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Development of Radioimmunotherapeutic and Diagnostic Antibodies: An Inside-Out View†

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Abstract

Only a handful of radiolabeled antibodies (Abs) have gained FDA approval for use in clinical oncology, including four immunodiagnostic agents and two targeted radioimmunotherapeutic agents. Despite the advent of non-immunogenic Abs and availability of a diverse library of radionuclides, progress beyond early Phase II RIT studies in solid tumors has been marginal. Furthermore, ^{18}F -FDG continues to dominate the molecular imaging domain, underscored by a decade-long absence of any newly approved antibody-based imaging agents (none since 1996!). Why has the development of clinically successful Abs for RIT been limited to lymphoma? What obstacles must be overcome to allow the FDA-approval of immunoPET imaging agents? How can we address the unique challenges that have thus far prevented the introduction of Ab-based imaging agents and therapeutics for solid tumors? Many poor decisions have been made regarding radiolabeled Abs, but useful insight can be gained from these mistakes. The following review addresses physical, chemical, biological, clinical, regulatory, and financial limitations that impede the progress of this increasingly important class of drugs.

Overview

The following review illustrates key components of a successful radiolabeled diagnostic or therapeutic antibody (Ab) from the *inside out* — that is, starting from the unstable nucleus itself and moving outwards. For each sequential topic, relevant theory will be examined and related to the practical use of various radiolabeled Abs that have succeeded to varying degrees in the clinic. This approach will present each important aspect of a rather complex multidisciplinary phenomenon in a logical, stepwise manner:

- I. The radionuclide itself
Physical properties of the unstable nucleus
- II. The radionuclide's chemical surroundings
Chemical attachment of the radiometal or radiohalogen
- III. The antibody
Biological issues of the radioimmunoconjugate

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IV. The target antigen and associated tumor(s)

Clinical efficacy of a given target receptor or antigen

V. Challenges and Prospects

Overcoming failures through wiser choices

The *inside-out* theme is also preserved within Section I–Section IV by initially analyzing the scientific efforts inside the laboratory, followed by a glimpse out into the real world, where *commercial, regulatory, and financial* obstacles are encountered that impede the accessibility of drugs to the target patient population.

I. The radionuclide itself

Radionuclides in Targeted Radioimmunotherapy

The commercially successful Genentech/Roche antibodies (Abs) (e.g. rituximab, trastuzumab, and bevasizumab) and most of the monoclonal Abs on the market or in late-stage development are naked, or unconjugated Abs that function by targeting tumor-expressed proteins. An alternative strategy is to use the Abs for targeted delivery of a cytotoxic drug or radionuclide thereby enhancing the therapeutic efficacy of the Ab.^{1–4}

Ehrlich conceived the idea of "magic bullets" targeting compounds and eradicating disease⁵, but it was not until the early 1950s that an Ab was conjugated to a radionuclide.⁶ Pioneering clinical studies by Mach⁷ and Goldenberg⁸ with anti-CEA Abs demonstrated the feasibility of specific targeting. Kohler isolated the first monoclonal antibodies in 1975.⁹ Nearly two decades later, Leichner and co-workers studied the use of ¹¹¹In/⁹⁰Y-labeled anti-ferritin for RIT in patients with hepatoma.¹⁰ Finally, the FDA approvals for two radiolabeled anti-CD20 mAbs, ⁹⁰Y-labeled Zevalin® (ibritumomab tiuxetan) in 2002 and ¹³¹I-labeled Bexxar® in 2003 for the treatment of non-Hodgkin's lymphoma (NHL) were landmark events in the developmental history of therapeutic radiolabeled mAbs (RIT) (see Section IV).¹¹

Radionuclides in Molecular Imaging

Molecular imaging is a rapidly emerging field and a powerful tool in the clinical diagnosis of disease.¹² Radioimmunoimaging has been traditionally developed in parallel with RIT for evaluating targeting and dosimetry. MAb-based tracers are gaining acceptance for identification of specific molecular targets and visualization of tumors at primary and metastatic sites. Drug development is also being revolutionized by molecular imaging probes, especially in the field of oncology.¹³

The most sensitive imaging modalities utilize radiotracers, including gamma (γ)- camera (planar) scintigraphy, single-photon emission computerized tomography (SPECT), and positron emission tomography (PET) with PET operating at 10-fold sensitivity superiority to SPECT. Radioactive modalities coupled with non-radioactive modalities such as optical imaging, magnetic resonance imaging (MRI), and computed tomography (CT) provide *multimodal* imaging approaches with significant advantages combining the strengths of complementary modalities (e.g., PET-CT couples high sensitivity of PET with detailed anatomical information from CT).

Inherent Nuclear Properties: Half-Life, Decay Energy, and Range

A successful diagnostic or radio immunotherapeutic Ab must be radiolabeled with a radionuclide matched for the intended use.^{14, 15} The half-life ($t_{1/2}$) must be adequate for target accumulation and non-specific clearance. Considering these requirements, labeling an intact IgG with ¹⁸F is an impractical endeavor. Copper-64 ($t_{1/2} \sim 12$ h) would be a more reasonable

choice for radiolabeling antibodies.^{16–18} On this basis, ^{89}Zr ($t_{1/2} \sim 3.3$ d) is perhaps even better suited for immunoPET.^{19–23} Several other radionuclides (Table 1) also possess half-lives compatible with mAb biological half-lives.

Radionuclide emissions are critical in the design of a given imaging or therapeutic application. Early RIT studies used ^{131}I due to advantages of commercial availability, potential direct dosimetry, and simple familiarity. However, the $t_{1/2}$ is unnecessarily long, the γ -emissions require patient isolation, and absorbed doses are compromised by a short biological half-life resulting from dehalogenation within blood and tumor sites.

Methods for attaching metal chelating groups have facilitated study of metallic radionuclides (e.g., ^{90}Y , ^{177}Lu , and ^{67}Cu) that are potentially far better suited for RIT. Yttrium-90 lacks interfering γ -emissions altogether, and, while ^{177}Lu and ^{67}Cu emit γ -rays, they are of much lower energy with respect to ^{131}I . Yttrium-90 provides advantages over ^{131}I because it delivers on average a more energetic tumor-killing β^- (935 keV versus 182 keV for ^{131}I) and concomitantly, a longer mean range (3.78 mm versus 0.36 mm for ^{131}I) (Table 1). These characteristics improve the ability of a radiolabeled Ab to kill both targeted and neighboring cells and offer a particular advantage in treating bulky or poorly vascularized disease. In addition, being a pure β^- -emitting radionuclide, ^{90}Y can be administered on an outpatient basis.

Radionuclide characteristics affect dose deposition. Lutetium-177 is not optimal for treatment of a large-diameter solid tumor as low energy β particles lack sufficient tumor penetration while conversely, ^{90}Y is not suited for treating disseminated microscopic tumor burden. Use of ^{131}I , ^{177}Lu , or ^{67}Cu is more appropriate in an adjuvant or small tumor setting. In contrast, ^{90}Y seems suited for use in patients with clearly detectable lesions due to its higher energy and consequent better penetration. Successful matching of β -emission energies to tumor volumes in RIT has been well defined.^{24, 25}

Choosing an appropriate radionuclide is equally important for imaging applications. For example, a high energy positron (β^+) emission (e.g. ^{66}Ga) may produce PET images of relatively poor quality and resolution; lower energy β^+ emitters (e.g. ^{64}Cu) are more desirable. ^{111}In is a popular choice for SPECT imaging. Lower β^+ and γ energies are associated with higher PET and SPECT image quality, respectively.

Examples of matched β^+/β^- (imaging/therapy) pairs include $^{124}\text{I}/^{131}\text{I}$, $^{86}\text{Y}/^{90}\text{Y}$, and $^{64}\text{Cu}/^{67}\text{Cu}$. Bremsstrahlung imaging of ^{90}Y is sub-optimal, and although ^{111}In can be used as a surrogate, PET imaging using ^{86}Y provides the advantage of matched chemistry. Associated β^- , β^+ and Auger electron emissions render ^{64}Cu useful for both RIT and PET imaging. More efficacious therapeutic effects may be possible using the β^- -emitter ^{67}Cu .^{26, 27}

Radiobiology and Dosimetry

Radionuclide choice is ideally influenced by clinical parameters such as tumor location, size, morphology, physiology, and radiosensitivity. Important variables affecting tumor response include cumulative radiation dose delivered to the tumor(s), dose rate, penetration, and radiosensitivity. The extent of heterogeneity of dose deposition is highly dependent on Ab characteristics and radionuclide properties. Furthermore, enhanced therapeutic efficacy can be attained through selective dose delivery to radiosensitive areas of tumor. The percent of the injected dose per gram of tumor (%ID/g) expresses the tumor uptake of a given agent. Quite encouraging %ID/g values are often observed in rodents, but this trend is deceptively optimistic and does not apply to humans. Tumor uptake and penetration have been the most challenging limitations, with accretion at such low levels as 0.001–0.01 %ID/g often resulting in tumor

doses of <1,500 cGy. Bone marrow is typically the dose-limiting organ, with an apparent dose limitation of about 150–200 cGy.

Any particulate emission – including helium nuclei (α particles), negative electrons (negatrons, β^- particles), positive electrons, (positrons, β^+ particles), and Auger electrons – may be employed in RIT; however, β^- -emitters are most often employed for a variety of reasons. A β^- particle travels long distances before dissipating all of its kinetic energy, and this low-linear energy transfer (low-LET) emission can span up to 50 cell diameters. Single-strand DNA breaks typically occur in cells traversed by β^- particles, and bystander effects on antigen-negative neighboring cells can also be exerted. A unique β^- emission energy spectrum — *not* a discretely defined value — governs the observed path lengths for a given radionuclide. Normal tissue damage is one of the disadvantages associated with β^- emissions. Circulation through bone marrow provides opportunity for circulating radionuclide conjugates to irradiate the marrow cells leading to myelosuppression.

An alpha (α) particle has a very short path length (< 100 μm), but a very high LET²⁸, with a typical energy deposition of ~ 100 keV/ μm compared to 0.2 keV/ μm from a β^- emission. The relative biologic effectiveness (RBE) of high-LET radiation exhibits no dose rate dependence and is effective even under hypoxic conditions. An individual cancer cell can be killed by interaction with only a few and possibly with only a single α -particle. Moreover, the range of α particles is short enough to minimize normal tissue damage. Alpha particles (i.e. helium nuclei) are quite large, have high energies of several MeV, and are associated with high probability of inflicting irreparable and cytotoxic DNA double-strand breaks.²⁹ Consequently, α -emitters are well suited for hematologic disease, micrometastatic disease, and tumor cells near the surface of cavities.

Alpha-emitting radionuclides with ideal characteristics for RIT include ^{212}Bi 30–34, $^{225}\text{Ac}/^{213}\text{Bi}$ 35–42, ^{211}At 43–45, and ^{149}Tb 41, 46 (Table 1). Alpha-targeted therapy using ^{212}Bi or ^{213}Bi may be achieved either directly or by using an *in vivo* generator through injection of the appropriate parent radionuclide, ^{212}Pb or ^{225}Ac , respectively. An attractive longer-lived α -emitter is ^{211}At , with a $t_{1/2}$ of 7.2 hours. The successful application of α -emitters in RIT has awaited developments in radionuclide production, protein labeling chemistry, dosimetry, and the development of appropriate pre-clinical models. A number of preclinical studies have concluded that α -emitters may be more effective than β^- emitters administered at comparable doses in RIT.^{47, 48}

Auger and conversion electron emitters' emissions are highly localized and are only effective post-localization in the cell nucleus.^{49, 50} Auger emitters have the shortest range of energy deposition, typically submicrometer, that is, less than one cell diameter. Indium-111 releases approximately 7 electrons per decay, whereas ^{125}I and $^{195\text{m}}\text{Pt}$ release 20–35 electrons per decay. Auger-emitting radionuclides exhibit desirable characteristics, especially under appropriately selected conditions including single-cell systems like leukemia, adjuvant settings, and post-surgical treatment of residual disease.

