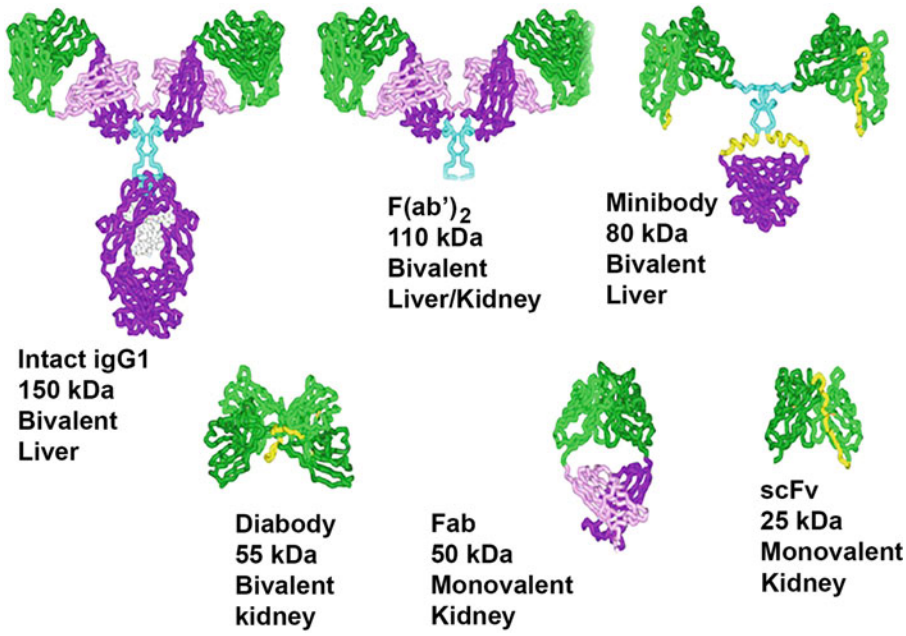

Fig. 9.3 Schematic structures of an intact antibody vs. antibody fragments. For each construct, the molecular size, valency, and route of clearance are shown. Each of the domains is color coded to allow for comparisons of their structures. The variable regions are shown in green, constants regions in magenta, and hinge region in cyan. $F(ab')_2$ and Fab fragments are usually made via the enzymatic digestion of intact IgGs, while the diabodies, minibodies, and scFv fragments are genetically engineered

and expression of intact antibodies are rather complicated. The production of engineered antibodies from a single gene construct has advantages over approaches that require separate genes for the heavy and light chains. An example of such a construct is the minibody, in which an scFv is fused to the C_H3 domain of a heavy chain [11].

Before we move on, it is important to touch upon the use of unusual immunoglobulins from other species in RPT. Camelid antibodies or nanobodies are antibodies that originate from the *Camelidae* family that includes llamas, camels, and alpacas [12]. These nanobodies are attractive in that they are heavy-chain only constructs that don't require light chains. However, they are inherently foreign and likely to produce immune responses when given to humans. This drawback may limit the repeat administrations of these constructs or necessitate humanization. A recent review of the use of nanobodies in the clinic is given by Yang et al. [13].

9.2 Details

Imaging with Radiolabeled Antibodies Although this chapter is focused on RPT, nuclear imaging is a logical complementary approach that can allows one to determine the extent of antibody targeting prior to therapy, monitor the response of a patient to therapy, and, most importantly, perform dosimetry calculations in advance of RPT. The relatively new field of *theranostics* refers to the use of different versions of the same agent for both imaging and therapy. In this context, an antibody radiolabeled with a gamma emitting radionuclide could be an imaging agent for the same mAb when radiolabeled with a beta- or alpha-emitting radionuclide. Several imaging modalities are available in the clinic, including planar imaging, single photon emission computerized tomography (SPECT), and positron emission tomography (PET). Although the ability to do three-dimensional imaging with SPECT and PET is

Table 9.1 Radionuclides for radioimmunoimaging

Radionuclide	Half life	Emission	Modality	Production
Ga-68	68 min	Positron	PET	Generator
F-18	109.7 min	Positron	PET	Cyclotron
Tc-99 m	6 h	Gamma	SPECT	Generator
Cu-64	12.7 h	Positron	PET	Cyclotron
I-123	13.2 h	Gamma	SPECT	Cyclotron
In-111	2.8 da	Gamma	SPECT	Cyclotron
Zr-89	3.3 da	Positron	PET	Cyclotron
I-124	4.2 da	Positron	PET	Cyclotron

preferred, it should be noted that considerable information can be obtained from planar imaging, too. Since it is very likely that the reader is familiar with all three modalities, they will not be discussed here. Instead, we will focus on the some of the most commonly used radionuclides for radioimmunoimaging.

The radionuclides most commonly used for radioimmunoimaging are presented in increasing order of their half-lives in Table 9.1. Since both SPECT and PET instruments are available at most institutions, the first consideration in choosing of radionuclide is typically its half-life. The general rule is to match the biological half-life of the agent with the radiological half-life of the radionuclide. Thus, Ga-68 and F-18 are typically restricted to rapidly clearing antibody fragments, while In-111, Zr-89 and I-124 are most suitable for intact antibodies or larger fragments. In contrast, Cu-64 and I-123 have been successfully used to image tumors with both intact antibodies *and* antibody fragments [14, 15].

One advantage of radioimmunoimaging prior to radioimmunotherapy is the ability to predict the clearance and retention of the radiolabeled antibody, which can affect targeting and dosimetry. Surrogate radioimmunoimaging describes the concept of choosing a positron-emitting radionuclide with similar/comparable characteristics (half-life, metabolism) to the radionuclide that will be used for therapy. Surrogate radioimmunoimaging can be used to profile the pharmacokinetics, biodistribution, and clearance of the mAb and thereby provide support in assembling the best radionuclide, labeling chemistry, and antibody or antibody fragment for RPT. Several good examples of radioisotopic pairs for surrogate imaging and RPT are I-124/I-131; Cu-64/Cu-67 and Zr-89/Lu-177. Figure 9.4 depicts surrogate radioimmunoimaging using an anti-prostate stem cell antigen (PSCA) minibody that correctly predicted that the bone marrow would be dose limiting for ^{131}I-based RPT and the kidneys would be dose-limiting for radiometal-RPT [16].

An example of PET imaging with a CEA-positive tumor and metastatic lymph node with a ^{64}Cu-labeled antibody is shown in Fig. 9.5. Both the primary tumor and a metastatic lesion in the lymph node can be visualized as early as 24 h after the administration of the radioimmunoconjugate.

A second example of PET imaging with a ^{64}Cu-labeled anti-Her2 antibody that detected liver metastases is given in Fig. 9.6 [17]. In this case, the physical dose of the antibody played a major role. While 5 mg of the radiolabeled antibody barely distinguished tumor from background tissue, pre-injection of 45 mg of unlabeled antibody dramatically improved imaging, presumably by saturating the non-specific uptake of the antibody-antigen complexes in normal hepatocytes.

Examples of SPECT imaging with a minibody [18] of our anti-CEA antibody are given in Fig. 9.7. Given the reduced clearance time of the minibody compared to its parental mAb, it was possible to use two radionuclides with slightly shorter half-lives: ^{123}I and ^{111}In.

The availability of radionuclides is a second issue to consider when choosing a label for an antibody. The production of Ga-68 requires an in-house generator, while the longer half-life of

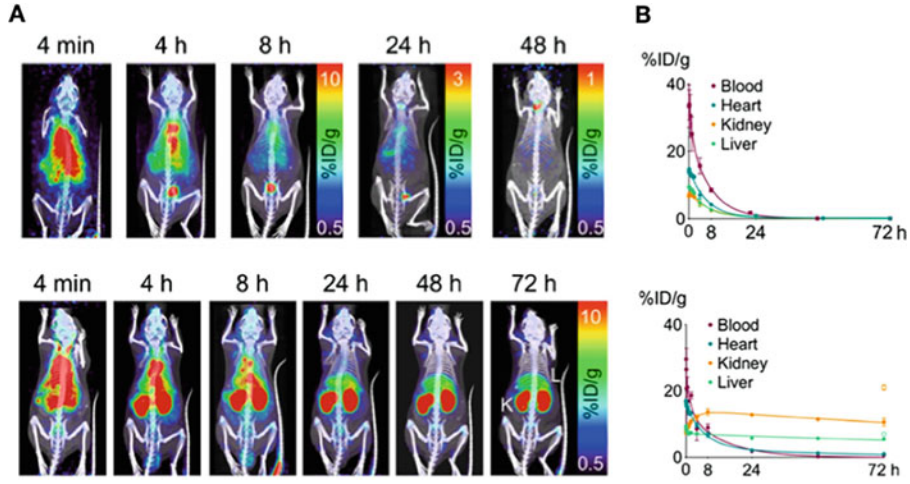

Fig. 9.4 Surrogate PET imaging of prostate cancer with ^{124}I- and ^{89}Zr-labeled anti-PSCA minibodies. (**a**) ImmunoPET images over time. (**b**) Blood clearance curves. *Upper:* The minibody was radio-labeled with I-124 as a surrogate for I-131 therapy. *Lower:* The minibody was radiolabeled with Zr-89 as a surrogate for Lu-177 therapy

