

Review

Targeting of Radio-Isotopes for Cancer Therapy

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KEY WORDS

radioimmunotherapy, radioimmunoconjugates, monoclonal antibody, bifunctional chelating agent

ABBREVIATIONS

BCA	bifunctional chelating agent
CR	complete response
DFS	disease-free survival
DLT	dose-limiting toxicity
HAMA	human anti-murine immunoglobulin antibody
mAb	monoclonal antibody
MTD	maximum tolerated dose
PFS	progression-free survival
PR	partial response
RIC	radioimmunoconjugate
RIT	radioimmunotherapy

ABSTRACT

Treatment of patients with therapeutic monoclonal antibodies (mAbs) such as rituximab (Rituxan), an anti-CD20 mAb, or trastuzumab (Herceptin), an anti-HER2 mAb, have shown efficacy in clinical trials and have gained approval from the Food and Drug Administration (FDA). Mylotarg, an anti-CD33 mAb conjugated with the antibiotic calicheamicin has proven efficacious in the treatment of patients with acute myeloid leukemia and has also received FDA approval. However, the use of radionuclides to either augment inherent activity or to exploit the specific targeting properties has been a major development in mAb therapeutics. Radionuclide-bearing mAbs have recently been approved by the FDA; Zevalin, an anti-CD20 mAb armed with ^{90}Y and Bexxar, an anti-CD20 mAb armed with ^{131}I . This overview presents some background and some of the strategies pertaining to radiolabeled monoclonal antibody therapies with a focus on experiences reported for radiolabeled mAbs as evaluated in clinical trials.

INTRODUCTION

Kohler and Milstein's seminal publication provides the foundation of this review and describes the fusion of a plasmacytoma with spleen cells and the subsequent isolation of hybrids that secreted antibody with a predefined specificity.¹ The development of methods, eg., hemolytic plaque assays, cytotoxicity assays, and radioimmunoassays, to screen large numbers of hybridomas for their antigen specificity quickly ensued. Monoclonal antibodies (mAb) became tools to identify molecules which were then characterized and their function determined. Thus, hybridoma technology and resultant mAb rekindled interest in Ehrlich's concept that antibodies could serve as "magic bullets".²

The 1980's marked a flurry of activity that focused on generating murine mAbs against tumor-associated antigens (TAA). A multitude of preclinical studies in animal models ensued and have been invaluable in providing evaluations of the potential of mAb for therapeutic applications in cancer treatment. However, inherent limitations of these models demonstrated that they were not always predictive of a mAb's potential therapeutic efficacy. Preclinical and the subsequent clinical investigations with murine mAbs provided evidence that there were several constraints that required addressing before success in the treatment and management of cancer could be achieved. The foremost of these was the seemingly inevitable development of human anti-murine immunoglobulin antibodies (HAMA) by the majority of patients after one to two treatments with a xenogeneic antibody that limited further treatments.³ The list of general obstacles to therapeutic antibodies, dependent upon the strategy, also include

1. inadequate (low) therapeutic doses delivered to tumor lesions;
2. insufficient activation of effector functions;
3. slow clearance of the mAb from the blood compartment;
4. mAb affinity and avidity;
5. trafficking to, or targeting of, the mAb to normal organs;
6. heterogeneous distribution of antigen on tumor cells; and
7. insufficient penetration of tumor tissue.³

Initially, some of these limitations were addressed by chemical modifications of the immunoglobulin molecule, but many of these manipulations are now accomplished through genetic engineering techniques.⁴ However, in regards to therapeutic applications this effort has primarily been applied to eliminating the HAMA response by either production of chimeric mAbs, CDR grafting, or complete humanization of the protein.⁴

Advances have been significant to where investigators are finally able to fully explore the real therapeutic potential of mAbs. With the elimination of many obstacles and a better understanding of the inherent limitations of mAbs, renewed interest and support from