Commercialization of Radionuclide Production

The commercial availability of radionuclides such as ^{131}I , ^{125}I , ^{111}In , ^{90}Y , and $^{99\text{m}}\text{Tc}$ has directly contributed to the pace at which diagnostic and therapeutic immunoconjugates have been developed and tested in clinical trials, although the converse may also be true.⁵¹ A number of generator-produced radionuclides are readily available (e.g., ^{188}Re , ^{212}Bi , ^{90}Y , $^{99\text{m}}\text{Tc}$), making them ideal candidates for widespread hospital-based applications. MDS Nordion has focused on supplying radionuclides for the emerging RIT business. Nordion extended its deal with Corixa to radiolabel the Ab in Bexxar® with ^{131}I , and also produced sterile ^{90}Y for IDEC's Zevalin®.

Regulatory, Financial, and Commercial Barriers

Radioactive decay, an inherent property that is crucial to the action of both imaging and RIT, is ironically one of the biggest hurdles to overcome. The fear of radioactive materials is a great obstacle that increases commercial production costs and influences public perception, industrial reluctance, and governmental regulation. While the development of non-radioactive cancer Ab immunotherapy is very much a growing industry, seemingly minimal effort is being expended in RIT, except by academic researchers.

Procedural and logistic hurdles in conducting RIT are often substantial.⁵² MDS Nordion has a unique position at the crossroads of the nuclear and drug research industries, experiencing both sides with stringent and often contradictory regulations. Beyond satisfying FDA guidelines, radiation safety issues abound. In the clinical setting, highly specialized teams are necessary, and for some high-dose protocols, major physical plant radiation shielding alterations can be required. Acquisition and radionuclide use can necessitate hospital-based care, making RIT more complicated than other therapies.

High cost and limited or unresolved availability are major obstacles that have limited the clinical evaluation of Abs radiolabeled with ^{213}Bi , ^{212}Pb , ^{64}Cu , ^{67}Cu , ^{177}Lu , and ^{86}Y . Some β^- -emitting radionuclides have not been available in adequate amounts (e.g., ^{177}Lu), while others await the development of improved bifunctional chelators (e.g., ^{67}Cu). Alpha emitters (e.g., ^{211}At , ^{213}Bi) possess great potential for RIT, but face equally complex challenges due to limited production and availability status.

Decisions concerning choice of radionuclide have been made regardless of real appropriateness; selection criteria have instead been based on availability, popularity, familiarity, or historical trends. Limited access to facile radiolabeling chemistry also contributes to this problem. A grand total of only four radionuclides, ^{111}In , $^{99\text{m}}\text{Tc}$, ^{131}I , and ^{90}Y , have been integrated into FDA-approved radiolabeled Abs (Table 2). All four of these radionuclides are widely available, relatively inexpensive, and in some small part this status reflects the development and existence of actual products. Two of these radionuclides, ^{90}Y and ^{131}I , are components of approved therapeutics, while ^{111}In and $^{99\text{m}}\text{Tc}$ are approved for traditional γ scintigraphic imaging. Strikingly, no Abs labeled with β^+ emitting (PET) radionuclides have been approved despite the superior image resolution and sensitivity of PET. There are also no approved α -emitting radionuclides which seem obviously far better suited for treating small tumor burden and micrometastases than high-energy β^- -emitters. Several promising lower-energy β^- -emitters (e.g., ^{177}Lu and ^{67}Cu) also await successful commercialization.

Although radionuclide cost can significantly elevate the cost of RIT agents *per dose* relative to non-radioactive therapies; fewer administered doses of RIT are typically necessary to achieve comparable results. Average costs per year were determined to be \$17,529 for ^{90}Y -Zevalin® RIT,⁵³ \$23,099 for a four-dose rituximab regimen,⁵⁴ and \$25,559 for an eight-dose rituximab regimen.⁵⁴ The average durations of remission were 14.4 months with ^{90}Y -Zevalin® RIT,⁵³ 6.2 months with a four-dose rituximab regimen,⁵⁴ and 11.4 months with an eight-dose rituximab regimen.⁵⁴ In this scenario, clinical data has validated that RIT is both more cost effective *and* more efficacious than nonradioactive immunotherapy.

Sadly, a serious conflict of interest may prevent cancer patients from receiving appropriate treatment. In some cases, patients are prescribed drugs that are unnecessary or insufficient simply because they are more profitable for the physician. In a nationally televised broadcast, Brian Williams and his colleague, Rehema Ellis, recently addressed this moral and ethical dilemma on the NBC Nightly News on September 21, 2006.⁵⁵ In the context of RIT, the referring physician (i.e. medical oncologist) may lose profits by referring his patient to a nuclear

medicine physician (i.e. radiation oncologist). The referring oncologist, often lacking the proper licensing for injection of radiopharmaceuticals, does not ultimately administer the drug and hence does not gain revenue. The economics of medical fees and billing as structured by modern medicine may determine the choice of administered therapy despite the proven clinical advantages of RIT.

Beyond the argument of cost effectiveness, quality of life issues should be weighed. Non-radioactive immunotherapy or chemotherapy can be followed by relapses, complications, and debilitating side effects, deleteriously impacting emotional health and personal finances. In contrast, RIT treatment is administered in two doses, one week apart, usually with minimal side effects. The real cost of current RIT therapies must be placed in context with financial burden, survival, and quality of life in addition to efficacy.

II. The radionuclide's chemical surroundings

Immediate chemical environment

Ideally, the cytotoxic/imaging agent should be linked to the carrier without impairing functionality of either while maintaining a stable linkage. Stable attachment of radiometals to Abs has been pursued to circumvent the pitfalls of radioiodine — especially dehalogenation of internalizing Abs — that preclude their use for RIT. The benefits of ^{90}Y and ^{111}In over ^{131}I have been demonstrated by clinically comparing tumor uptake kinetics of ^{90}Y - and ^{131}I -labeled Abs and by imaging studies with ^{111}In and ^{131}I .

The clinical application supposedly guides radionuclide choice, which, in turn, determines the radiolabeling procedure. No single radionuclide is suitable for all clinical applications. Likewise, no single chelator is compatible with the full spectrum of available metal ions. Fundamental differences in coordination chemistry define that different metals vary in their preference of ligand.⁵⁶

Radiolabeling Protocols

Radiolabeling procedures must be simple, efficient, reproducible, and affordable. Currently, only ^{131}I and ^{90}Y appear to fulfill the present requirements for commercial development. Direct radioiodination (^{131}I , ^{125}I , ^{124}I , and ^{123}I) is well established, and tositumomab, the Ab in Bexxar®, is radiolabeled with ^{131}I by this means.

Metallic radionuclides such as ^{90}Y , ^{111}In , ^{67}Cu , and ^{177}Lu require chelation chemistry for radiolabeling to an Ab. Radiometal complexation must occur under practical conditions. Some complexation reactions can be quite slow, requiring elevated temperature and/or high pH values over extended time periods and therefore compromising the use of such radiolabeling methodologies. Generally, DTPA (diethylenetriaminepentaacetic acid) derivatives and other *acyclic* chelates exhibit fast complex association rates, whereas 1,4,7,20-tetraazacyclododecane *N,N',N'',N'''*-tetraacetic acid (DOTA) derivatives and other *macrocyclic* chelates have slower complex dissociation rates.

Method and degree of connection to the antibody

The conjugation of a radionuclide to an Ab must fulfill a single, critical requirement: maintain the affinity/avidity of the Ab for its target antigen. Chemical linkage methodologies comprised of thiourea, thioether, amide, ester, and disulfide bonds are available. As for the extent of conjugation, an optimal 'happy medium' must be established wherein degree of protein modification is optimized without reducing binding activity. This key variable is generally controlled by adjusting reaction stoichiometry and reaction conditions (e.g., pH, temperature, and time).

Metal Complex Stability

Choosing a bifunctional chelating agent that forms an adequately kinetically and thermodynamically stable complex within the context of radionuclide choice and application is also critical. For example, in Zevalin®, the ^{90}Y is stably chelated by tiuxetan, which is covalently linked to the mAb, ibritumomab, *via* thiourea bonds to lysine residues of the IgG. Inadequate stability would lead to loss of the radionuclide into inherent proteins *in vivo*, increasing background in diagnostic imaging and/or imparting radiation doses to normal organs during RIT.

Available bifunctional chelators

Acyclic DTPA-based and macrocyclic DOTA-based chelators represent the most commonly utilized classes of agents used in RIT. While DTPA-analogs boast rapid complexation kinetics for Y(III) and other lanthanides, a compromise is accepted, as *in vivo* kinetic stabilities are lower than for macrocyclic chelators. This is especially detrimental when using ^{90}Y , which leaches from the DTPA chelate *in vivo* over time and accumulates in skeletal tissue in close proximity to highly radiosensitive bone marrow, although it is difficult to deconvolute this effect from circulatory exposures. Conversely, DOTA-based chelators tend to exhibit high *in vivo* stability for lanthanides but require somewhat elevated temperatures and/or longer complexation reaction times.

The structurally reinforced CHX-DTPA family of chelators is a significant improvement over the traditional DTPA chelators. These preorganized chelators form complexes with high *in vivo* stability without sacrificing rapid complexation kinetics. Furthermore, CHX-A", a specific stereoisomer of the CHX-DTPA family, is an efficacious carrier of ^{86}Y and ^{90}Y for PET imaging⁵⁷ and radiotherapy⁵⁸, respectively, and is the premier chelator for stable complexation of ^{213}Bi .⁵⁹

The development of improved chelators is an active pursuit for non-lanthanides as well. Crossbridged macrocyclic chelators are currently being investigated for Cu(II) complexes, as these complexes are quite inert relative to those formed with either DOTA or 1,4,8,11-tetraacetic acid (TETA), both of which have exhibited *in vivo* instability with copper radionuclides.⁶⁰ While the crossbridged cyclam chelator 4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (CB-TE2A) has shown promising results with peptides, it is ill-suited for Ab radiolabeling due to somewhat harsh radiolabeling requirements.⁶¹ Further refinements must be performed to develop an optimal Ab-compatible Cu chelator.

Commercialization of Chelate Production

To date, no company can boast that it has played a primary role in developing a bifunctional chelate for a marketed radiolabeled protein. Bexxar® does not require a chelating agent, and tiuxetan (MXDTPA), the chelating agent used in Zevalin®, was developed years ago originally at the NCI. Established in 2000, Macrocyclics is a small firm that has been developing cGMP capabilities and commercializing chelant technologies emerging from the Sherry lab at the University of Texas, Dallas.