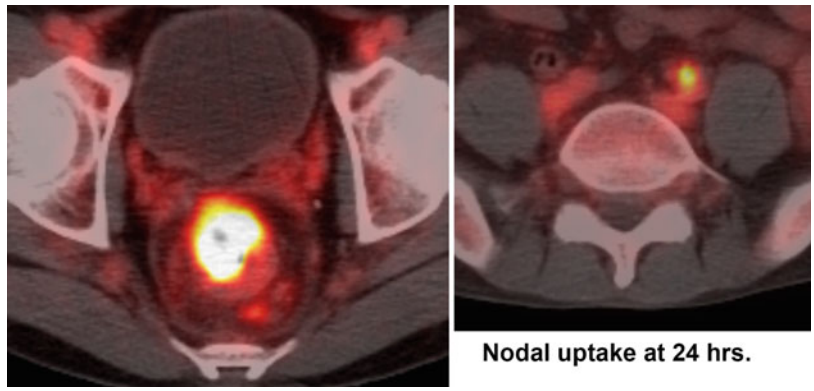

Fig. 9.5 PET imaging of rectal cancer at 24 h with ^{64}Cu-labeled anti-CEA antibody DOTA-M5A. DOTA-M5A (3 mg) radiolabeled with 555 MBq of Cu-64 was administered and PET imaging performed at 24 and 48 h. The patient had recurrent tumor growth at both the primary site and a lymph node. *Left:* primary site. *Right:* metastatic lymph node. Both sites were confirmed by subsequent biopsies

Tc-99 m means it can be shipped. Both ^{99m}Tc- and ^{18}F-labeled agents can be ordered from a variety of suppliers, and ^{18}F can even be generated in-house using a cyclotron. The remaining radionuclide options—i.e., ^{89}Zr, ^{111}In, etc.—have half-lives that are long enough to allow for shipping that can often take 1–2 days. In this respect, orders of ^{64}Cu can be a bit tricky, as they require shipments of larger amounts to compensate for their significant decay during shipping. Facilities with in-house cyclotrons can produce their own radionuclides, including those for which higher energy beams are required.

A third consideration in the selection of a radionuclide is the possible requirement for pre-clinical imaging. Preclinical studies are usually a requirement for filing an IND on a new radiotherapeutic. In this respect, access to animal

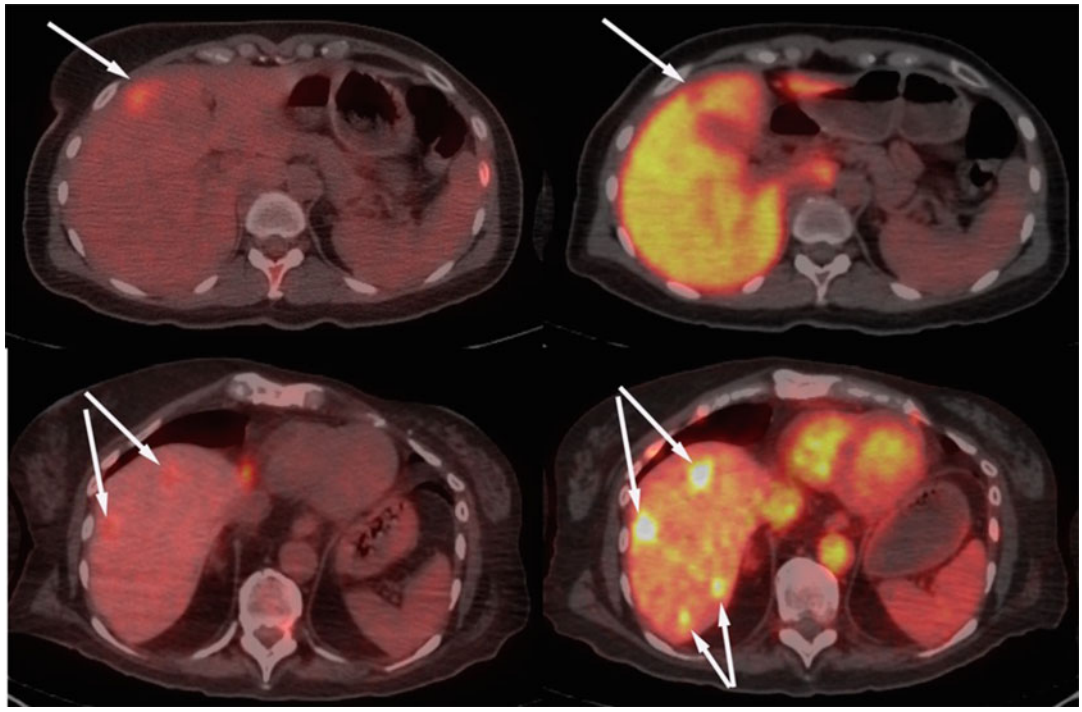

Fig. 9.6 PET imaging of liver metastasis in breast cancer with ^{64}Cu-DOTA-trastuzumab compared to ^{18}F-FDG. (**a, b**): FDG PET/CT of liver metastasis in breast cancer patient at 24 (**a**) and 48 h (**b**). (**c**, **d**): PET/CT at 24 h with 5 mg (551 MBq) radiolabeled trastuzumab (**c**) or 5 mg (551 MBq) radiolabeled trastuzumab plus 45 mg of unlabeled trastuzumab (**d**). Arrows indicate sites of metastasis

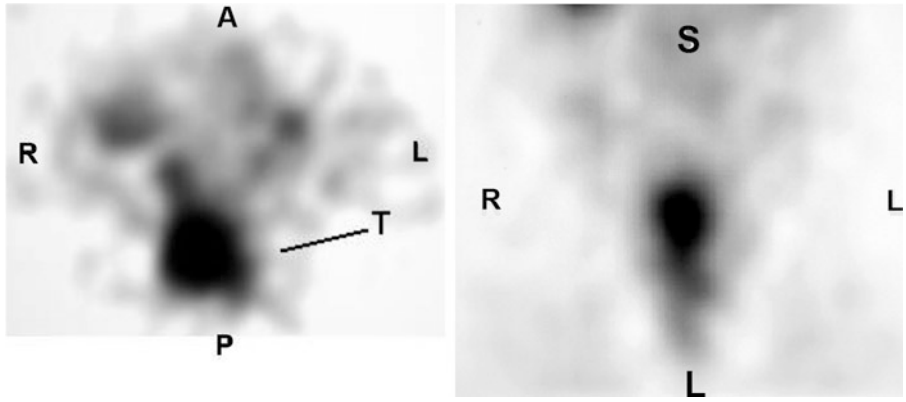

Fig. 9.7 SPECT images of rectal cancer with ^{123}I- or ^{111}In-labeled anti-CEA minibody. *Left:* SPECT image with ^{123}I-labeled antibody. *Right:* ^{111}In-labeled DOTA-antibody

imaging instruments is essential. PET/CT animal imaging instruments are perhaps the most versatile, allowing anatomical imaging with CT alongside PET. Instruments that also allow SPECT are desirable as well, as are PET/MR instruments. An example of preclinical PET images acquired with a ^{64}Cu-labeled anti-CEA antibody is shown in Fig. 9.8. Multiple imaging times can be used to

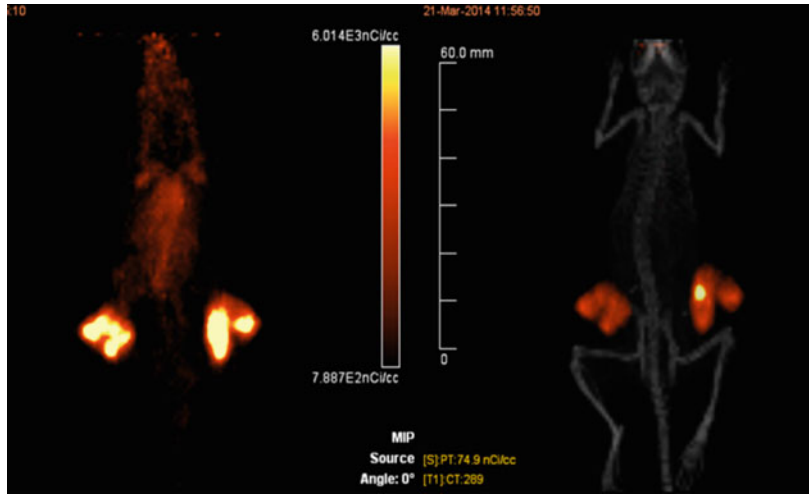

Fig. 9.8 PET imaging of CEA-positive flank xenografts in a nude mouse with the ^{64}Cu-labeled anti-CEA antibody DOTA-M5A. *Left:* image at 23 h, *Right:* image at 44 h. 30 μg of DOTA-anti-CEA antibody was radiolabeled with 3.7 MBq of Cu-64

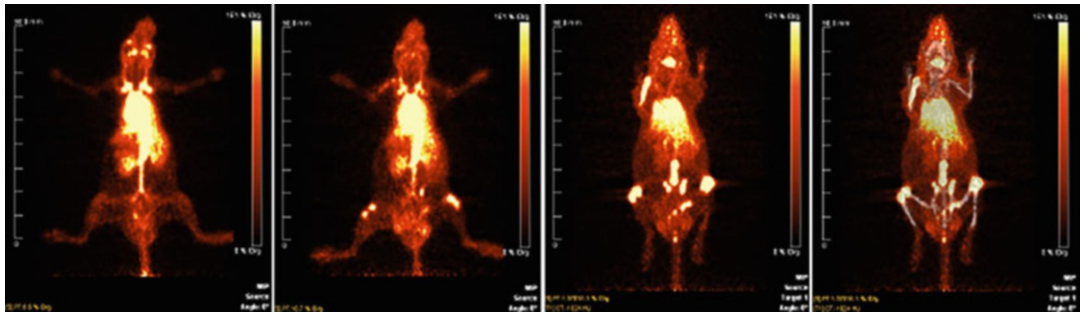

Fig. 9.9 PET imaging of multiple myeloma in NSG mice with ^{124}I-labeled daratumumab. *Left to right:* PET images at 4, 21, and 44 h as well as a fused PET/CT image from 44 h. The early images show uptake in the blood and liver, while the later images illustrate clearance from the blood and uptake into bony sites

demonstrate the clearance of the radioimmunoconjugate from the blood and liver.