Table 1 **MONOCLONAL ANTIBODIES IN ADVANCED RADIOIMMUNOTHERAPY CLINICAL TRIALS**

Antibody	Antibody Form	Radionuclide	Antigen	Disease	Clinical Trial
Bexxar	mu IgG2a	¹³¹ I	CD20	NHL	FDA Approved
Lymphocide	hu IgG1 (LL2)	⁹⁰ Y	CD22	NHL	Phase III
CEA-Cide	hulgG1	⁹⁰ Y	CEA	colorectal, breast, lung, pancreatic, stomach ca	Phase III
Cotara	hu IgG	⁹⁰ Y	DNA	glioblastoma multiforma, anaplastic astrocytoma	Phase II/III
Oncolym	hu IgG1	¹³¹ I	HLA-DR10	NHL, CLL	Phase II/III
Theragyn	mu IgG1	⁹⁰ Y	PEM	ovarian, gastric ca	Phase II/III
Zevalin	mu IgG1	⁹⁰ Y	CD20	NHL	FDA Approved

The abbreviations used are: Ca, carcinoma; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; PEM, polymorphic epithelial mucin.

industry, several mAbs have been, or are currently being evaluated in Phase III clinical trials (Table 1). In the past 2–3 years, the Food and Drug Administration has approved two radiolabeled mAbs for the treatment of nonHogkin's lymphoma, Zevalin and Bexxar, thereby setting the path for additional targeted radiation therapy products to follow.⁵

THE PROTEIN

The protein is present, albeit in lowered concentration in most targeted radiation therapies, and may contribute to active therapeutic action that cannot be overlooked or discounted. Thus, this section provides some background into the source of mAb activity that may also be present during targeted radiation therapy and clearly provides a source of additional efficacy.

Therapy employing a naked mAb may be achieved through several mechanisms. Direct, tumor cell killing may be achieved via antibody-dependent cell cytotoxicity (ADCC) or via complement-dependent cytotoxicity (CDC). ADCC is triggered when the Fcγ receptor on effector cells such as neutrophils, macrophages and natural killer cells engage the Fc region of an antibody bound to a tumor cell. CDC is induced when the complement component C1q binds to the Fc region of antibody bound to the tumor cell surface. In either situation, cell killing may then occur through a cell-dependent (phagocytosis) or a cell-independent mechanism (lysis). The murine immunoglobulin isotypes IgG2a and IgG3 and human IgG1, IgG3 and IgG4 isotypes have been found to induce ADCC or CDC.^{6,7} The Fc-Fcγ receptor interaction has been found to play a role in the action of Trastuzumab and Rituximab.^{8,9} It has been demonstrated in animal models that there is a reduced therapeutic effect against breast cancer and lymphoma by these mAbs when the Fc receptor activation is absent.⁸

Tumor cell death mediated by a mAb might occur through other mechanisms. Interactions with its cognate antigen may induce cell cycle arrest or apoptosis by triggering or blocking signaling events within the cell.¹⁰ Additionally, cells are dependent upon receptors for proliferation, cellular functions and interactions with other cells. Thus, triggering and/or blocking of these receptors can inhibit cell growth and/or cause death.¹¹ Evidence exists that the effectiveness of Rituximab and Trastuzumab is not only due to recruiting effector cells, but also within an ability to trigger signaling events in cells when engaging the target ligand.¹² The chimeric anti-CD20 mAb, Rituximab, approved for the therapy of patients with relapsed, low grade NHL, appears to invoke a series of signaling events including increased phosphorylation, phospholipase Cγ activation, and upregulation of

c-myc upon crosslinking CD20.¹³ Rituximab, specifically, has been determined to induce apoptosis in B lymphocytes.¹⁴ The efficacy of mAb anti-Tac is achieved through interference of cellular signaling and functioning. Anti-Tac recognizes the 55 kD α-chain of the IL-2 receptor also known as CD-25 and blocks IL-2 receptor binding resulting in inhibition of tumor cell proliferation.¹⁵ Humanization of anti-Tac rendered it able to support ADCC.¹⁶

mAbs that bind to members of the epidermal growth factor receptor family, e.g., Trastuzumab binds HER2, also cause alterations in cellular signaling. These mAbs have been postulated to have a direct anti-proliferative signaling effect and block receptor-ligand interactions causing downregulation of the receptor.^{17,18} Therapy with Trastuzumab, now approved as Herceptin, has resulted in stabilization of metastatic breast cancer patients.¹⁷