Dow Chemical Company established ChelaMed radiopharmaceutical services to integrate its chelating agent operations into current Good Manufacturing Practices (cGMP)-compliant pharmaceutical manufacturing.⁶² MeO-DOTA, Dow's premier chelating agent, can form stable complexes with radiometals like ^{177}Lu or ^{90}Y . Dow has collaborated with Memorial Sloan-Kettering Cancer Center in the development of ^{225}Ac -labeled mAbs targeted to a variety of cancer types. Avidex Limited has applied Dow's technology to radiolabel Avidex's monoclonal T cell receptors (mTCRs). Cytogen Corporation has partnered with Dow in the development of Quadramet® (^{153}Sm - EDTMP), a radiopharmaceutical for treatment of pain

associated with bone cancer. NeoRX Corporation had an exclusive worldwide license from Dow to ^{166}Ho -DOTMP for bone marrow ablation. Dow has also participated in collaborations with Kereos, Human Genome Sciences (HGS), Proxima Therapeutics, Barnes Jewish Hospital/ Washington University School of Medicine, and Michigan Research Institute.

III. The antibody

Nature of origin

Monoclonal Abs were initially derived from mouse B-cell hybridomas. These murine derived Abs have been useful in the *in vitro* diagnostics market. However, injection into humans as *in vivo* imaging agents or for therapeutic purposes induces a human anti-mouse antibody (HAMA) response. To minimize HAMA, chimeric Abs were developed through fusion of the variable region of the mouse Ab with the constant region of a human Ab. Further reductions in HAMA were realized by developing humanized Abs retaining only the CDR region of the murine Ab, while more recent methods have generated fully human monoclonal Abs.⁶³

Zevalin® and Bexxar® are of murine origin and are indicated for therapy of lymphoma, a disease characterized by decreased host-immune recognition. In contrast, more severe immunogenicity of murine Abs in solid tumors has precluded repeat administration and hence meaningful therapy. Effective solid tumor RIT can be achieved only through the continual development of non-immunogenic Abs. A compromise is involved, however, as humanized Abs tend to remain in circulation longer, effectively decreasing the relative tumor residence time and increasing hematopoietic toxicity.

Size, Penetration, and Clearance Rate

Size is one factor that impacts the circulation time of Abs. A full IgG Ab is a large, 150-kDa protein that can remain in circulation for 3–4 weeks while being metabolized slowly by the reticuloendothelial system. A 25-kDa monovalent fragment (i.e., scFv) has a blood clearance time of less than 10 hours with primarily renal excretion in 2–4 hours. Molecules with MWs above ~70 kDa, the glomerular filtration threshold, remain in circulation much longer than smaller, more rapidly eliminated molecules. Smaller molecules tend to exhibit faster uptake, but consequently in lower amounts and with shorter target residence times. Monovalent fragments (i.e. Fab) illustrate this condition. As a result, intermediate-sized minibodies and related constructs are also being pursued to address these limitations.

Solid tumor radiation-absorbed doses are generally far less than the levels that are predicted to result in durable clinical responses. The best uptake is approaching ~0.1% injected dose per gram of tumor. This limitation may be rationalized by the inherent barriers of macromolecular entry into solid tumors. Tumor-to-blood ratios as high as 30:1 have been reported at late time points, but therapeutic indices have generally been disappointing due to slow uptake and clearance. Improvements leading to faster target accumulation, complementary rapid clearance, and a more favorable therapeutic index deem well for translation to targeted immunotherapy, especially when coupled with short half-lived nuclides.

To image disease, probes must provide detection specificity, and therefore use of Ab based tumor targeting molecular probes is appropriate. However, the images obtained using Abs have typically been sub-optimal due to long circulation times and concomitant low tumor-to-background ratios. Small-size tumor imaging requires high image contrast. A systemically delivered radiotracer must accumulate at high levels within target sites and clear from the blood in a time frame compatible with the radionuclide's $t_{1/2}$.

Increased tumor uptake has been observed for intermediate-sized bivalent Ab formats, such as 75-kDa triabodies and 80-kDa minibodies (scFv-CH3), however, slower blood clearance was

evident.^{64, 65} Faster clearance rates can lead to increased tumor-to-background ratios, enhanced contrast, and improved tumor delineation. For instance, an ¹²³I-anti-CEA minibody allowed imaging of tumors ≥ 1 cm in size by SPECT⁶⁶, and an ¹²⁴I-labeled anti-HER2 diabody showed promising results in PET imaging of mouse tumor xenografts.⁶⁷ Significantly improved blood clearance (from 12 days to 8 hours) was achieved through point mutations in the FcRn receptor of a 105-kDa scFv-Fc molecule, yielding high tumor-to-blood ratios and impressive imaging results.⁶⁸

Pre-Targeting Approaches

Multistep targeting strategies continue to be investigated and have demonstrated impressive *in vitro* improvement (factors of 10 or more) over traditional infusion routes.^{69–71} The biotin-avidin chemical complex may be exploited in multistep approaches.^{72, 73} For instance, a three-step pre-targeting approach involves biotinylation of the Ab prior to infusion and cell targeting. Injection of avidin thereafter results in exposed avidin sites on the tumor, permitting injection of radiolabeled biotin that either rapidly binds at the tumor complex or concomitantly rapidly clears. Encouraging results have been achieved in pre-clinical settings and, while tumor-to-tissue ratios improved, renal accumulation and toxicity became a potential problem.

NeoRX extensively investigated a three-step strategy of systemic injection of a streptavidin-anti-Ep-CAM Ab conjugate (SA-NR-Lu-10) and radiolabeled biotin with a chase step using either HSA-galactose-biotin^{73, 74} or biotin-LC-NM-(Gal-NAc)₁₆.^{75, 76} Despite impressive pre-clinical results, very disappointing results were obtained in a Phase II clinical trial of ⁹⁰Y-DOTA-biotin pre-targeted by NR-LU-10 Ab/streptavidin in metastatic colon cancer patients.⁷⁷ The overall response rate was only 8%, and both hematological (8% grade 3 neutropenia, 8% grade 3 thrombocytopenia) and non-hematological (16% grade 3 diarrhea) toxicities were observed.⁷⁷ The expression of the Ep-CAM receptor in healthy bowel suggests that a poor choice of target receptor, rather than the pre-targeting strategy itself, was the major pitfall generating these negative results.

Despite the lackluster NeoRX results, pre-targeting has produced encouraging results in other cases. Signal amplification by pre-targeting a trivalent, bispecific Ab resulted in a > 40 -fold tumor-to-blood ratio increase relative to arcitumomab (^{99m}Tc-antiCEA- F(ab').⁷⁸ Binding of a divalent hapten labeled with ¹¹¹In or Gd(III) to scFv receptors allowed for noninvasive assessment by γ or MR imaging of transgenic gene expression.⁷⁹

Pharmacokinetic behavior

High renal uptake can lead to renal toxicity thereby limiting the amount of administered dose. Similarly, slow clearance from the blood compartment can limit the window during which optimal diagnostic/therapeutic conditions are achieved. It is of critical importance, however, to correctly identify and delineate underlying causes of non-target uptake. The overall charge, hydrophobicity/hydrophilicity, shape, and size can impact pharmacokinetics and clearance as will the presence of endogenous target receptors in non-target organs. Structural modification (e.g., PEGylation) and residue mutation are both useful strategies in reducing chemically- or physically-derived non-target organ uptake of antibodies, but these methods do not reduce uptake due to receptor expression within non-target organs.

In vivo, radioimmunoconjugates accumulate in the liver and kidneys where protein catabolism and elimination occur, and non-specific radioactivity retention results from the transfer of radioactive metal to endogenous metalloproteins. This undesirable behavior can be minimized through various strategies in an effort to increase the tumor-to-normal tissues radiation ratio (i.e., therapeutic index). Cleavable linkers can reduce renal and hepatic doses. Renal retention can also be minimized by the administration of lysine, a cationic amino acid.⁸⁰

Factors Affecting Tumor Uptake

Studies involving human tumor heterografts in immunosuppressed mice have created unrealistic expectations for RIT. Radionuclide conjugates routinely target established transplants with great efficiency and produce long-term regressions in mice, but such success is rarely observed in cancer patients. Valency, shape, size, isoelectric pH (pI), blood pool concentration, and receptor binding affinity are all parameters of a given Ab that govern the magnitude and depth (penetration) of tumor uptake. Studies with iodine-radiolabeled scFvs demonstrated that a threshold affinity between 10^{-7} and 10^{-8} M was required to observe detectable tumor uptake in mice 24 hours post-injection, whereas no gain in tumor accumulation was observed with affinities $> 10^{-9}$ M.⁸¹ Affinities $> 10^{-9}$ M were detrimental to rapid and uniform tumor penetration due to stable binding at first pass of tumor antigens forming a binding site barrier.⁸¹ A 16-amino-acid-long basic cell-penetrating peptide, penetratin, improved solid tumor uptake and distribution when co-injected with an ^{125}I -labeled covalent dimeric scFv.⁸²

Physiological Side Effects

Ideally, a given Ab would exhibit only a single physiological action: binding to its antigen. This is, of course, far from reality. Physiological side effects following Ab infusion are typically less of a concern in the context of radiolabeled diagnostic and RIT Abs since the amounts of protein administered are significantly lower with respect to non-radioactive immunotherapy applications. Nonetheless, unwanted biological effects are real, are often difficult to predict, and must be addressed.

Within 90 minutes following a single Ab infusion, serious physiological complications — including severe pain, extreme swelling, headache, nausea, diarrhea, myalgias, vasodilation, and hypotension — occurred in six healthy individuals in TeGenero's Phase I clinical trial with an unconjugated Ab, anti-CD28 mAb, in 2006.^{83–85} One individual remained in a coma for three weeks with heart, liver, and renal failure, pneumonia, septicemia, and gangrene. Radiolabeled antibodies can also produce undesirable side effects. A non-oncologic radioimmunodiagnostic agent, $^{99\text{m}}\text{Tc}$ -fanolesomab (NeutroSpec™), was approved in the U.S. for use in patients with equivocal presentation of appendicitis. However, following serious adverse events, including two fatalities, the agent was withdrawn in late 2005.⁸⁶

Antibody Production and Commercialization

Antibodies have emerged as an important drug class as underscored by 18 current FDA-approved therapeutic Abs (17 on the market; one withdrawn) across diverse clinical settings.^{87–93} Among these are 14 unmodified IgG molecules, two radioimmunoconjugates, one Ab-drug conjugate, and one Fab. Cancer therapy represents one major area in which mAbs have been successful, with eight of the 18 being approved for cancer, including treatment of both solid tumors and hematologic cancers (Table 2).^{94–100} Commercial development of Ab-based pharmaceuticals has become a burgeoning research area.¹⁰¹ Over 400 Abs are currently in clinical trials (www.ClinicalTrials.gov), and sales of recombinant protein therapeutics are projected to reach \$15 billion by 2010.¹⁰²

Three Abs — rituximab (anti-CD20; Rituxan®, marketed as MabThera® outside the U.S.) for treatment of non-Hodgkin's lymphoma (NHL), trastuzumab (anti-HER2/*neu*; Herceptin®) for treatment of breast cancer, and bevacizumab (anti-VEGF, Avastin®) for treatment of metastatic colorectal — represented three of Roche's top five selling pharmaceutical products, comprise half of the six Abs having greater than \$1 billion in individual sales in 2005, and had combined sales greater than \$6 billion worldwide in 2005. Genentech developed and currently markets these drugs in the U.S.; Roche holds marketing rights for the rest of the world.