An example of preclinical PET using an ^{124}I-labeled radioimmunoconjugate of the anti-CD38 mAb daratumumab in systemic model of multiple myeloma (MM) is shown in Fig. 9.9. These preclinical studies helped to demonstrate the utility of imaging with radiolabeled anti-CD38 antibodies. A clinical trial later illustrated the feasibility visualizing MM in patients using ^{64}Cu-labeled DOTA-daratumumab [19].

Conjugation Chemistry of Radionuclides to Antibodies The chemistry of the attachment of radionuclides to antibodies or antibody fragments is similar whether the final radioimmunoconjugates are used for imaging or RPT. The chief issue with radiolabeling any antibody is the potential loss of immunoreactivity. Thus, an assay must be established to compare the immunoreactivity of the agent before and after radiolabeling. Several potential assays are discussed in the next section. Since traditional methods of bioconjugation introduce the radiolabel at random sites on the immunoglobulins, the most care must be taken with smaller fragments. Concerns regarding the loss of immunoreactivity can be eliminated using site-specific conjugation methods. Along these lines, conjugation into the hinge region of antibodies is popular, since cysteines in this area can be selectively reduced (and then modified) without disturbing the less

Table 9.2 Common methods for the conjugation of radionuclides to antibodies

Radionuclide	Conjugation method	Chelator[a]	Ref
I-131, I-125, I-124, I-123	Iodogen (tyrosine)	None (direct)	[21]
Tc-99 m	Active ester (lysine) Maleimide (cysteine)	SHNH	[22]
Ga-68	Active ester (lysine) Maleimide (cysteine)	NOTA, DOTA	[23]
Cu-64, Cu-67	Active ester (lysine) Maleimide (cysteine)	NOTA, DOTA sarcophagine	[24–26]
In-111	Active ester (lysine) Maleimide (cysteine)	DOTA	[27]
Lu-177	Active ester (lysine) Maleimide (cysteine)	DOTA	[28]
Y-90	Active ester (lysine) Maleimide (cysteine)	DOTA	[29, 30]
Zr-89	Thiourea (lysine) Maleimide (cysteine)	DFO	[31]

[a]SHNH (hydrazino nicotinamide), NOTA (2,2′,2″-(1,4,7-triazacyclononane-1,4,7-triyl)triacetic acid), DOTA (2,2′,2″,2‴-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid), DFO (deferoxamine)

accessible internal disulfides elsewhere in the immunoglobulin. The mild reduction in antibodies usually does not disturb the overall structure or activity of the antibody, with one exception: $F(ab')_2$ fragments will be irreversibly reduced to monovalent Fab' fragments. Since there are a wide variety of conjugation methods that are reviewed well elsewhere [20], only the most common methods will be mentioned here (Table 9.2).

Radioiodination is perhaps the oldest method for the radiolabeling of antibodies. It is still commonly used because it is rapid and several isotopes of iodine are available that differ in their emissions and half-lives (see Chap. 7 for a more detailed discussion). The most common method of radioiodination is the iodogen method, which incorporates radioiodine into tyrosine residues in a matter of minutes at room temperature. Importantly, iodinated tyrosines are targets of tissue-specific metabolic scavenging that is most prevalent in organs such as the liver and kidney. Once the radiolabeled antibody is metabolized, iodotyrosine is rapidly taken up and transported to the thyroid and, less so, the stomach. This process will reduce the amount of radioactivity in the liver, and is an advantage if liver metastases are the target for imaging and/or therapy [32]. However, this process may also reduce the total uptake in the target, requiring the use of rather high doses of the radioimmunoconjugate for both imaging and therapy. This process also results in unwanted uptake in the thyroid that must be blocked via the co-administration of iodide over several days to reduce the very real prospect of radiodamage to the thyroid. To circumvent some of these problems, alternative methods have been developed in which a prosthetic group is radioiodinated first and *then* conjugated to lysines on the antibody [33].

The use of radiometals requires careful attention to the choice of chelator (see Chap. 6 for a more comprehensive discussion of radiometal chelates). Well-developed methods for labeling immunoglobuliins with Tc-99 m also exist. Given the short half-life of Tc-99 m (6 h), its use is largely limited to labeling antibody fragments. To this end, the unusual chemistry of Tc requires special chelators that can be attached to either lysine or cysteine residues in the antibody. There are also a large number of radiometals that can be coordinated by amine- or thiol-reactive variants of the promiscuous metal ion chelator DOTA. These derivatives are termed "bifunctional chelators," since they facilitate both the coordination of the radiometal and its conjugation to antibodies via lysine or cysteine residues. Since the ionic radius of the radiometal is a factor in stable binding to DOTA, the smaller ring size of NOTA for $^{68}Ga^{3+}$ has been utilized.

Besides DOTA and NOTA, the cage-like chelate sarcophagine has also been used for $^{64}Cu^{2+}$ and $^{67}Cu^{2+}$ (see Table 9.2).

Radiometals—including most of those in Table 9.2—are often termed "residualizing radionuclides" because once they are internalized and metabolized, they are often slowly lost from cells that have no specific mechanisms for their excretion (especially while still bound to a chelate). This retention of radiometals in target tissue is an advantage, as less radioactivity is lost over time compared to radioiodinated probes. On the other hand, the retention of radioactivity in non-target organs like the liver and kidney can lead to a high background signal in the context of imaging and high background dose rates in the context of RPT. The radioisotopes of copper are a possible exception to the trend of residualized radiometals, since it can be metabolically labile in the liver [34]. As a result, the field has worked to develop more metabolically stable chelators for Cu^{2+}, including—but not limited to—the sarcophagine family [35]. Nonetheless, there is a real advantage to the use bifunctional derivatives of DOTA, since DOTA-conjugated antibodies may be radiolabeled with radiometals suitable for either imaging or therapy.

Zr-89, like Tc-99 m, requires a special chelator due to its unique coordination chemistry. The natural iron-chelating siderophore deferoxamine (DFO) has emerged as the chelator of choice for ^{89}Zr and can be conjugated to lysine or cysteine residues via isothiocyanate- or maleimide-bearing bifunctional variants, respectively. These derivatives are more metabolically labile than the active ester derivatives that produce amide bonds within the antibody. Furthermore, the chemical linkage between the chelate and antibody can be fine-tuned to be more or less metabolically stable. This consideration is critically important for antibody-drug conjugates, for which the release of the drug from the antibody may be key to its function [36]. Extensive linker chemistry is available for bifunctional chelates [37].

Once the radionuclide and bioconjugation method are chosen, a number of fundamental steps are required before commencing preclinical or clinical studies with a radioimmunoconjugate. Along these lines, radioiodinated radioimmunoconjugates are simplest as they lack a chelator. In the case of radiometallated radioimmunoconjugates, things are slightly more complicated, as the stability of the chelator-antibody bond must be assessed, and methods must be developed to radiolabel the chelator-bearing immunoconjugate with the radiometal of choice. Further details are described below.

Characterization of Radiolabeled Antibodies The properties of radiolabeled antibodies must be systematically characterized prior to their use in animals or humans. The most important properties to assess are summarized below:

- Percent radiolabeling
- Specific activity (radioactivity/mg)
- Immunoreactivity
- Antibody purity (SEC)
- Radiolabel stability (from production to patient)
- Serum stability (incubation with human serum over time)

The ability of a given radiolabeling method to provide a product in sufficient yield and specific activity will vary with parameters such as antibody concentration, reaction volume, pH, time of incubation and the nature of the radionuclide, especially in the case of a radiometal. The best approach is to first decide on the desired specific activity for the application in mind. For animal studies in which cohorts of 40 mice are imaged or treated with 10–30 μg of antibody per mouse, the amount of antibody to be labeled for a given study may be in the range of 1–2 mg, and the amount of radioactivity required may be in the range 3–15 MBq. In order to optimize a radiolabeling procedure, trials should be executed with a fixed amount of antibody and increasing amounts of radionuclide and monitored via instant thin layer chromatography (iTLC) to determine the efficiency of radiolabeling. In most cases, >95% is the target efficiency at the desired specific activity. An example of a scanned iTLC strip is shown

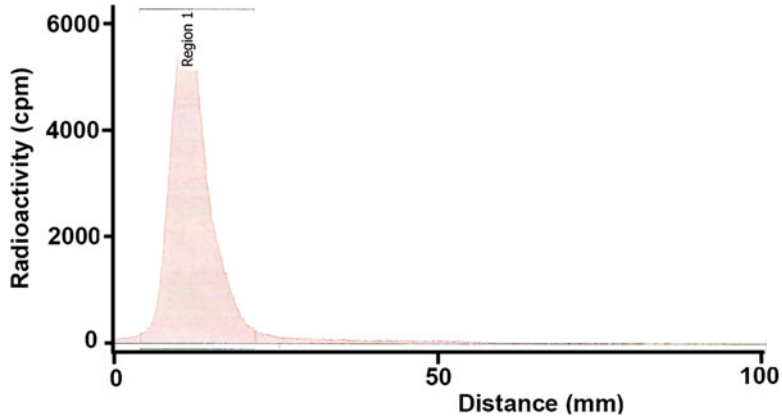

Fig. 9.10 Percent radiolabeling determination by iTLC. DOTA-anti-CEA antibody M5A (5 mg/mL) in 0.25 M pH 6.0 ammonium acetate with 50 mM ascorbic acid was radiolabeled with 16.5 MBq of Ac-225 at 43 °C for 40 min, and excess radiometal was scavenged via the addition of 10 mM DTPA for 10 min. An aliquot was applied to an iTLC strip, developed in saline, and scanned for radioactivity. The counts at the origin (region 1) were > 99% of those applied. Any excess unbound radiometal would appear at the end of the strip

in Fig. 9.10. The concentration of antibody, the volume and pH of the reaction, and the formulation of the radionuclide itself can all affect the radiolabeling reaction and thus must be optimized. In addition, since radiolysis is a potential problem (especially at high concentrations of therapeutic radionuclides), it may be necessary to add a radioprotectant such as ascorbate to the reaction buffer to protect the radioimmunoconjugate [38].