Four radiolabeled Abs have been FDA-approved for cancer diagnosis: CEA-Scan® (arctumomab), Verluma® (nofetumomab merpentan), OncoScint® (satumomab pentetide), and ProstaScint® (capromab pentetide) (Table 2). Arctumomab, or carcinoembryonic antigen (CEA) scan (Immunomedics; Morris Plains, NJ), is a ^{99m}Tc anti-CEA murine F(ab') approved for SPECT imaging of colorectal cancer patients. Verluma® is a ^{99m}Tc Fab fragment approved for identification of advanced-stage small-cell lung cancer (SCLC). ProstaScint® (Cytogen Corp.; Princeton, NJ) is an ^{111}In anti-prostate-specific membrane antigen (PSMA) murine IgG currently used for SPECT imaging of post-prostatectomy patients with increasing PSMA. Among these four agents, only ProstaScint® remains available; the others are obsolete.

In addition, two radiolabeled Abs have been FDA-approved for use in nononcologic medical diagnostic imaging: ^{111}In -imiciromab pentetate (MyoScint™, 1996) for myocardial infarctions and ^{99m}Tc -fanolesomab (NeuroSpec™, 2004) for equivocal appendicitis. Four additional immunodiagnostic agents have been approved exclusively in Europe: ^{111}In -igovomab (Indimacis 125, 1996) for ovarian adenocarcinoma, ^{99m}Tc -murine anti-melanoma fragments (Tecnemab K1, 1996) for cutaneous melanoma lesions, ^{99m}Tc -sulesomab (LeukoScan®, 1997) for bone infection and inflammation in osteomyelitis patients, and ^{99m}Tc -votumumab (Humaspect, 1998) for colorectal carcinoma.

Regulatory and Financial Barriers

Despite a steady increase in earned revenues and newly approved products, there are concerns about the failure to obtain FDA approval on the part of several well-publicized and promising mAb candidates at the Phase II and Phase III levels. Several likely reasons for negative Phase III trials of molecular-target-based drugs were highlighted in a review by Saijo: (i) poor predictability of pre-clinical models, (ii) low enrichment levels of molecular target within tumors, (iii) molecular target is not essential for tumor survival, (iv) drug produces inadequate antitumor activity, (v) inappropriate clinical decision-making in Phase I/II → Phase III transition, and (vi) inappropriate clinical study design.¹⁰³ Among these, items (iii) and (iv) are typically not applicable to RIT, as the radionuclide ultimately provides tumor cytotoxicity. The remaining items, however, *are* quite relevant and represent challenges that must be overcome in order for radiolabeled antibodies to reach their full potential.

Clinical trials involving RIT have demonstrated partial, short-lived responses in some patients with advanced, solid tumors, but most of the significant advances have occurred in the treatment of hematologic neoplasms. The nature of patient population and strict developmental requirements for radiolabeled Abs are complicating factors contributing to a lackluster representation in the clinic. The 10-year absence of any newly approved imaging agents (none since 1996!) is also a sluggish trend that challenges molecular imaging researchers.^{104, 105}

IV. The target antigen and associated tumor(s)

Choice of Target Receptor/Antigen

A variety of promising targets are currently being evaluated in clinical trials at various stages (Table 3). Optimization of an Ab-based pharmaceutical requires several phases of development including choice of target antigen, binding-site selection, choice of effector functions, molecular design, delivery strategy, and administration route. Tumor-associated antigens and receptors present on the tumor cell surface include CD20 (where CD denotes cluster of differentiation), CD22, prostate-specific membrane antigen (PSMA), mucin 1 (MUC1), carcinoembryonic antigen (CEA), pancarcinoma antigen (TAG-72), sialyl Lewis antigen, HER2/*neu* receptor, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor, and epidermal growth factor receptor (EGFR). In contrast, vascular endothelial growth factor (VEGF) and integrins (e.g., $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, and $\alpha_v\beta_5$) are more abundant on vascular

endothelial cells within newly sprouting blood vessels that nourish the nearby tumor during angiogenesis.

The ideal antigen is readily accessible, highly over-expressed and expressed only within the desired target tissue. One of the limitations of the obsolete ^{111}In -capromab pentetide scan was pooling of the Ab and decreased sensitivity as a result of the epitope's cytoplasmic location. In the context of RIT, internalization of the receptor after binding carries the radionuclide intracellularly and may lead to infliction of greater damage to nuclear DNA. However, this same action may compromise repeat dosing due to disappearance of the target. Low receptor numbers can yield poor image intensity and limit therapeutic efficacy.

Some targets are less optimal because of shedding or secretion from the cell surface, circulation in the blood, and residence in the interstitial compartment. Freely circulating antigen binds administered Ab, diverting it from cancer cells. Non-Hodgkin's lymphoma (NHL) idiotype is an example of a shed antigen found within hematologic neoplasms. Many antigens studied extensively as targets for RIT in solid tumors (e.g, CEA and TAG-72) are also secreted, and Abs with these targets will bind circulating target antigen, compromising Ab binding to the tumor. In contrast, CD20 is an excellent target for immunotherapy because it is neither shed from the cell surface nor internalized, and it is expressed by nearly all B-cell tumors.

Antigens found in multiple cancer types

Some target antigens are disease specific, while others are more broadly expressed. Antigens ubiquitous across several tumor types are attractive targets from a commercial viewpoint due to a more populated customer base. Several currently approved immunotherapeutic Abs are targeted towards fairly ubiquitous antigens, many of which are discussed herein, including CEA, TAG-72, HER2/*neu*, EGFR and vascular endothelial growth factor (VEGF). TAG-72 is expressed on human carcinomas including colorectal, gastric, pancreatic, ovarian, endometrial, breast, non-small cell lung, and prostate. CEA is expressed on colorectal, pancreatic, gastric, non-small cell lung, and breast carcinoma. Because RIT requires differential targeting to minimize hematopoietic toxicity, antigenic distribution would ideally be limited to cancer cells.

A particularly noteworthy example of an Ab directed against a pancarcinoma antigen is nimotuzumab (TheraCIM, YM BioSciences), a humanized mAb directed against a widely expressed transmembrane receptor tyrosine kinase — namely, the EGF receptor (i.e. EGFR, HER-1, or ErbB-1).¹⁰⁶ A ^{188}Re -labeled version of the Ab (RadioTheraCIM) showed promising results in a Phase I trial for the radiation treatment of gliomas *via* an in-dwelling catheter into the post-operative cavity following resection.¹⁰⁷ Radioimmunoscinigraphy of nodal metastatic disease using $^{99\text{m}}\text{Tc}$ -nimotuzumab (DiaCIM) was also performed in a Phase I trial, but little correlation between EGFR expression and positive tumor imaging was observed.¹⁰⁸ In addition, unconjugated nimotuzumab (TheraCIM) demonstrated very encouraging results in pediatric pontine (brainstem) and adult gliomas, is undergoing clinical trials in non-small cell lung cancer, and is currently undergoing a Phase II monotherapy trial in Europe in patients with advanced metastatic pancreatic cancer.¹⁰⁶ Other potential indications under investigation include nasopharyngeal cancer, head and neck cancer, esophageal cancer, breast cancer, prostate cancer, uterine cervical cancer, and colorectal cancer.¹⁰⁶

Solid Tumors vs. Hematologic Burden

Contrary to clinical responses obtained for hematologic malignancies, solid carcinomas demonstrate an inconsistent response record.¹⁰⁹ Investigators are now exploring various enhancement mechanisms designed to increase clinical responses to the levels achieved for B-

cell lymphomas. Strategies include increased target tissue localization, decreased normal tissue exposure, and development of more cytotoxic radionuclides and ligand constructs.

Inhomogeneous targeting may be more significant for solid tumors, which are often poorly vascularized¹¹⁰ and have high interstitial pressure due to poor lymphatic drainage, all of which serve to impede entry of macromolecules. Diffusion from blood vessels and tumor penetration are the initial barriers encountered by systemically delivered Abs, and increased interstitial pressure in solid tumors correlates to penetration of a scFv being 6-fold greater than for an IgG.

Higher radioresistance of solid tumors has been proposed as an obstacle to successful RIT, since actual targeting has been shown to be as good, if not better, than that observed in lymphoma. Based on external radiation, solid tumor radiation absorbed dose has been far less than that considered necessary for solid tumor control. Clearly, radioresponse characteristics of indolent B-cell NHL heavily impacted clinical results. Also noteworthy is that the current lymphoma RIT success is as much a feature of the tumor-killing properties of the Ab itself as the radiation-absorbed dose, with no clear relationship between absorbed dose and response.

Following this pathway, other radiolabeled Abs specific to hematopoietic surface antigens are in clinical trials, and one or two additional RIT agents may receive FDA approval in the near term for the treatment of lymphoid malignancies. However, the larger question remains whether RIT will ever become a significant factor in the treatment of the more commonly occurring solid tumors. For several well-vascularized target sites (e.g., breast, ovarian, and brain tumors), intravenous Ab infusion has been the preferred mode of administration. For other regional tumor sites in more enclosed spaces, such as the peritoneum or the CNS, intracompartmental treatments including intraperitoneal (IP), intrathecal (IT), and even intracranial (IC) modes of administration have been employed to increase the accessibility and amount of Ab targeted to tumors. Realistically, adjuvant treatment of compartmental or microscopic residual disease is probably the only attainable indication for solid tumors in the near term.

Clinically significant anti-tumor responses remain quite rare. Efforts to increase administered radiation doses through bone marrow or peripheral stem cell support have not significantly improved solid tumor responses. Combinations of high-dose RIT with chemotherapy have also fallen short of desirable effects. Again, RIT as a stand-alone therapy seems more likely to succeed in a minimal disease burden arena or when used as an adjuvant treatment.

Lymphoma

The development of RIT for lymphoma is more advanced than for any other tumor type. Abs that target specific antigens on B cells include anti-B1 (anti-CD20), LYM-1 (anti- HLA-DR10 β), LL2 (anti-CD22), and ibritumomab tiuxetan (anti-CD20).^{11, 111}

The only two RIT agents approved for commercial use, Bexxar® and Zevalin® both target CD20 and are indicated for treatment of indolent B cell lymphoma and related conditions.¹¹ Both are administered intravenously in a multi-step process over a 1–2 week period. Excellent clinical results are obtained (20–40% complete response rates and 60–80% overall response rates), and toxicity is typically quite mild. Patients with bone marrow involvement by lymphoma (>25%), however, are generally excluded to avoid potential damage to hematopoietic stem cells.

Zevalin® (ibritumomab tiuxetan) was approved in 2002.⁵³ The Zevalin® kit contains a murine mAb and materials for the preparation of both ¹¹¹In-Zevalin® (for imaging) and ⁹⁰Y-Zevalin® (for therapy). Zevalin® is co-administered with the therapeutic Ab, Rituxan®. The private

insurance industry's bellwether, Medicare, reimbursed hospitals for Zevalin® treatment after October 2002. Nevertheless, sales have been limited and in 2004 were only \$18.7 million as compared to \$19.6 million in 2003.

Bexxar®, approved in 2003,¹¹² is an ¹³¹I labeled Ab also with limited sales. Bexxar®, originally developed by Corixa, transferred all rights to its partner GlaxoSmithKline in December 2004. Corixa indicated belief in Bexxar®, but stated that commercial acceptance was “too slow” to continue its funding. These problems illustrate that, even with the blessing of FDA approval, there remain obstacles to real financial success.

LL2 has been evaluated for treating NHL. Immunomedics originally tested several candidate murine mAbs labeled with ¹³¹I, and ImmuRAIT-LL2 advanced through Phase I/II trials. However, the murine Ab prevented repeat administration, so ImmuRAIT-LL2 was halted in favor of a humanized Ab to obviate immunogenicity. Epratuzumab (hLL2) has been evaluated in Phase I/II studies labeled with ⁹⁰Y (complexed by DOTA) or ¹³¹I with considerable success.¹¹³ The ⁹⁰Y-labeled hLL2 (LymphoCide-Y-90™) was still recruiting in Phase II trials in France and Germany according to ClinicalTrials.gov at the date of submission.¹¹⁴

Immunomedics has also developed ^{99m}Tc labeled (Fab') bectumomab (^{99m}Tc-labeled IMMU-LL2, Lymphoscan®), which has shown promise as a pre-RIT imaging probe for B-cell NHL.^{115, 116} While it excelled at defining small volume, low-grade disease, performance was variable as a purely diagnostic agent. FDG-PET has also been accepted for imaging NHL and has been directly compared to Lymphoscan®.¹¹⁵ Fifteen of the 21 (71%) sites detected by FDG-PET were also positive using bectumomab. In one patient, additional sites were observed by bectumomab that were not delineated by FDG-PET or by CT.¹¹⁵

Lym-1, which targets the HLA-DR10β subunit expressed on most malignant B cells, has been extensively studied by DeNardo and co-workers and has shown efficacy in treating NHL when labeled with ¹³¹I or ⁶⁷Cu.¹¹⁷ Following treatment with ¹³¹I-Oncolym®, the overall response rate in 130 indolent and aggressive NHL patients was 26% (five complete remissions and 13 partial remissions). Commercial development of Oncolym® by Peregrine Pharmaceuticals, Inc. was suspended in 2002. The company stated that this was due to the failure to successfully develop the product coupled with the emergence of superior or equivalent products, such as Bexxar®, Zevalin®, and Rituxan®.¹¹⁸

In May 2002, HGS launched Phase I clinical trials on LymphoRad-131, a radio-iodine-labeled (by MDS Nordion) version of B-lymphocyte stimulator (BLyS) intended for treatment of multiple myeloma and NHL. LymphoRad-131 is potentially applicable in the treatment of a broad range of B-cell tumors, including multiple myeloma, large B-cell lymphomas, follicular B-cell lymphomas, chronic lymphocytic leukemia, and Burkitt's lymphoma. The distribution of LymphoRad-131 receptors on multiple myelomas broadens the potential patient population, since CD20 receptors are absent in this condition. In addition, pre-B cells needed to replenish the normal B-cell population lack the LymphoRad-131 receptor, but carry CD20, suggesting that pre-B cells might escape killing by LymphoRad-131. HGS discontinued clinical development of LymphoRad-131 in 2005, despite only mild to moderate reversible toxicity and tumor deposited doses up to 3600 cGy being observed in the Phase I trial.¹¹⁹ The anticipated competition from Zevalin® and Bexxar® were likely heavily weighed in this decision.

Colorectal Cancer

Some of the most advanced efforts relate to both imaging and RIT of gastrointestinal disease.^{120–122} Heavily pursued antigens for colorectal cancer include Ep-CAM, A33, TAG-72, and CEA.

The NR-LU-10 Ab targets the Ep-CAM receptor. Knox and colleagues reported results of a Phase II clinical trial of ^{90}Y -DOTA-biotin pre-targeted by NR-LU-10 Ab/streptavidin in patients with metastatic colon cancer; however, the results were overshadowed by significant bowel toxicity resulting from antigen expression by normal bowel (see section III).⁷⁷

Iodine-131 has been targeted to the A33 antigen, a transmembrane glycoprotein of the IgG superfamily. In an open-label, dose escalation, biopsy-based Phase I trial, colorectal patients were treated with a combination of ^{131}I -huA33 and ^{125}I -huA33 one week before surgery.¹²³ No dose-limiting toxicity was observed, and excellent tumor uptake was demonstrated.¹²³ Higher doses were administered in a corresponding Phase I dose escalation trial of ^{131}I -huA33 RIT wherein the maximum tolerated dose was determined to be 40 mCi/m² with excellent targeting resulting in four of the 15 patients having stable disease.¹²⁴

CC49 is a murine Ab that binds to TAG-72. Iodine-131-CC49 and the chimeric version of the Ab have been used to treat patients with colorectal cancer, but failed to produce significant clinical responses.¹²⁵ Dehalogenation was an issue such that radiolabeling with ^{90}Y was evaluated in a Phase I clinical trial, but both tissue biopsies and imaging using the surrogate ^{111}In confirmed high levels of ^{90}Y deposition in the liver.¹²⁶ In addition, estimates of absorbed hepatic dose equaled or exceeded that which could be achieved in metastatic tumor sites and raised doubts about the potential of ^{90}Y -CC49.¹²⁶

Goldenberg and colleagues originally used ^{131}I -labeled polyclonal anti-CEA Abs to identify and treat small groups of patients with recurrent CEA-positive adenocarcinomas.¹²⁷ Goldenberg founded Immunomedics in 1982 with a focus on anti-cancer Ab products. CEA-Scan® is a murine mAb fragment linked to $^{99\text{m}}\text{Tc}$ indicated with other standard diagnostic modalities for the detection of recurrent and/or metastatic colorectal cancer. In conjunction with CT, the agent provided information about the presence, location, and extent of disease. CEA-Scan® was approved June 26, 1996, however, Immunomedics ceased commercialization during the 2006 fiscal year, consistent with the company's de-emphasis on their diagnostic business.¹²⁸

Immunomedics completed a series of Phase I/II clinical trials of ^{90}Y -labeled-hMN14 (labetuzumab, CEA-cide®), a humanized radiolabeled Ab targeting CEA, in patients with colorectal (2000–2004)¹²⁹ and pancreatic (2000–2003)¹³⁰ cancers. More recently, the company's focus has shifted towards a radiohalogenated version of the same Ab, ^{131}I -labetuzumab, obtaining impressive results in a German Phase II trial in 19 colorectal cancer patients after salvage resection of liver metastases.¹³¹ The majority of patients received only a single infusion of 50–60 mCi/m², and a better than predicted outcome was observed: the survival rate was 51% at 5 years, as compared with the expected rate of 28% derived from an analysis of 1,596 patients.¹³¹ However, a similar clinical trial in the U.S. using ^{90}Y -labetuzumab was terminated for unspecified reasons, although one might speculate that the choice of ^{90}Y for treating limited residual disease after surgery may have been a factor.¹³²

Another anti-CEA Ab, T84.66, has undergone a Phase I RIT trial in which the ^{90}Y -labeled chimeric Ab was combined with the radiation-enhancing chemotherapy agent, 5-fluorouracil.¹³³ No objective responses were observed; however, more than half of patients shifted from progressive to stable disease.¹³³

Breast Cancer

Breast cancer imaging and therapy is being aggressively studied in a variety of pre-clinical and clinical models. Targets include MUC1, CEA, and L6. Although some clinical responses have been observed, the level of response is again not nearly as compelling as RIT for hematopoietic malignancies. Studies with $^{99\text{m}}\text{Tc}$ -labeled anti-CEA monoclonal antibodies and with ^{111}In -

labeled or radioiodinated anti-mucin antibodies (e.g., HFMG, B72.3, and anti-TF) have demonstrated the ability of radioimmunoscintigraphy in detecting over 80% of breast cancer lesions, but showed lower sensitivity and specificity for accurate staging of the axillae. Non-specific localization of radiolabeled mAbs in tumor-negative nodes, even following lymphoscintigraphy, appears to be the major factor limiting widespread clinical application of radioimmunoscintigraphy in staging newly diagnosed breast cancer patients.

Monoclonal and, more recently, humanized anti-MUC1 Abs, such as BrE-3, have been developed. A Phase I trial was performed to explore the use of ^{90}Y -BrE-3 murine Ab.¹³⁴ Although responses were observed, an immune response prevented further use of this Ab.¹³⁴ A humanized version has been evaluated in a clinical trial, and 8 of 17 patients (47%) showed responses despite failing previous conventional therapies.¹³⁵ Anti-MUC1 Ab, m170, radiolabeled with ^{90}Y and combined with paclitaxel has progressed to dosimetric studies with measurable tumor regression and partial responses.¹³⁶ A Phase I trial using ^{90}Y -BrE-3 combined with stem cell support gave objective partial responses.¹³⁷

First believed to be a specific colon cancer marker, CEA was later shown to be expressed in normal tissues and in cancers from other sites, including breast carcinomas.¹³⁸ NP-4, a murine anti-CEA Ab, labeled with ^{131}I resulted in therapeutic responses in a Phase I/II study. When 57 patients were treated with ^{131}I -NP-4, modest anti-tumor activity was seen in 12 of 35 assessable patients with 1 partial remission, 4 minor/mixed responses and 7 instances of stabilization of progressing disease.¹³⁹ Preliminary studies with $^{99\text{m}}\text{Tc}$ -labeled CEA-Scan® appear to indicate a useful role for this agent in distinguishing between benign and malignant breast lesions in patients with indeterminate mammographic findings.¹⁴⁰

The L6 cell surface antigen is highly expressed in breast cancer and is related to a number of cell surface proteins with similar predicted membrane topology implicated in cell growth. Yttrium-90-DOTA-peptide-ChL6 resulted in excellent tumor targeting and an effective therapeutic index pre-clinically.¹⁴¹ Combined modality RIT was also performed using the same radioimmunoconjugate combined with an $\alpha_v\beta_3$ -integrin inhibitor¹⁴², with paclitaxel¹⁴³, with an anti-EGFR mAb⁵⁸, or with a bcl-2 antisense oligonucleotide. Both positive and negative influences on cure rate and toxicity were observed with these various combinations.¹⁴⁴

Prostate Cancer

Prostate-specific membrane antigen (PSMA) is perhaps the most highly studied and well-established target for prostate cancer. The most well-known radiolabeled Ab to PSMA is commercially available ProstaScint® (^{111}In -capromab pendetide, Cytogen Corp, Princeton, NJ), which was FDA approved ten years ago for imaging soft-tissue, but not bone sites, of metastatic prostate cancer for presurgical staging or evaluation of PSMA relapse after local therapy.^{145, 146} Surgical resection of the prostate is not indicated for patients whose disease has spread outside the prostatic bed. For pre-surgical patients with high-risk disease but negative bone, CT, and MRI scans, capromab was able to identify some patients with positive nodes, thereby sparing them unnecessary surgery. No follow-up studies are available that indicate that high-risk patients with a negative capromab scan have a lower failure rate after surgery.

Capromab is severely compromised by its inability to image bone metastases because it is directed toward an intracellular epitope (*N*-terminus) of the PSMA molecule that is inaccessible to circulating Ab. This is quite an unfortunate scenario, as bone is the first site of metastatic prostate cancer in 72% of patients. Other prostate cancer targets have also been pursued with variable success. No major responses were observed in therapeutic studies targeting TAG-72 in prostate cancer patients.¹⁴⁷ More advanced Abs such as J591 target the *extracellular* domain

of PSMA and therefore may provide significant benefits in the imaging of prostate cancer. Promising results have been obtained using ^{90}Y to treat hormone-refractory metastatic prostate cancer.¹⁴⁸ Patient recruitment is ongoing for a Phase II trial to study the efficacy of ^{177}Lu -DOTA-J591 in the treatment of metastatic prostate cancer.¹⁴⁸ The additive effects of another radioimmunoconjugate, ^{90}Y -DOTA-peptide-ChL6, combined with taxanes yielded a 67% cure rate, whereas no mice were cured with RIT alone or chemotherapy alone.¹⁴⁹

Ovarian Cancer

Success for ovarian cancer has been elusive.¹⁵⁰ There are no radiolabeled Abs in late stage clinical development for ovarian cancer, although a number are currently in Phase I/II clinical trials including ^{90}Y -HU3S193 at Memorial Sloan-Kettering and ^{90}Y -CC49 at University of Alabama.