Once a reproducible radiolabeling method has been established, the immunoreactivity of the product must be determined. Along these lines, access to the cognate antigen or a cell line expressing the cognate antigen is essential. In the case of cell surface receptors, many are available as recombinant Fc fusion products or can be prepared by gene synthesis and cell expression systems that include post-translation modifications such as glycosylation. An example of an immunoreactivity assay for CEA is shown in Fig. 9.11a, b. In this assay, the radiolabeled antibody is mixed with a 10- to 20-fold molar excess of CEA, incubated for 1–2 h, and injected on a size exclusion column monitored with a radioactivity detector. Both the radiolabeled antibody and CEA must be diluted in 1% serum albumin to prevent the small amounts of protein present from sticking to the sides of the assay tubes. The size of the antibody-antigen complex will be greater than the starting antibody, thereby allowing one to measure the area under the curves of the bound and unbound antibody. An alternative measure of immunoreactivity is the cell-binding assay. For cell-based immunoreactivity, trace amounts of radiolabeled antibody are incubated with excess antigen-expressing cells (along with antigen-negative controls). The cell-bound and supernatant fractions are separated by centrifugation and gamma counted. A double-reciprocal plot is created by plotting the inverse bound antibody fraction (1/ (bound/[[bound + free]) as a function of the inverse cell number [(1/(cells/mL)] and extrapolated to "infinite antigen excess". The reciprocal of the immunoreactive fraction is given at the *y*-intercept. The example given in Fig. 9.11c is for an ^{124}I-labeled cys-diabody binding to human CD20 [39].

Another example can be given for the determination of the immunoreactivity of an anti-CD38 antibody daratumumab to its cognate antigen, the extracellular domain of CD38. An Fc fusion protein with CD38 is commercially available, and since it is fused to a Fc domain, both its valency and molecular size are increased, leading to a

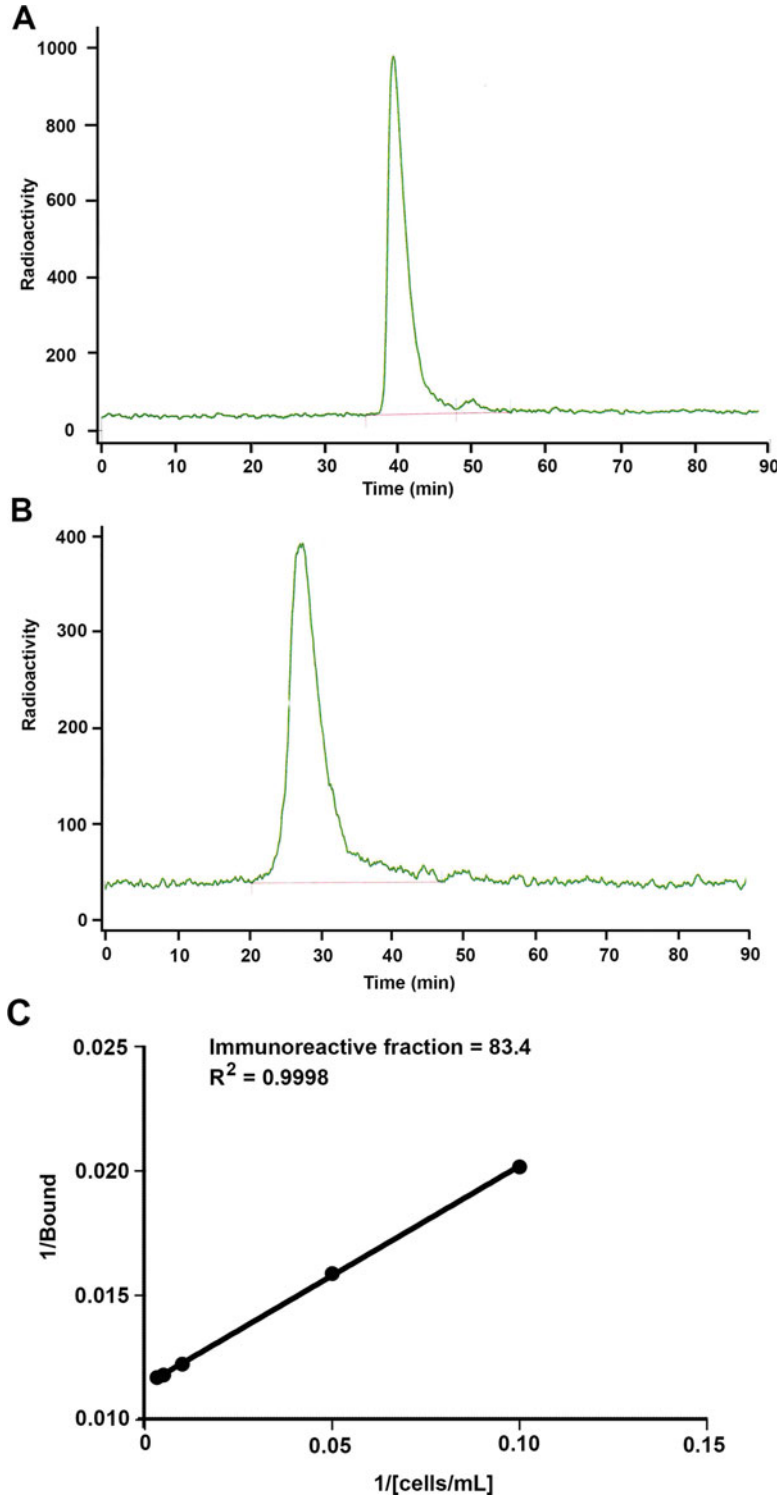

Fig. 9.11 Immunoreactivity of radiolabeled antibodies. (**a, b**) An aliquot of ^{225}Ac-labeled DOTA-anti-CEA (see Fig. 9.10) was injected onto a Superose 200 column and eluted with PBS. Radiochromatograms before (**a**) and after (**b**) mixing with a 20-fold excess of CEA. Note the shift to higher molecular size after the addition of CEA. The percent immunoreactivity = bound/(bound plus unbound) x 100. In this example the % immunoreactivity was >98%. (**c**). An example of a double reciprocal plot stemming from a cell-based immunoreactivity assay in which an ^{124}I-labeled anti-CD20 antibody binds to 38C13 murine B-cell lymphoma cells expressing human CD20

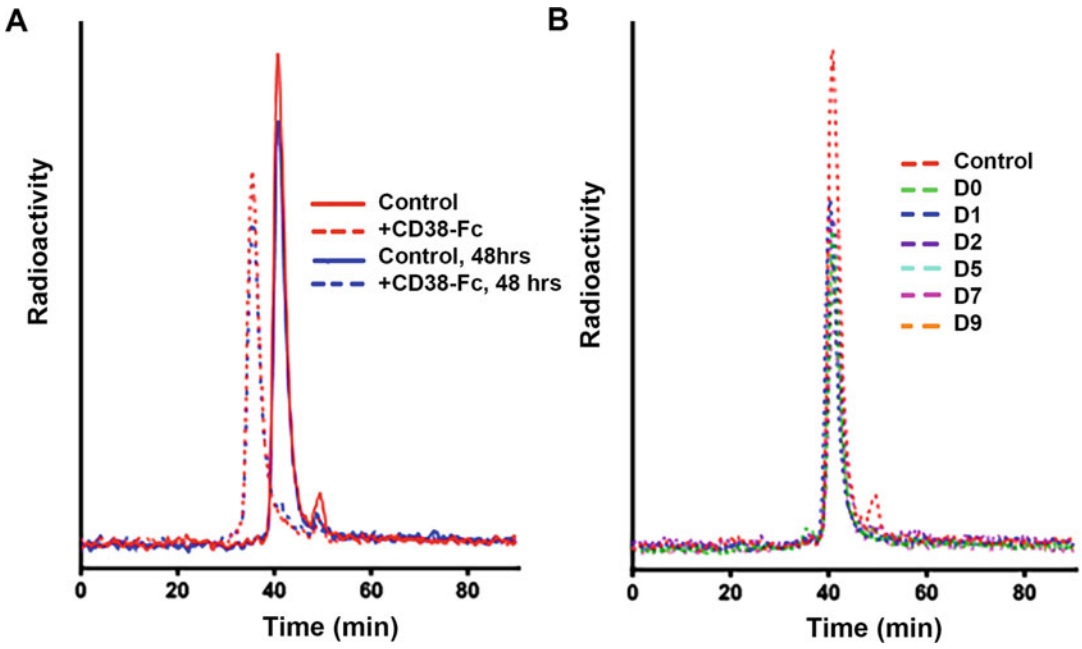

Fig. 9.12 Immunoreactivity and serum stability of ^{225}Ac-labeled DOTA-daratumumab. (**a**). Immunoreactivity: the radioimmunoconjugate was analyzed via SEC before and after incubation with CD38-Fc. (**b**). Serum stability: SEC chromatograms were collected over 9 days during which the radioimmunoconjugate was incubated in serum

substantial size shift upon binding the radiolabeled antibody (Fig. 9.12a). An advantage of using SEC for the immunoreactivity assay is that the injection of the radiolabeled antibody alone is required. Thus, this radiochromatogram will serve as an indication of the purity of the radiolabeled antibody as well. In some cases, the method of radiolabeling may increase the odds of aggregation, which can be easily observed and calculated from the radiochromatogram. In most cases, aggregation should be kept below 5–10%; if the radioimmunoconjugate contains aggregates >5–10%, it may be necessary to remove the aggregates on a preparative SEC column.