Primary and metastatic ovarian tumors have been detected by radioimmunoscintigraphy using two radio-iodinated anti-MUC-1 mAbs, human milk fat globule antigen 1 and 2 (HMFG1 and HMFG2). Y-90-labeled HMFG-1 murine mAb (pemtumomab) has been used to treat patients with advanced ovarian cancer following conventional therapy.¹⁵¹ Encouraging results were obtained in patients with minimal residual disease with 50% complete remission several years post-treatment.¹⁵¹ Following surgery, chemotherapy, and intraperitoneal RIT, 78% of the 21 patients in complete remission survived for longer than 10 years.¹⁵¹ Unfortunately, ^{90}Y -HMFG-1 then failed to demonstrate a therapeutic effect in a multi-institution international randomized concurrently controlled Phase III clinical trial.¹⁵²

Clinical evaluation of intravenously administered ^{131}I -labeled chimeric mAb MOv18 directed against the folate receptor¹⁵³ showed that therapeutic doses could be achieved without normal organ toxicity.¹⁵⁴ Immuno-specific localization of ^{90}Y -labeled c-MOV18 on FR-expressing tumors has been demonstrated, suggesting that further studies are warranted.¹⁵⁵ The same Ab labeled with the α -emitting halogen, ^{211}At , has also been investigated.⁴⁴

Lung Cancer

Approved in 1996 but presently clinically obsolete, Verluma® is a $^{99\text{m}}\text{Tc}$ labeled Fab fragment for identifying advanced stage disease in patients with small cell lung cancer (SCLC).¹⁵⁶,¹⁵⁷ In a clinical trial involving 89 confirmed SCLC patients, Verluma® accurately determined whether the disease was limited or extensive 82% of the time. However, if the test indicated limited disease, it failed to image tumors in some body organs in ~23% of patients. Additional standard diagnostic tests, such as bone or CT scan, or a bone marrow biopsy are needed to compensate for these false negative readings.

In 2005, a Phase I study of ^{90}Y -CC49 in advanced non-SCLC patients yielded very disappointing results, warranting the development of a humanized version of CC49.¹⁵⁸ There were no objective tumor responses, and both immunogenicity and hematologic toxicities were problematic.¹⁵⁸

Brain Cancer

Glioma, the most common and lethal form of primary brain tumor, has been treated with cytoreductive surgery, external-beam irradiation, and systemic chemotherapy. Results have been disappointing owing to tumor invasion into functional brain, chemoresistance, and the blood-brain barrier for systemically delivered therapy. Because brain tumors in adults rarely metastasize outside the cranium, locoregional RIT has been evaluated to treat glioblastoma multiforme (GBM), a malignancy with an extremely poor prognosis that generally kills through local invasion and regional metastasis.¹⁵⁹

One new delivery strategy and target is tumor necrosis therapy (TNT) *via* convection-enhanced delivery (CED) of Cotara® (¹³¹I-chTNT, Peregrine Pharmaceuticals, Inc., Tustin, CA), an ¹³¹I-labeled chimeric mAb that recognizes a universal, intracellular antigen exposed in the necrotic core of malignant solid tumors for the treatment of malignant glioma. Chimeric chTNT was shown to target tumors by binding to DNA exposed in necrotic zones. The clinical experience to date with ¹³¹IchTNT was recently reviewed by Shapiro et al.¹⁶⁰

Tenascin-C (TN-C) is an extracellular matrix (ECM) glycoprotein expressed ubiquitously in high-grade gliomas, but not in normal brain.¹⁶¹ The murine IgG_{2b} Ab, 81C6, binds to an epitope within the alternatively spliced fibronectin type III region of TN-C. Intratumoral administration of ¹³¹I-81C6 has shown promise in a phase I trial.¹⁶² In a more recent phase II study at Duke, the efficacy and toxicity of this ¹³¹I-81C6 infused directly into the resection cavity (intracavitary injection) was assessed in 33 patients with previously untreated malignant glioma.¹⁶³ Dosimetry and SPECT imaging were also performed.¹⁶⁴ Median survival for all glioma patients was 86.7 weeks, and for GBM patients was 79.4 weeks, exceeding historic controls obtained by treating with conventional radiotherapy and chemotherapy.¹⁶⁵ Reversible hematologic toxicity was observed in 27% of patients, and symptomatic neurologic toxicity occurred in 15%, but only a single patient required re-operation for radionecrosis.¹⁶³

A three-step pre-targeting approach was evaluated in an Italian trial treating 24 patients with recurrent high-grade glioma after a second surgical debulking and implantation of a catheter into the resection cavity.¹⁶⁵ Biotinylated anti-TN-C Ab was infused initially, and avidin was administered after 24 hours, followed 18 hours thereafter by ⁹⁰Y-biotin.¹⁶⁵ In a subsequent Phase II study, 37 patients with high-grade glioma, including 20 patients with GBM, were treated similarly.¹⁶⁶ Among 12 historical controls with GBM, median survival was 8 months as compared to 33.5 months in the pretargeted RIT group.¹⁶⁶

Renal Cancer

Advanced renal cell carcinoma (RCC) resists traditional therapies and is the subject of intense investigation. Metastatic renal cell carcinoma in 33 patients was treated with an ¹³¹I-labeled mouse mAb, ¹³¹I-G250.¹⁶⁷ Antibody immunogenicity (HAMA) within four weeks post-therapy in all patients restricted therapy to a single infusion.¹⁶⁷ In external imaging, ¹³¹I-labeled mouse mAb G250 showed excellent localization to all tumors that were ≥ 2 cm.¹⁶⁷ Tumor reduction was observed in only 2 patients, while 17 patients had stable disease.¹⁶⁷ A follow-up Phase I dose escalation trial showed that fractionation did not significantly improve dose limiting hematopoietic toxicity.¹⁶⁸

V. Challenges and Prospects

Failure to Launch

The impressive targeting of a broad range of tumors by diverse Abs combined with the relatively good radiosensitivity of certain cancer cells *should* have encouraged considerable advances in Ab imaging and RIT development. However, despite the significant advances in immunology, including relatively nonimmunogenic chimeric and humanized Abs, and stable radiolabeling with a variety of radionuclides — halides, metals; α-emitters, β-emitters, Auger electron emitters — progress beyond early Phase II RIT studies in solid tumors has been marginal.^{109, 169} Furthermore, ¹⁸F-FDG continues to dominate the molecular imaging domain. Several key challenges must be confronted to increase the presence and success rate of these increasingly important drugs.

Most of the clinical lymphoma histologies that have proven responsive to RIT are also responsive to other new investigational agents, making it difficult for RIT to become established and integrated into standard clinical practice. The competition for inclusion as a

standard of care is brisk. As illustrated earlier, financial disincentives are also present that may discourage patient referral by physicians who lack appropriate licensing to inject RIT agents.
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Current Challenges

Researchers in radioimmunodiagnosis and therapy must clearly identify real clinical needs, discover promising new target antigens, and create therapeutic Abs with superior clinical efficacy and safety. The synergy between molecular imaging and targeted radiotherapy will be strengthened by new generations of therapeutic radiopharmaceuticals coupled with advanced imaging systems in the creation personalized dose regimens. Treatment regimens are often lengthy and result in significant side effects, so an early assessment of treatment effectiveness is critical in patient care management.

Detection of simple anatomical changes in tumor size and shape is already feasible. Functional methods of anatomic imaging can assess tumor response before there are structural changes within the tumor. MRI can detect changes in tumor metabolism early during the course of radiation therapy to assess response or radio-resistance. FDG-PET is extremely sensitive to small changes in tumor metabolism; specifically, upregulation of hexokinase in response to the tumor's need for additional energy. This application of PET will no doubt see tremendous growth during the next decade. In 2006, advanced imaging techniques with CT, MR, PET, and SPECT represented 63% of imaging procedures for cancer patients.¹⁷⁰ Due to the expanding utility of these applications, the procedure volume for these modalities is predicted to increase 189% by 2016.¹⁷⁰

Radionuclide therapy requires injection of higher activities to reach efficient absorbed doses in radio-resistant solid tumors, while limiting exposure of vital organs (e.g., liver, lung, and kidney). Pre-therapeutic dosimetry studies using PET allow accurate quantification of absorbed radiation doses. Multimodal approaches such as PET/CT also show promise to routinely predict and thereafter measure early responses and non-responses. By screening patients for various tumor receptors and antigens, higher therapeutic efficiency is attainable through individualized (i.e. personalized) medicine.

Choosing Better Targets

Perhaps the most prevalent underlying cause of preclinical and clinical failures in both imaging and RIT is a poor choice of target receptor. Several instances of poorly chosen targets have been noted herein, including NeoRX's streptavidin-anti-Ep-CAM Ab that was plagued by normal bowel receptor expression^{73–77}, the obsolete ¹¹¹In-capromab pentetide scan whose cytoplasmic epitope in bone metastases is Ab-inaccessible^{145, 146}, and shed/secreted antigens such as NHL idiotype, CEA, and TAG-72. Significant amounts of time, effort, and financial resources have already been futilely exhausted on the pursuit of several targets that were predestined to fail in theory from their inception. Efforts need to be shifted away from such unsuitable targets and redirected toward the development of novel targets having more favorable properties for Ab imaging and RIT.

Although the prospect of developing a new generation of targets might initially seem to be a practically impossible endeavor, much of the groundwork may already be in place. Several potentially successful Ab-based imaging agents are often discarded — like fish back into the sea — simply because they have failed as non-radioactive therapeutic agents. It is a not uncommon for an Ab under pre-clinical evaluation to successfully target a given receptor but still fail to produce a biological therapeutic response. Although ineffective by themselves, such Abs, when coupled with a radionuclide, still hold great potential because specific and exclusive

receptor targeting is the only real prerequisite for both molecular imaging and RIT; the Ab is merely a vehicle, while the radionuclide carries the major workload.

Radioimmunotracers vs. FDG-PET

Despite the initial promise of several radioimmunoimaging agents discussed herein, ^{18}F FDG is often preferentially used in the clinic owing to convenience, availability, improved accuracy, and lack of immunogenicity. Strengthening this trend is the fact that FDG is approved for reimbursement for treatment-monitoring applications by the Centers for Medicare and Medicaid Services (CMS) under the National Oncologic PET Registry program. Although FDG-PET has progressively asserted itself as the gold standard of imaging for early tumor detection and assessment of treatment response, it is important to consider its mechanism of action. FDG targets upregulation of hexokinase in rapidly growing tumors; as a result, it may fail to target slower growing tumors, and it may also non-specifically accumulate in any lesion of increased metabolism, including inflammatory processes, resulting in a limited diagnostic specificity in some cases.

To illustrate a scenario in which FDG-PET may not be the optimal imaging agent, consider the case of renal cell carcinoma (RCC). FDG has a fairly low sensitivity as a prognostic and diagnostic agent for RCC. At least 25% of metastatic lesions fail to take up FDG.¹⁷¹ In contrast to FDG, tumor targeting by radiolabeled Abs is not governed by a tumor's metabolic status. These considerations warrant the development of immunoPET agents for conditions such as kidney cancer wherein FDG fails to provide useful diagnostic information.

In contrast to the case of RCC, FDG imaging is superior to current Ab-based imaging agents in the clinical diagnosis of colon carcinoma (CC). In CC patients with a rising CEA level but having negative results on conventional imaging studies, an FDG scan is used to determine the likelihood of value from a second-look laparotomy. Clinical investigators comparatively evaluated FDG versus anti-CEA Ab immunoscintigraphy to localize disease before patients underwent second-look laparotomy.¹⁷² FDG detected disease in 23 of the patients, while the CEA scan detected disease in only 4.¹⁷² FDG successfully predicted unresectable disease in 90% of patients confirmed at laparotomy, but CEA scan failed to predict unresectable disease in any patient.¹⁷² Of 16 patients found to have resectable disease at laparotomy, FDG-PET scan predicted it in 81%, but CEA scan predicted it in only 13%.¹⁷² These results lead many clinicians to choose FDG-PET over CEA scans as standard practice. It is critical to realize, however, that although these findings raise doubts regarding the efficacy of CEA as a target antigen, one may *not* infer that FDG is the preferred diagnostic agent in all situations.

Combination Therapies

Despite common perceptions, RIT should *not* be considered exclusively as a stand-alone therapy. The clinical advantages and increases in efficacy obtained using combination therapies are becoming more evident. Both unconjugated antibodies and radioimmunoconjugates have shown greater effectiveness when used in combination with a broad range of other therapies including chemotherapeutics (e.g. paclitaxel & docetaxel (taxanes), capecitabine), integrin inhibitors, antisense oligonucleotides, and other antibodies. The rationale behind these improvements can encompass simple additive as well as synergist effects, but the notion that tumor receptor expression can significantly vary from patient to patient is sufficient cause to pursue a multi-pronged attack-strategy against tumor burden.

Despite the several instances of favorable results with combination therapies (several have been noted herein^{58, 123, 137, 142–144, 149}), certain limitations severely discourage a smooth translation into the clinic. As mentioned earlier, the combination of RIT with an $\alpha_v\beta_3$ -integrin inhibitor¹⁴², paclitaxel¹⁴³, an anti-EGFR mAb⁵⁸, or a bcl-2 antisense oligonucleotide¹⁴⁴ had

a significantly variable impact on cure rate and toxicity. These results emphasize the clinical potential of combined modality RIT, but also highlight the intrinsic complexity of such multi-component systems. This leads to complicated questions that must be systematically answered to satisfy FDA requirements.

Several criteria must be carefully considered, including the likelihood that different patients will respond to different combination therapies due to individual differences in receptor expression, a situation that may be probed by imaging. In addition, the added cost of multiple drug combinations (i.e. 'cocktails') can push them financially out of reach by challenging the healthcare system and third-party payers.¹⁷³ Weighing the benefits of personalized medicine against practical and financial realities is a difficult challenge that looms in the future of RIT.

Regulatory Barriers

A critical factor to consider is the overall cost and time required to obtain approval for new radiopharmaceuticals. The exploratory investigational new drug (xIND) in the U.S. is intended to decrease the barriers to execute preliminary toxicology-pathology studies. Despite the xIND pathway, study completion times and pathological tissue examination costs continue to rise, and safety concerns contribute to this effect. Nevertheless, the increased use of radiotracers can provide a better understanding of the pharmacologic effect of the parent compound, leading to faster approval processes for first in man studies and, eventually, full clinical approval.

Economic Barriers: Supply and Demand

In the United States, kidney cancer accounts for about 3% of all cancers, with approximately 12,000 deaths each year. With early diagnosis, the survival rate for RCC patients ranges from 79 to 100 percent. As highlighted by Choyke¹⁷⁴, however, it is unreasonable to expect the development of new molecular imaging methods that will be able to identify histologic subtypes of RCC in the near term. With only 35,000 new cases of RCC per year in the US and 700,000 worldwide, the commercialization economics of such agents is unfavorable; insufficient numbers of cases exist to justify the required monetary investment associated with the development of a stand-alone diagnostic agent.

For comparison, over 20 million Americans alone take the cholesterol-lowering agent Lipitor® (atorvastatin; Pfizer, New York, NY) every day, and annual worldwide sales are over \$12 billion. Comparison to such blockbuster drugs places profits derived from stand-alone diagnostic imaging agents at a severe competitive disadvantage. Molecular imaging agents that identify targets amenable to specific molecular therapies hold more promise. Like their matched treatments, these agents span more than one type of cancer and therefore offer a larger, more viable market for drug developers. Examples include the angiogenic inhibitor and mAb against VEGF, Avastin® (bevacizumab), the mAb against HER2/*neu* receptors, Herceptin® (trastuzumab; Genentech) and the mAb against HER1 receptors, cetuximab (Erbix®; ImClone Systems, New York, NY).

Concluding Remarks

The multidisciplinary fields of Ab-based molecular imaging and RIT have yet to reach their full potentials in both pre-clinical and clinical domains. In contrast to the body of impressive pre-clinical data derived from murine models, the limited clinical response rate in RIT has been quite disappointing. This trend extends across several tumor types, antigens, delivery platforms, and radiochemical formats. The only exceptions, hematopoietic neoplasms, are uniquely susceptible to RIT due to high radiosensitivity combined with the immunobiological response to Ab binding. Furthermore, radiolabeled antibodies possess an overwhelming

potential in molecular target-based imaging for clinical diagnosis and therapy monitoring, yet FDG continues to be the agent of choice.

Possible solutions to the current problems at hand involve selection of *better and new target antigens, humanized or chimeric Abs or fragments, and wiser selection of radionuclides and delivery mechanisms*. Molecular imaging will play a pivotal role in shaping the future of targeted RIT, as will *advances in imaging equipment and data reconstruction*. The creation of *personalized treatments and dose regimens* will allow therapies to be specifically tailored to match the unique receptor expression of a given patient's tumor burden. In the context of RIT, *post-surgical adjuvant treatment* of residual disease appears to be the most reasonable short-term clinical goal wherein significant success might be realized. In the future, however, additional technical advances may also help penetrate the more difficult barriers impeding *treatment and control of gross disease* deposits associated with solid tumors. Finally, the integration of RIT from a stand-alone therapy into a component of standard combination therapies taking advantage of synergistic activities should also be pursued.

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Glossary

Abbreviations

Ab	antibody
FDA	U.S. Food and Drug Administration
RIT	radioimmunotherapy
FDG	fluorodeoxyglucose
PET	positron emission tomography
$t_{1/2}$	half-life
CEA	carcinoembryonic antigen
NHL	non-Hodgkin's lymphoma
CD	cluster of differentiation
mAb	monoclonal antibody
γ	gamma ray
SPECT	single-photon emission computerized tomography
MRI	magnetic resonance imaging
CT	computed tomography
eV	electron volts
β or β^-	beta particle (negatron)
β^+	positron
Gy	gray (absorption of 1 joule of radiation energy by 1 kilogram of matter)
LET	linear energy transfer
DNA	deoxyribonucleic acid

α	alpha particle (helium nucleus)
RBE	relative biologic effectiveness
MDS	medical device sterilization
NBC	National Broadcasting Company
DTPA	diethylenetriaminepentaacetic acid
DOTA	1,4,7,20-tetraazacyclododecane <i>N,N',N'',N'''</i> -tetraacetic acid
Ig	immunoglobulin
CHX-DTPA	2-(p-isothiocyanato-benzyl)-cyclohexyl-diethylenetriaminepentaacetic acid
TETA	1,4,8,11-tetraacetic acid
SOD	superoxide dismutase
CB-TE2A	4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane
cGMP	current Good Manufacturing Practices
MeO-DOTA	methoxy-DOTA
mTCR	monoclonal T cell receptor
EDTMP	ethylenediamine-tetra (methylene phosphonic acid)
DOTMP	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene-phosphonate
HGS	Human Genome Sciences
MX-DTPA	combined 1-p-isothiocyanatobenzyl 3-methyl- and 1-p-isothiocyanatobenzyl 4-methyldiethylenetriamine pentaacetic acid
HAMA	human antimouse antibody
CDR	

	complementarity determining region
scFv	single-chain variable fragment
Da	Dalton
Fab	antigen-binding fragment
FcRn	neonatal Fc receptor
SA	streptavidin
Ep-CAM	epithelial cellular adhesion molecule
HSA	human serum albumin
Gal-NAc	<i>N</i> -acetyl galactosamine
PEG	polyethylene glycol
HER	human epidermal growth factor receptor
VEGF	vascular endothelial growth factor
PSMA	prostate-specific membrane antigen
MUC	mucin
TAG-72	pancarcinoma antigen
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
EGFR	epidermal growth factor receptor
ErbB	epidermal growth factor receptor
IP	intraperitoneal
IT	intrathecal

IC	intracranial
BLyS	B-lymphocyte stimulator
Lym	lymphoma
CC	colorectal carcinoma
HMFG	human milk fat globule antigen
FR	folate receptor
SCLC	small cell lung cancer
GBM	glioblastoma multiforme
TNT	tumor necrosis therapy
CED	convection-enhanced delivery
RCC	renal cell carcinoma
ECM	extracellular matrix
xIND	exploratory investigational new drug
CMS	Centers for Medicare and Medicaid Services

Table 1

Physical Data of useful Radionuclides for Immunodiagnosis (γ/β^+) and for RIT ($\beta^-/\beta^+/\alpha/\text{Auger}$). Information was obtained from Kocher¹⁷⁵ (1981) and Milenic et al¹ (2004).