Another IND requirement is the determination of the serum stability of the radioimmunoconjugate. This can be performed via repeat ITLC assays at various time points after radiolabeling. For most studies, a stability of >95% out to 24–48 h should be sufficient. Alternatively, serum stability measurements can be performed by incubating the radioimmunoconjugate with human serum at 37 °C and assaying the construct's integrity over time via SEC. An example of an acceptable serum stability by SEC for ^{225}Ac-labeled DOTA-daratumumab is shown in Fig. 9.12b.

In addition to the studies above, animal studies demonstrating in vivo targeting are often required when filing an IND application. In the case of cancer studies, animals bearing human xenografts must be studied by both imaging and tissue biodistribution. In vivo targeting—that is, the antigen-mediated accumulation of radioactive signal in the target tissue (xenograft)—can be observed over time. Antigen specificity should be confirmed by including a blocking control (i.e., via the co-injection of excess cold antibody) or by including an antigen-negative xenograft or a non-specific antibody (isotype) control group. This is shown in Fig. 9.13, in which the anti-CD20 cys-diabody (^{124}I-GAcDb) demonstrates high uptake in a CD20-positive B-cell lymphoma, resulting in high-contrast images at early time points [40]. The addition of excess blocking antibody and the antigen-negative tumor control groups show significantly lower uptake, confirming the specificity of this radiotracer.

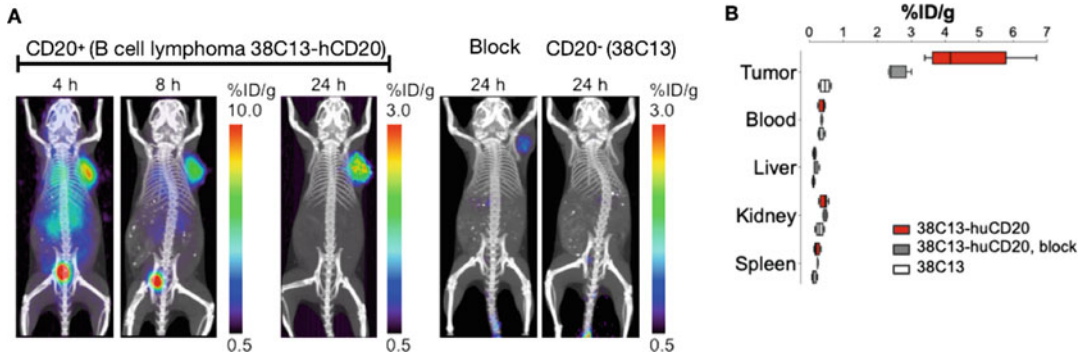

Fig. 9.13 In vivo targeting specificity. (**a**). Serial imaging of ^{124}I-labeled anti-CD20 cys-diabody antibody in mice bearing huCD20 transfected 38C13 tumors with and without a blocking injection of unlabeled anti-CD20. (**b**). The biodistribution of the radioimmunoconjugate 24 h after injection in mice bearing xenografts of antigen-expressing cells (38C13-huCD20), xenografts of cells that do not express antigen (38C13-huCD20), and antigen-expressing xenografts after blocking (block)

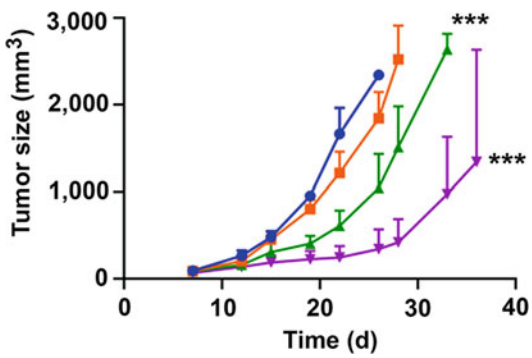

Fig. 9.14 Dose response of CEA positive tumors to ^{225}Ac-DOTA-anti-CEA M5A. Murine breast tumors transfected with CEA and implanted in the mammary glands of CEA Tg mice ($n = 6$ per group) were untreated (blue) or treated with 3.7 kBq (orange), 7.4 kBq, (green), or 11.1 kBq (purple) of ^{225}Ac-labeled DOTA-anti-CEA-M5A. Tumor growth curves were analyzed ANOVA, $^{***}p < 0.001$

For therapeutic radioimmunoconjugates, the preclinical demonstration of therapeutic efficacy is also essential prior to any attempts at clinical translation. An example of the dose-dependent inhibition of tumor growth by an ^{225}Ac-labeled DOTA-anti-CEA M5A is shown in Fig. 9.14. In the study, the lowest dose of radioimmunoconjugate has a minimal effect on tumor growth compared to the untreated saline control. The increasing doses exhibit statistically significant reductions in tumor growth that suggest even higher doses may be effective.

Since treatment of mice with ^{225}Ac-labeled antibody could have hematological toxicity due to circulating radiolabeled antibody, selected animals were euthanized at 12 days post-injection to probe for evidence of early toxicity, and the remainder were analyzed after the terminal time point. The transient depression of white blood cell (mainly neutrophils) and platelet levels were observed, with complete recovery at the terminal time point (Fig. 9.15).

IND applications usually include an estimate of radiation doses extrapolated from an animal study using programs such as OLINDA [41]. This can be accomplished by choosing a single dose of radioimmunoconjugate and performing a biodistribution study in tumor-bearing mice over a time period that covers one to two half-lives of the radionuclide and/or circulating antibody. A typical biodistribution study using the same ^{225}Ac-labeled antibody is shown in Fig. 9.16. In this case, counting the alpha emissions from Ac-225 is difficult, so the gamma emissions from its final daughter radionuclide—Bi-213—were used.

These data clearly show that the accumulation of radioactivity in the tumor increases over time. By integrating the activity concentration in each organ over time, it is possible to obtain total doses per organ and extrapolate these doses to human organs. It is also important to note that the relative biological effect (RBE) of different particle

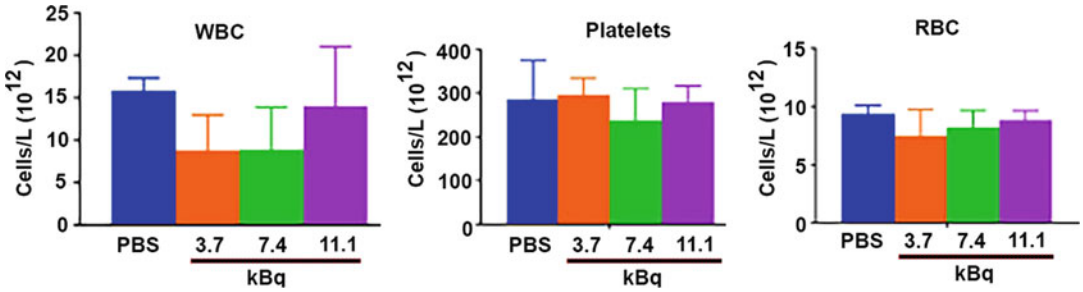

Fig. 9.15 Hematological toxicity of ^{225}Ac-DOTA-anti-CEA m5A in tumor-bearing mice. Tumor-bearing animals treated or not with ^{225}Ac-DOTA-anti-CEA M5A were euthanized at the terminal time point (Fig. 9.12), and blood was collected and analyzed for white blood cells (WBC), platelets, and red blood cells (RBC)

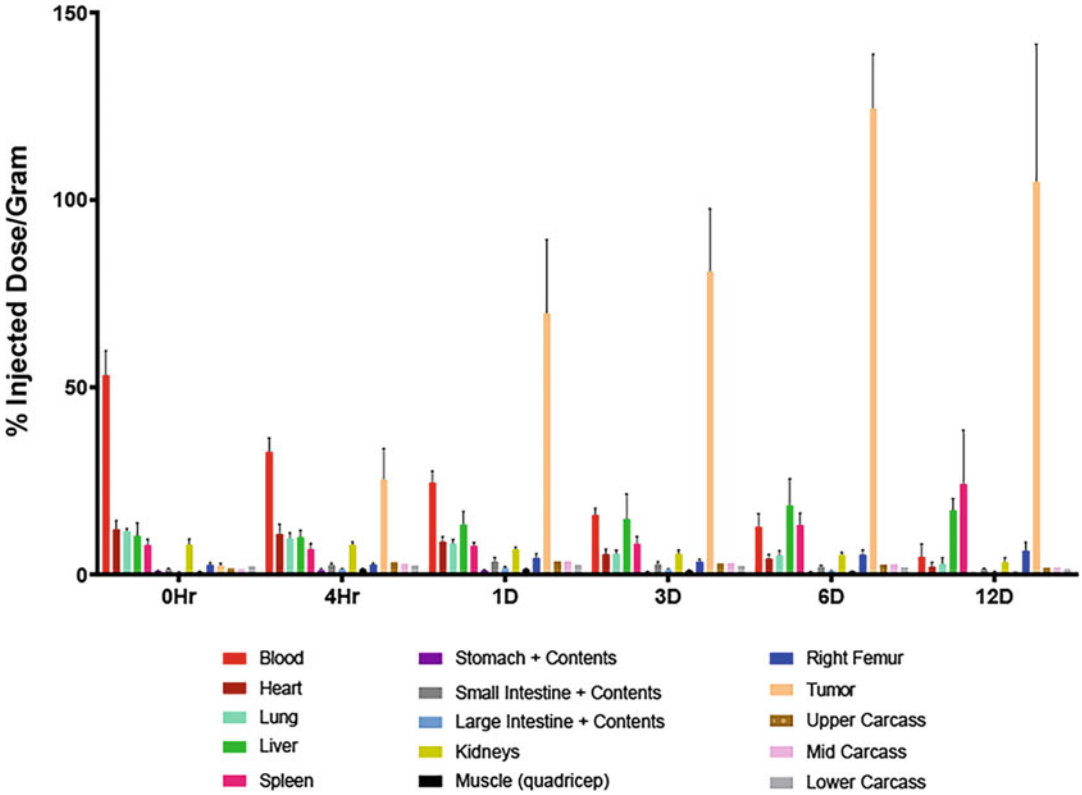

Fig. 9.16 Tissue biodistributions of ^{225}Ac-DOTA-anti-CEA M5A. Biodistribution data collected from nude mice bearing CEA positive LS174T that were treated with 11.1 kBq of ^{225}Ac-labeled anti-CEA M5A (30 μg, 6 mice per group) and euthanized at the indicated times

emissions must be considered. For example, the RBE of gamma and beta emissions are taken as 1.0, while alpha emissions have a much higher RBE [42]. Yet these data, while useful, cannot substitute for actual measurement in patients. In this respect, we were able to extrapolate human organ doses from our previous imaging studies with an ^{111}In-labeled variant of the radioimmunoconjugate in colorectal cancer patients. A comparison of the results obtained

Table 9.3 Dosimetry of ^{225}Ac-DOTA-anti-CEA M5A[a]

Organ	Animal biodistribution	In-111 patient imaging
Heart wall	65	1020
Kidneys	150	607
Liver	3004	3292
Lungs	63	108
Red marrow	119	181
Osteogenic cells	833	1281
Spleen	1959	1936
Total body	**149**	**205**

[a]Mean doses in mSv/MBq with the biological effect of the alpha emission as 5.0

using these two methods is shown in Table 9.3. There was good agreement between the methods for the liver and spleen, the two healthy organs with the highest uptake of intact antibodies. In contrast, higher doses were calculated for heart wall, red marrow, and kidneys from the clinical In-111 data. Based on these analyses, we are planning a phase 1 trial for ^{225}Ac-DOTA-M5A in rectal cancer.

Clinical Considerations for Radiopharmaceutical Therapy The first Food and Drug Administration (FDA)-approved radioimmunoconjugate was Zevalin, a ^{90}Y-labeled anti-CD20 antibody approved in 2002 for the treatment of patients with non-Hodgkin's lymphoma [43]. Since then, the number of FDA-approved radioimmunoconjugates remains low despite many phase 1–2 trials with radiotherapeutics bearing beta-emitting radionuclides. However, there is renewed interest in arming antibodies with alpha-emitting radionuclides that have high linear energy transfer compared to beta-emitters. Whether or not these studies will lead to new FDA-approved drugs remains to be seen.

Once the necessary characterization and preclinical studies are completed, the starting points for a phase 1 radioimmunotherapy human trial are patient selection, choosing the starting dose, planning the dose escalation, and deciding on safety protocols. Patient selection typically includes a biopsy or blood test to determine if they are positive for the antigen of interest. The patients in a phase I RPT trial will have likely failed standard-of-care therapies and may have low performance indices that may affect their ability to withstand expected toxicities. Since the expected toxicities of a radioimmunoconjugates are hematological, a patient's platelet and WBC counts should be within the normal range before starting therapy and then monitored throughout the trial. The starting dose for the trial can be extrapolated from the animal dosimetry studies and/or similar published trials. The overall goal will be to test safety and determine the maximum tolerated dose (MTD). A standard trial design may include at least three doses with three patients at each dose until adverse effects are observed. An additional three patients may be added at the final dose to accrue more data. The increments of the dose escalation may be 1.5–2.0 times the starting dose depending on the expected toxicity in the patient population selected.

During the trial, regular blood draws are essential to monitor the pharmacokinetics of the radiolabeled antibody, the potential generation of an immune response to the antibody, changes in biomarker levels, and the hematological toxicity (if any) of the treatment. Response to therapy can be monitored by standard radiological imaging. This can be accompanied by radioimmunoimaging if the therapeutic radioimmunoconjugate also produces "imageable" emissions or if a variant of the radioimmunoconjugate labeled with a different radionuclide suitable for imaging is available. Decreasing levels of serum biomarkers—especially the antigen of interest—often represents an additional important readout for response to therapy.

Table 9.4 Therapeutic radionuclides suitable for radiolabeled antibodies

Radionuclide	Particle	Emax (MeV)	Range max	Half-life	Gamma emissions
Cu-67	Beta	0.58	2 mm	2.6 d	185, 93 keV
Y-90	Beta	2.28	120 mm	2.7 d	n/a
I-131	Beta	0.60	2 mm	8.0 d	364 keV
Lu-177	Beta	0.50	0.6 mm	6.7 d	208 keV
Pb-212	Alpha	7.8	0.09 mm	10.6 hr	239 keV
Ac-225	Alpha	8.4	0.09 mm	10.0 d	(435 keV Bi-213)

Examples of commonly used therapeutic radionuclides as well as their emissions and energy characteristics are given in Table 9.4. A general rule for choosing a radionuclide is to match its half-life with the biological half-life of the immunoglobulin vector. Most intact humanized antibodies have a half-life of 10–12 days in the circulation [44] that can be confirmed by collecting regular blood collections and counting radioactivity starting from the time of infusion. It should be noted that blood clearance times will be affected by the presence of circulating antigen levels that may vary from patient to patient. As mentioned in an earlier section, the blood residence time of a radioimmunoconjugate can be changed by using smaller antibody fragments or engineering the Fc region of the antibody to manipulate its interaction with FcRn receptors [45]. Based on these general guidelines and the need to deliver the maximum amount of radionuclide to the target tissue, intact antibodies are often the first choice for therapeutic radioimmunoconjugates, since their longer residence time translates into higher target tissue uptake. Nonetheless, this feature is offset by increased hematological toxicity, since the radiolabeled antibody can irradiate the bone marrow during its extended circulation in the body. The doses to both the target and non-target tissues—calculated as described in the previous section—will also strongly depend on the tissue pathlength of the emission. Indeed, there are several choices of beta-emitting radionuclides that vary in tissue penetration. Y-90 exhibits the greatest tissue pathlength and thus—all other things being equal—will have more hematological toxicity than Cu-67, I-131, or Lu-177. I-131 and Lu-177 are commercially available, as is (more recently) Cu-67 [46, 47]. Alpha emitters, with their short tissue penetration but high linear energy transfer (LET), were originally thought to be suitable only for the treatment of hematological malignancies but have more recently enjoyed success in treating solid tumors, presumably due to their cytotoxic effects on tumor vasculature [48]. Pb-212 and Ac-225 are available from the DOE and at least one commercial supplier. As their use increases, their availability can be expected to increase as well.

A large number of therapeutic clinical trials with radiolabeled antibodies have been performed. No attempt will be made to review them all, since most have the same overall goal: delivering a maximum dose with minimal toxicity. Examples of beta radionuclide therapies shown in Table 9.5 indicate a range of about 0.1–6.7 Gy delivered to tumors. In most cases, MTDs were reached with hematologic dose limiting toxicities. The best responses were observed in lymphomas, while radiation resistant solid tumors were less responsive to therapy. The treatment of solid tumors was often improved by multiple cycles [49] or the addition of radiosensitizers [50] or chemotherapeutics [51].

As mentioned earlier, most trials have employed intact monoclonal antibodies or larger bivalent fragments. Since it is well known that full-length antibodies penetrate only a few cell diameters beyond the tumor vasculature of solid tumors [52], it is likely that the tissue penetration of the antibody and its radionuclide emission do not play as large a role in tumor killing as once thought. Thus, alpha emitters with their low tissue penetration and high LET may have a pronounced effect on tumor vasculature (TV) by binding to the tumor cells in contact with the TV. The mechanisms of cytotoxicity to the TV may also include the production of ROS [53].

Table 9.5 Examples of endoradiotherapy trials and tumor doses

Radiolabeled antibody	Tumor type	Tumor dose (cGy/cycle)	Ref
^{131}I-CC49	Prostate, ovary	208–1083	[54]
^{131}I-NP4 F(ab')2	Colorectal, lung, pancreas, thyroid	511–6476	[55]
^{131}I-chL6	Breast	120–3700 (~1300 mean)	[56]
^{131}I-cMOv18	Ovary	600–3800	[57]
^{186}Re-NR-CO-2 F(ab')2	Lung, colorectal, breast, ovary, renal	500–2100	[58]
^{186}Re-hu anti-CD44v66	Head and neck	380–7610 (1240 median)	[59]
^{90}Y-BrE-3	Breast	442–1887	[60]
^{90}Y-chT84.66	Colorectal, breast	46–6400 (1320 mean)	[61]
^{131}I-huA33	Colorectal	1173–3273 (2119 mean)	[62]
^{90}Y-2B8	Non-Hodgkin's lymphoma	580–6700 (1700 median)	[63]
^{131}I-anti-B1	Non-Hodgkin's lymphoma	795 mean	[64]
^{131}I-anti-B1	Non-Hodgkin's lymphoma	141–2584 (925 mean)	[65]
^{131}I-Lym-1	Non-Hodgkin's lymphoma	16–1485 (241 median)	[66]
^{131}I-LL2	Non-Hodgkin's lymphoma	166–861	[67]

Beyond phase 1 safety trials in which determining the MTD is the major goal, many trials include clinical correlates, including monitoring changes in tumor volume, changes in biomarkers, and organ toxicities. In the case of the trials with our ^{90}Y-labeled anti-CEA chimeric antibody, serum CEA levels were used as a reliable biomarker. In a trial with the addition of the radiation sensitizer 5-FU, an MTD of 614 MBq/m^2 combined with 1000 mg/m^2/day 5-FU was reached, with patients eligible to receive up to three cycles of the combination treatment every 6 weeks. Radiological stable disease of 3–8 months duration was observed in 11/21 patients with progressive disease entering the study, while one patient demonstrated a mixed response [61]. In a more recent study with the humanized anti-CEA antibody M5A in which 16 patients received ^{90}Y-DOTA-M5A, the maximum doses were one patient at 614 MBq/m^2 with gemcitabine (150 mg/m^2 days 1 and 3), three patients at 518 MBq/m^2 with gemcitabine, six patients at 440 MBq/m^2 without gemcitabine, and six patients at 370 MBq/m^2 without gemcitabine. Prolonged cytopenias resulted in the discontinuation of dose escalation with gemcitabine. A single agent MTD of 370 MBq/m^2 was established based on the dose-limiting hematopoietic toxicities, and a human anti-human antibody response was observed in 2 of 16 patients (12.5%). Stable disease at 3 months was seen in 10 patients, and 2 patients demonstrated an 88 and 64% decrease in serum CEA levels. In two patients that had concurrent ^{111}In-DOTA-M5A imaging, previously unknown brain metastases were revealed.