Radio-nuclide	$t_{1/2}$ (h)	Imaging Decay	RIT Decay	R_{mean} $\bar{r}$ (mm)	E_{α} (keV)	E_{β} [avg] (keV)	E_{γ} (keV)	Production
⁹⁰ Y	64.08	----	β^-	3.78	----	934.8	----	⁹⁰ Si/ ⁹⁰ Y generator ⁸⁹ Y(n, γ) ⁹⁰ Y
¹³¹ I	192.96	γ	β^-	0.36	----	181.7	364.5	¹³¹ Te(n, γ) ¹³¹ I
¹⁷⁷ Lu	161.04	γ	β^- EC	0.22 <0.02	----	133.0	208.4 113.0	¹⁷⁷ Lu(n, γ) ¹⁷⁷ Lu
⁶⁷ Cu	61.92	γ	β^- EC	0.24 <0.02	----	141.0	184.6 93.3 91.3	⁶⁸ Zn(p,2p) ⁶⁷ Cu ⁶⁷ Zn(n,p) ⁶⁷ Cu
¹⁶⁶ Ho	26.80	γ	β^- EC	2.43 <0.02	----	665.7	80.6 1379.4	¹⁶⁶ Dy/ ¹⁶⁶ Ho generator
¹⁸⁵ Re	90.64	γ	β^- EC	0.98 <0.02	----	349.3	137.1 122.3	¹⁸⁵ Re(n, γ) ¹⁸⁶ Re
¹⁸⁸ Re	16.98	γ	β^- EC	2.91 <0.02	----	764.2	155 633.1	¹⁸⁸ W/ ¹⁸⁸ Re generator ¹⁸⁷ Re(n, γ) ¹⁸⁸ Re
¹⁵³ Sm	46.27	γ	β^-	0.50	----	223.6	103.2	¹⁵² Sm(n, γ) ¹⁵³ Sm
¹¹¹ Ag	179.28	γ	β^-	0.98	----	349.8	342.1	¹¹⁰ Pd(n, γ) ¹¹¹ Pd(β^-) $\rightarrow$ ^{111m} Ag(γ) ¹¹¹ Ag
¹⁰⁹ Pd	13.45	----	β^-	1.03	----	361.0	----	¹⁰⁸ Pd(n, γ) ¹⁰⁹ Pd
¹⁶⁵ Dy	2.33	γ	β^- EC	1.37 <0.02	----	440.2	94.7	¹⁶⁴ Dy(n, γ) ¹⁶⁵ Dy
¹⁶⁶ Dy	81.6	γ	β^- EC	0.18 <0.02	----	118.3	82.47	¹⁶⁴ Dy(n, γ) ¹⁶⁵ Dy ¹⁶⁵ Dy(n, γ) ¹⁶⁶ Dy
¹⁶⁹ Er	225.6	γ	β^-	0.13	----	99.4	8.4	¹⁶⁸ Er(n, γ) ¹⁶⁹ Er ¹⁶⁹ Tm(n,p) ¹⁶⁹ Er
¹⁷⁵ Yb	100.56	γ	β^- EC	0.20 <0.02	----	125.5	369.3 282.5	¹⁷⁴ Yb(n, γ) ¹⁷⁵ Yb
¹⁹⁸ Au	64.70	γ	β^- EC	0.83 <0.02	----	311.5	411.8 675.9	¹⁹⁷ Au(n, γ) ¹⁹⁸ Au
²¹² Pb	10.64	γ	β^- EC	0.13 <0.02	----	99.4	238.6	²²⁴ Ra/ ²¹² Pb generator
²¹² Bi	1.01	γ	α β^- EC	0.04-0.1 2.68 <0.02	6,090 6,051	717.3	727.2 39.8	²¹² Pb/ ²¹² Bi generator ²²⁸ Th/ ²¹² Bi generator
²¹³ Bi	0.76	γ	α β^-	0.04-0.1 1.32	5,870 5,549	430.0	440.4	²²⁵ Ac/ ²¹³ Bi generator

Radio-nuclide	$t_{1/2}$ (h)	Imaging Decay	RIT Decay	$R_{\text{mean}}^{\pm}$ (mm)	E_{α} (keV)	E_{β} [avg] (keV)	E_{γ} (keV)	Production
^{225}Ac	240.00	γ	α EC	0.04-0.1 <0.02	5,829 5,792 5,731	----	99.8 86.1	n-capture of $^{232}\text{Th} \rightarrow$ $^{233}\text{U} \rightarrow ^{225}\text{Ac}$ or $^{226}\text{Ra}(p,2n)^{225}\text{Ac}$
^{211}At	7.21	γ	α EC	0.04-0.1 <0.02	5,867	----	687.0	$^{209}\text{Bi}(\alpha,2n)^{211}\text{At}$
^{149}Tb	4.12	β^+, γ	α β^+ EC	0.04-0.1 3.71 <0.02	3,967	921.0	352.2 165.0 3883.	(Ta(p,spall)) $^{152}\text{Gd}(p,4n)^{149}\text{Tb}$ or $^{142}\text{Nd}(^{12}\text{C},5n)^{149}\text{Tb}$
$^{195\text{m}}\text{Pt}$	96.48	γ	IT/EC	<0.02	----	----	98.9 129.8	$^{195}\text{Pt}(n,n')^{195\text{m}}\text{Pt}$ $^{194}\text{Pt}(n,\gamma)^{195\text{m}}\text{Pt}$
^{203}Pb	52.02	γ	EC	<0.02	----	----	279.2 401.3	$^{203}\text{Tl}(p,n)^{203}\text{Pb}$ $^{203}\text{Tl}(d,2n)^{203}\text{Pb}$
^{203}Tl	73.06	γ	EC	<0.02	----	----	167.4 135.3	$^{203}\text{Tl}(p,3n)^{201}\text{Pb}^{201}\text{Tl}$
^{125}I	1443.4	γ	EC	<0.02	----	----	35.49	$^{124}\text{Xe}(n,\gamma)^{125}\text{Xe}/^{125}\text{I}$
^{67}Ga	78.26	γ	EC	<0.02	----	----	93.3 184.6 300.2	$^{68}\text{Zn}(p,2n)^{67}\text{Ga}$
^{111}In	67.31	γ	EC	<0.02	----	----	171.3 245.4	$^{111}\text{Cd}(p,n)^{111}\text{In}$
^{153}Gd	5.798	γ	EC	<0.02	----	----	97.4 103.2	$\text{Eu}(n,\gamma)^{152}\text{Eu}/^{152}\text{Gd}$ $^{152}\text{Gd}(n,\gamma)^{153}\text{Gd}$
^{123}I	13.13	γ	EC	<0.02	----	----	159.0	$^{124}\text{Te}(p,2n)^{123}\text{I}$
^{124}I	100.22	β^+, γ	β^+ EC	3.25 <0.02	----	830.5	602.7	$^{214}\text{Te}(p,n)^{124}\text{I}$
^{66}Ga	9.40	β^+, γ	β^+	8.06	----	1739.1	1039.3 2752.1	$^{66}\text{Zn}(p,n)^{66}\text{Ga}$
^{55}Co	17.53	β^+, γ	β^+ EC	5.74 <0.02	----	1306.8	1408.4 477.2 931.2	$^{54}\text{Fe}(d,n)^{55}\text{Co}$ $^{56}\text{Fe}(p,2n)^{55}\text{Co}$
^{77}Br	57.04	β^+, γ	β^+ EC	0.27 <0.02	----	151.7	238.9 520.7	$^{77}\text{Se}(p,n)^{77}\text{Br}$
^{76}Br	16.00	β^+, γ	β^+ EC	5.07 <0.02	----	1180	559.1 657.0 1853.7	$^{76}\text{Se}(p,n)^{76}\text{Br}$
^{89}Zr	78.43	β^+, γ	β^+ EC	1.18 <0.02	----	396.9	909.1	$^{89}\text{Y}(p,n)^{89}\text{Zr}$
^{64}Cu	12.70	β^+, γ	β^+ β^-	0.70 0.39	----	β^+ 278.1 β^- 190.2	1345.8	$^{64}\text{Ni}(p,2p)^{64}\text{Cu}$

Radio-nuclide	$t_{1/2}$ (h)	Imaging Decay	RIT Decay	$R_{\text{mean}}^{\pm}$ (mm)	E_{α} (keV)	E_{β} [avg] (keV)	E_{γ} (keV)	Production
^{86}Y	14.74	β^+,γ	EC	<0.02	----	672	1076.7 627.8 1153.1	$^{86}\text{Sr}(\text{p,n})^{86}\text{Y}$
$^{99\text{m}}\text{Tc}$	6.02	γ	IT/EC	----	----	----	140.5	$^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator

$^{\pm}$ Mean β ranges (R_{mean} , mm) were calculated from mean β energies (E_{β} [avg], MeV) using the following equation 176 ; $R_{\text{mean}} = 4.12 \cdot E_{\text{mean}}^{1.265} - 0.094 \cdot \ln E_{\text{mean}}$

Table 2

Current FDA-Approved Antibodies for the Parenteral Use in Detection and Treatment of Cancer. Derived from Milenic et al¹ (2004) and Walsh⁹³ (2006).

Generic Name	Trade Name	Agent/Target	Cancer Indication	Approval
<i>Unconjugated</i>				
Rituximab	Rituxan®	chimeric anti-CD20 IgG1	B-cell lymphoma	1997
Trastuzumab	Herceptin®	humanized anti-HER2 IgG ₁	breast	1998
Alemtuzumab	CamPath®	humanized anti-CD52	chronic lymphocytic leukemia	2001
Cetuximab	Erbitux®	chimeric anti-EGFR	colorectal head/neck	2004 2006
Bevacizumab	Avastin®	chimeric anti-VEGF	colorectal	2004
<i>Radioconjugates</i>				
Satumomab pendetide	OncoScint®*	¹¹¹ In-murine anti-TAG-72 IgG	colorectal, ovarian	1992
Nofetumomab merpentan	Verluma®*	^{99m} Tc-murine anti-EGP-1 Fab'	small cell lung	1996
Arcitumomab	CEA-Scan®*	^{99m} Tc-murine anti-CEA Fab'	colorectal	1996
Capromab pendetide	ProstaScint®	¹¹¹ In-murine anti-PSMA	prostate	1996
Ibritumomab tiuxetan	Zevalin®	⁹⁰ Y-murine anti-CD20 IgG + rituximab	B-cell lymphoma	2002
Tositumomab "Anti-B1"	Bexxar®	¹³¹ I-murine anti-CD20 IgG + unlabeled tositumomab	B-cell lymphoma	2003
<i>Drug conjugates</i>				
Gemtuzumab ozogamicin	Mylotarg®	humanized anti-CD33 IgG ₄ conjugated to colicheamicin	acute myelogenous leukemia	2000

* No longer commercially available.

Table 3

Selected Radiolabeled Antibodies Currently Undergoing FDA Clinical Trials for the Detection and Treatment of Cancer (accessed at www.ClinicalTrials.gov).

Sponsor	Agent/Target	Cancer Indication	Phase
GlaxoSmithKline	¹³¹ I-tositumomab vs. ibritumomab tiuxetan	non-Hodgkin's lymphoma	III
Radboud University / Immunomedics, Inc.	hMN-14xm734 + ¹¹¹ In-IMP-205 (pretargeted)	colorectal carcinoma	I
Immunomedics, Inc.	^{99m} Tc-LL2 (LymphoScan)	non-Hodgkin's lymphoma	III
Immunomedics, Inc.	⁹⁰ Y-HuPAM4	pancreatic cancer	I
Immunomedics, Inc.	⁹⁰ Y-epratuzumab (hLL2)	non-Hodgkin's lymphoma	II
Ludwig Institute for Cancer Research	¹³¹ I-huA33 (in combination with capecitabine)	metastatic colorectal carcinoma	I
Ludwig Institute for Cancer Research / Wyeth	¹¹¹ In-CMD-193 (a humanised mAb linked to the toxin, calicheamicin)	advanced tumors expressing the Lewis-Y antigen	I
Ludwig Institute for Cancer Research	¹¹¹ In-cG250 (phase I) and ¹⁷⁷ LuG250 (phase II), (a chimeric mAb)	advanced renal cell carcinoma	II
Sidney Kimmel Comprehensive Cancer Center / NCI	¹¹¹ In-labeled humanized PAM4 IgG	pancreatic cancer	I
Memorial Sloan-Kettering Cancer Center / NCI	¹³¹ I-8H9	CNS or leptomeningeal cancer	I
Memorial Sloan-Kettering Cancer Center / NCI	²¹³ Bi-M195 (humanized anti-CD33 mAb)	advanced myeloid cancer	II
Fred Hutchinson Cancer Research Center	¹³¹ I-BC8	acute myeloid leukemia or myelodysplastic syndromes	II
Duke University / NCI	²¹¹ At-81C6 (antitenascin human/mouse chimeric mAb)	primary or metastatic brain tumors	II
Weill Medical College of Cornell University / Columbia University	¹⁷⁷ Lu-J591	prostate cancer	II
NCI	^{99m} Tc-arctumomab (IMMU-4), comparison study with FDG	colorectal carcinoma	II
NCI	¹³¹ I-TNT-1/B	glioblastoma multiforme	I