9.3 The Future

The future of radiolabeled antibodies for therapy remains bright. Given the importance of antibody therapies in so many areas of cancer research, it seems obvious that their use as vectors for the delivery of radionuclides will be explored. The advantages of radioimmunotherapy over other targeted treatments include the ability to tailor the radionuclide half-life and emissions to the disease and the ability to calculate doses, especially when the radionuclide or a surrogate radionuclide has gamma emissions. This advantage cannot be over-estimated because calculating doses is difficult for most types of biologically based therapies.

9.4 Bottom Line

- Antibodies are promising vectors for the delivery of therapeutic radionuclides to tumor tissue due to their exquisite selectivity and affinity for their molecular targets.
- Full-length immunoglobulins have long circulation half-lives. This can result in high

radiation dose rates to healthy tissues, so smaller format antibody fragments with more rapid pharmacokinetic profiles—i.e., minibodies, diabodies, etc.—have also been explored as vectors for radioimmunotherapy.

- When designing a radioimmunoconjugate, care must be taken with respect to the selection of the radionuclide and the approach to radiolabeling.
- A wide variety of chemical characterization, biological characterization, and preclinical in vivo evaluation steps must be performed as a radioimmunoconjugate is ushered toward clinical translation.
- Radioimmunoimaging studies can complement both the preclinical development and the clinical deployment of therapeutic radioimmunoconjugates.
- Although patients have been treated with FDA-approved radiolabeled antibodies for over 20 years, the number of approved therapeutic radioimmunoconjugates remains low. There is optimism that this may change in the coming years, as new antibodies radiolabeled with high LET alpha emitters are evaluated in clinical trials.

References

1. Lu RM, Hwang YC, Liu IJ, Lee CC, Tsai HZ, Li HJ, et al. Development of therapeutic antibodies for the treatment of diseases. J Biomed Sci. 2020;27(1):1.
2. Yazaki PJ, Sherman MA, Shively JE, Ikle D, Williams LE, Wong JY, et al. Humanization of the anti-CEA T84.66 antibody based on crystal structure data. Protein Eng Des Sel. 2004;17(5):481–9.
3. Freise AC, Wu AM. In vivo imaging with antibodies and engineered fragments. Mol Immunol. 2015;67 (2 Pt A):142–52.
4. Wagener C, Clark BR, Rickard KJ, Shively JE. Monoclonal antibodies for carcinoembryonic antigen and related antigens as a model system: determination of affinities and specificities of monoclonal antibodies by using biotin-labeled antibodies and avidin as precipitating agent in a solution phase immunoassay. J Immunol. 1983;130(5):2302–7.
5. Wagener C, Yang YH, Crawford FG, Shively JE. Monoclonal antibodies for carcinoembryonic antigen and related antigens as a model system: a systematic approach for the determination of epitope specificities of monoclonal antibodies. J Immunol. 1983;130(5):2308–15.
6. Neumaier M, Shively L, Chen FS, Gaida FJ, Ilgen C, Paxton RJ, et al. Cloning of the genes for T84.66, an antibody that has a high specificity and affinity for carcinoembryonic antigen, and expression of chimeric human/mouse T84.66 genes in myeloma and Chinese hamster ovary cells. Cancer Res. 1990;50(7):2128–34.
7. Tsai SW, Li L, Williams LE, Anderson AL, Raubitschek AA, Shively JE. Metabolism and renal clearance of 111In-labeled DOTA-conjugated antibody fragments. Bioconjug Chem. 2001;12 (2):264–70.
8. Wu AM, Chen W, Raubitschek A, Williams LE, Neumaier M, Fischer R, et al. Tumor localization of anti-CEA single-chain Fvs: improved targeting by non-covalent dimers. Immunotechnology. 1996;2 (1):21–36.
9. Li L, Olafsen T, Anderson AL, Wu A, Raubitschek AA, Shively JE. Reduction of kidney uptake in radiometal labeled peptide linkers conjugated to recombinant antibody fragments. Site-specific conjugation of DOTA-peptides to a Cys-diabody. Bioconjug Chem. 2002;13(5):985–95.
10. Kenanova V, Olafsen T, Williams LE, Ruel NH, Longmate J, Yazaki PJ, et al. Radioiodinated versus radiometal-labeled anti-carcinoembryonic antigen single-chain Fv-Fc antibody fragments: optimal pharmacokinetics for therapy. Cancer Res. 2007;67 (2):718–26.
11. Hu S, Shively L, Raubitschek A, Sherman M, Williams LE, Wong JY, et al. Minibody: a novel engineered anti-carcinoembryonic antigen antibody fragment (single-chain Fv-CH3) which exhibits rapid, high-level targeting of xenografts. Cancer Res. 1996;56(13):3055–61.
12. Muyldermans S. Nanobodies: natural single-domain antibodies. Annu Rev Biochem. 2013;82:775–97.
13. Yang EY, Shah K. Nanobodies: next generation of cancer diagnostics and therapeutics. Front Oncol. 2020;10:1182.
14. Caserta E, Chea J, Minnix M, Poku EK, Viola D, Vonderfecht S, et al. Copper 64-labeled daratumumab as a PET/CT imaging tracer for multiple myeloma. Blood. 2018;131(7):741–5.
15. Wong JY, Chu DZ, Williams LE, Yamauchi DM, Ikle DN, Kwok CS, et al. Pilot trial evaluating an 123I-labeled 80-kilodalton engineered anticarcinoembryonic antigen antibody fragment (cT84.66 minibody) in patients with colorectal cancer. Clin Cancer Res. 2004;10(15):5014–21.
16. Tsai WK, Zettlitz KA, Dahlbom M, Reiter RE, Wu AM. Evaluation of [(131)I]I- and [(177)Lu]Lu-DTPA-A11 minibody for radioimmunotherapy in a preclinical model of PSCA-expressing prostate cancer. Mol Imaging Biol. 2020;22(5):1380–91.
17. Mortimer JE, Bading JR, Park JM, Frankel PH, Carroll MI, Tran TT, et al. Tumor uptake of (64)Cu-DOTA-

Trastuzumab in patients with metastatic breast cancer. J Nucl Med. 2018;59(1):38–43.
18. Wong JY, Thomas GE, Yamauchi D, Williams LE, Odom-Maryon TL, Liu A, et al. Clinical evaluation of indium-111-labeled chimeric anti-CEA monoclonal antibody. J Nucl Med. 1997;38(12):1951–9.
19. Krishnan A, Adhikarla V, Poku EK, Palmer J, Chaudhry A, Biglang-Awa VE, et al. Identifying CD38+ cells in patients with multiple myeloma: first-in-human imaging using copper-64-labeled daratumumab. Blood Adv. 2020;4(20):5194–202.
20. Chomet M, van Dongen G, Vugts DJ. State of the art in radiolabeling of antibodies with common and uncommon radiometals for preclinical and clinical immuno-PET. Bioconjug Chem. 2021;32(7):1315–30.
21. Gupta S, Batra S, Jain M. Antibody labeling with radioiodine and radiometals. Methods Mol Biol. 2014;1141:147–57.
22. Boschi A, Uccelli L, Martini P. A picture of modern Tc-99m radiopharmaceuticals: production, chemistry, and applications in molecular imaging. Appl Sci (Basel). 2019;9(12):2526.
23. Wangler C, Wangler B, Lehner S, Elsner A, Todica A, Bartenstein P, et al. A universally applicable 68Ga-labeling technique for proteins. J Nucl Med. 2011;52 (4):586–91.
24. Grunberg J, Novak-Hofer I, Honer M, Zimmermann K, Knogler K, Blauenstein P, et al. 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 (14):5112–20.
25. Dearling JL, Paterson BM, Akurathi V, Betanzos-Lara S, Treves ST, Voss SD, et al. The ionic charge of copper-64 complexes conjugated to an engineered antibody affects biodistribution. Bioconjug Chem. 2015;26(4):707–17.
26. Cooper MS, Ma MT, Sunassee K, Shaw KP, Williams JD, Paul RL, et al. Comparison of (64)Cu-complexing bifunctional chelators for radioimmunoconjugation: labeling efficiency, specific activity, and in vitro/in vivo stability. Bioconjug Chem. 2012;23 (5):1029–39.
27. Rinne SS, Leitao CD, Mitran B, Bass TZ, Andersson KG, Tolmachev V, et al. Optimization of HER3 expression imaging using affibody molecules: influence of chelator for labeling with indium-111. Sci Rep. 2019;9(1):655.
28. Kang L, Jiang DW, Ehlerding E, Barnhart T, Ferreira C, Wang RF, et al. Safe and dosimetry evaluation of radimmmunotherapy using Lu-177-labeled antibodies in lymphoma. J Nucl Med. 2019;60
29. Crow DM, Williams L, Colcher D, Wong JYC, Raubitschek A, Shively JE. Combined Radioimmunotherapy and chemotherapy of breast tumors with Y-90-labeled anti-Her2 and anti-CEA antibodies with Taxol. Bioconjug Chem. 2005;16 (5):1117–25.
30. Ahlgren S, Orlova A, Rosik D, Sandstrom M, Sjoberg A, Baastrup B, et al. Evaluation of maleimide derivative of DOTA for site-specific labeling of recombinant affibody molecules. Bioconjug Chem. 2008;19(1):235–43.
31. Vugts DJ, Klaver C, Sewing C, Poot AJ, Adamzek K, Huegli S, et al. Comparison of the octadentate bifunctional chelator DFO*-pPhe-NCS and the clinically used hexadentate bifunctional chelator DFO-pPhe-NCS for (89)Zr-immuno-PET. Eur J Nucl Med Mol Imaging. 2017;44(2):286–95.
32. Pentlow KS, Graham MC, Lambrecht RM, Daghighian F, Bacharach SL, Bendriem B, et al. Quantitative imaging of iodine-124 with PET. J Nucl Med. 1996;37(9):1557–62.
33. Kim EJ, Kim BS, Choi DB, Chi SG, Choi TH. Improved in vivo stability of radioiodinated rituximab using an iodination linker for radioimmunotherapy. Cancer Biother Radiopharm. 2016;31(8):287–94.
34. Wadas TJ, Wong EH, Weisman GR, Anderson CJ. Copper chelation chemistry and its role in copper radiopharmaceuticals. Curr Pharm Des. 2007;13 (1):3–16.
35. Liu S, Li Z, Conti PS. Development of multi-functional chelators based on sarcophagine cages. Molecules. 2014;19(4):4246–55.
36. Hafeez U, Parakh S, Gan HK, Scott AM. Antibody-drug conjugates for cancer therapy. Molecules. 2020;25:20.
37. Guillou A, Earley DF, Klingler S, Nisli E, Nuesch LJ, Fay R, et al. The influence of a polyethylene glycol linker on the metabolism and pharmacokinetics of a (89)Zr-radiolabeled antibody. Bioconjug Chem. 2021;32(7):1263–75.
38. Takashima H, Koga Y, Manabe S, Ohnuki K, Tsumura R, Anzai T, et al. Radioimmunotherapy with an (211) At-labeled anti-tissue factor antibody protected by sodium ascorbate. Cancer Sci. 2021;112 (5):1975–86.
39. Zettlitz KA, Tavare R, Knowles SM, Steward KK, Timmerman JM, Wu AM. ImmunoPET of malignant and normal B cells with (89)Zr- and (124)I-labeled obinutuzumab antibody fragments reveals differential CD20 internalization in vivo. Clin Cancer Res. 2017;23(23):7242–52.
40. Zettlitz KA, Tavare R, Tsai WK, Yamada RE, Ha NS, Collins J, et al. (18)F-labeled anti-human CD20 cys-diabody for same-day immunoPET in a model of aggressive B cell lymphoma in human CD20 transgenic mice. Eur J Nucl Med Mol Imaging. 2019;46 (2):489–500.
41. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med. 2005;46(6):1023–7.
42. Sgouros G, Roeske JC, McDevitt MR, Palm S, Allen BJ, Fisher DR, et al. MIRD Pamphlet No. 22 (abridged): radiobiology and dosimetry of

alpha-particle emitters for targeted radionuclide therapy. J Nucl Med. 2010;51(2):311–28.
43. Forero A, Lobuglio AF. History of antibody therapy for non-Hodgkin's lymphoma. Semin Oncol. 2003;30 (6 Suppl 17):1–5.
44. Saunders KO. Conceptual approaches to modulating antibody effector functions and circulation half-life. Front Immunol. 2019;10:1296.
45. Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. J Pharm Sci. 2004;93(11):2645–68.
46. Novak-Hofer I, Schubiger PA. Copper-67 as a therapeutic nuclide for radioimmunotherapy. Eur J Nucl Med Mol Imaging. 2002;29(6):821–30.
47. Keinanen O, Fung K, Brennan JM, Zia N, Harris M, van Dam E, et al. Harnessing (64)Cu/(67)Cu for a theranostic approach to pretargeted radioimmunotherapy. Proc Natl Acad Sci U S A. 2020;117(45):28316–27.
48. Morgenstern A, Apostolidis C, Kratochwil C, Sathekge M, Krolicki L, Bruchertseifer F. An overview of targeted alpha therapy with (225)Actinium and (213)Bismuth. Curr Radiopharm. 2018;11(3):200–8.
49. Vallabhajosula S, Goldsmith SJ, Kostakoglu L, Milowsky MI, Nanus DM, Bander NH. Radioimmunotherapy of prostate cancer using 90Y- and 177Lu-labeled J591 monoclonal antibodies: effect of multiple treatments on myelotoxicity. Clin Cancer Res. 2005;11(19 Pt 2):7195s–200s.
50. Chang JE, Khuntia D, Robins HI, Mehta MP. Radiotherapy and radiosensitizers in the treatment of glioblastoma multiforme. Clin Adv Hematol Oncol. 2007;5(11):894–902, 7–15.
51. Magnes T, Wagner SM, Melchardt T, Weiss L, Rinnerthaler G, Huemer F, et al. Postoperative chemoradiotherapy with cisplatin is superior to radioimmunotherapy with cetuximab and radiotherapy alone : analysis of the Austrian head and neck cancer registry of the AGMT. Wien Klin Wochenschr. 2021;133(21–22):1131–6.
52. van Dongen G. Improving tumor penetration of antibodies and antibody-drug conjugates: taking away the barriers for Trojan Horses. Cancer Res. 2021;81(15):3956–7.
53. Behling K, Maguire WF, Di Gialleonardo V, Heeb LE, Hassan IF, Veach DR, et al. Remodeling the vascular microenvironment of glioblastoma with alpha-particles. J Nucl Med. 2016;57(11):1771–7.
54. Meredith RF, Bueschen AJ, Khazaeli MB, Plott WE, Grizzle WE, Wheeler RH, et al. Treatment of metastatic prostate carcinoma with radiolabeled antibody CC49. J Nucl Med. 1994;35(6):1017–22.
55. Juweid ME, Sharkey RM, Behr T, Swayne LC, Dunn R, Siegel J, et al. Radioimmunotherapy of patients with small-volume tumors using iodine-131-labeled anti-CEA monoclonal antibody NP-4 F(ab')2. J Nucl Med. 1996;37(9):1504–10.
56. Denardo SJ, O'Grady LF, Richman CM, Goldstein DS, O'Donnell RT, Denardo DA, et al. Radioimmunotherapy for advanced breast cancer using I-131-ChL6 antibody. Anticancer Res. 1997;17 (3B):1745–51.
57. van Zanten-Przybysz I, Molthoff CF, Roos JC, Plaizier MA, Visser GW, Pijpers R, et al. Radioimmunotherapy with intravenously administered 131I-labeled chimeric monoclonal antibody MOv18 in patients with ovarian cancer. J Nucl Med. 2000;41(7):1168–76.
58. Breitz HB, Weiden PL, Vanderheyden JL, Appelbaum JW, Bjorn MJ, Fer MF, et al. Clinical experience with rhenium-186-labeled monoclonal antibodies for radioimmunotherapy: results of phase I trials. J Nucl Med. 1992;33(6):1099–109.
59. Postema EJ, Raemaekers JM, Oyen WJ, Boerman OC, Mandigers CM, Goldenberg DM, et al. Final results of a phase I radioimmunotherapy trial using (186)Re-epratuzumab for the treatment of patients with non-Hodgkin's lymphoma. Clin Cancer Res. 2003;9 (10 Pt 2):3995S–4002S.
60. DeNardo SJ, Kramer EL, O'Donnell RT, Richman CM, Salako QA, Shen S, et al. Radioimmunotherapy for breast cancer using indium-111/yttrium-90 BrE-3: results of a phase I clinical trial. J Nucl Med. 1997;38 (8):1180–5.
61. Wong JY, Shibata S, Williams LE, Kwok CS, Liu A, Chu DZ, et al. A Phase I trial of 90Y-anti-carcinoembryonic antigen chimeric T84.66 radioimmunotherapy with 5-fluorouracil in patients with metastatic colorectal cancer. Clin Cancer Res. 2003;9(16 Pt 1):5842–52.
62. Chong G, Lee FT, Hopkins W, Tebbutt N, Cebon JS, Mountain AJ, et al. Phase I trial of 131I-huA33 in patients with advanced colorectal carcinoma. Clin Cancer Res. 2005;11(13):4818–26.
63. Wiseman GA, White CA, Stabin M, Dunn WL, Erwin W, Dahlbom M, et al. Phase I/II 90Y-Zevalin (yttrium-90 ibritumomab tiuxetan, IDEC-Y2B8) radioimmunotherapy dosimetry results in relapsed or refractory non-Hodgkin's lymphoma. Eur J Nucl Med. 2000;27(7):766–77.
64. Kaminski MS, Zasadny KR, Francis IR, Fenner MC, Ross CW, Milik AW, et al. Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. J Clin Oncol. 1996;14(7):1974–81.
65. Vose JM. Bexxar: novel radioimmunotherapy for the treatment of low-grade and transformed low-grade non-Hodgkin's lymphoma. Oncologist. 2004;9 (2):160–72.
66. Lamborn KR, DeNardo GL, DeNardo SJ, Goldstein DS, Shen S, Larkin EC, et al. Treatment-related parameters predicting efficacy of Lym-1 radioimmunotherapy in patients with B-lymphocytic malignancies. Clin Cancer Res. 1997;3(8):1253–60.
67. Vose JM, Wahl RL, Saleh M, Rohatiner AZ, Knox SJ, Radford JA, et al. Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. J Clin Oncol. 2000;18(6):1316–23.