Many reports document mAbs that alter cell signaling being used in combination with chemo- or conventional radiotherapy. Studies in animal models are demonstrating that anti-HER2 mAbs used in combination with external beam radiation generate a synergistic anti-tumor effect. Radiation or mAb alone had minimal effect on tumor xenografts whereas inhibition of tumor growth could be achieved with the combination.¹⁹ Trastuzumab combined with paclitaxel or doxorubicin also enhanced both the rates of response and the duration of response in patients with metastatic breast cancer.²⁰

THE RADIONUCLIDE AND CHEMISTRY

One of the other critical aspects pertaining to targeted radiation are those variables pertaining to the radiation, i.e., emission type, energy and thus range of emission, and the half-life of the source that in this case originates from a vast array of choices (Table 2).²¹ There are essentially three choices of emission; β-particles, α-particles, and Auger electrons. The first category has received the greatest attention for a variety of reasons, mainly convenience and familiarity, and includes ¹³¹I. The other choices of β-emitter are radio-metals such as ⁹⁰Y, ⁶⁷Cu, ¹⁸⁶Re, and more recently ¹⁷⁷Lu. There are several additional β-emitting radionuclides that have either been investigated or proposed, and while of interest, discussion of these will be limited here except to state that the range of possible emission energies and half-lives is realistically met with a relatively small sampling of those isotopes that are also readily available.²¹

There is an equally short list of available α-emitting radionuclides, high-energy particles (4–9 MeV), that qualify for targeted radiation therapy, specifically so within constraints of the half-life variable. Presently, only ²¹²Bi, ²¹³Bi, and ²¹¹At are generally being considered,

Table 2 THERAPEUTIC RADIONUCLIDES

Radiouclide	Type	Half-Life	E(max) (MeV)	Mean Tissue Range (mm)	Imageable Emissions
⁹⁰ Y	β	2.7 d	2.3	2.76	No
¹³¹ I	β, γ	8.0 d	0.81	0.40	Yes
¹⁷⁷ Lu	β, γ	6.7 d	0.50	0.28	Yes
¹⁵³ Sm	β, γ	2.0 d	0.80	0.53	Yes
¹⁸⁶ Re	β, γ	3.8 d	1.1	0.92	Yes
¹⁸⁸ Re	β, γ	17.0 hr	2.1	2.43	Yes
⁶⁷ Cu	β, γ	2.6 d	0.57	0.6	Yes
²²⁵ Ac	α, β	10 d	5.83	0.04–0.1	Yes
²¹³ Bi	α	45.7 min	5.87	0.04–0.1	Yes
²¹² Bi	α	1.0 hr	6.09	0.04–0.1	Yes
²¹¹ At	α	7.2 hr	5.87	0.04–0.1	Yes
²¹² Pb	β	10.6 hr	0.57	0.6	Yes
¹²⁵ I	Auger	60.1 d	0.035	0.001–0.02	Yes

however, recently ²²⁵Ac has shown promise despite concerns about the lengthy half-life and trafficking of decay products in vivo. The bismuth radioisotopes are available from generators, ²²⁴Ra and ²²⁵Ac, respectively. Both ²¹²Bi and ²¹³Bi decay via branched pathways that result in both α- and β-particles. ²¹³Bi lacks the high abundance of energetic and potentially hazardous photon emissions of ²¹²Bi and thus is a more attractive candidate for radioimmunotherapy (RIT).²²

Auger emitters have received the least attention in part due to the accepted premise that their extreme cytotoxicity and thus efficacy is limited to those emissions occurring within the cell nucleus.²³ Despite this apparent limitation, several investigations have demonstrated that Auger emitters may have a significant role as therapeutics.²⁴

The β-emitters have relatively lengthy, yet sparse emission path lengths (⁹⁰Y mean range of 275 μm, max. of 500–600 μm) of low linear energy transfer (LET), with energy deposition taking place at some distance from the actual decay event.²⁵ Thus, there are optimal ranges for therapy and therefore complementary sizes of disease for which each β-emitter might be most appropriate as a therapeutic.²⁶ The therapeutic benefit generated by these emitters is resultant from crossfire and the cell with the radionuclide attached either to the surface or internalized is not the effective target of that specific decay event. Advantages to this choice include the ability to obviate in part tumor antigen expression heterogeneity and differential penetrance of the targeting vector. If the emission range is greater than the radius of the targeted lesion then it becomes possible to uniformly target the entire lesion.²⁶ ⁹⁰Y has a pure β-emission which delivers ~4.5 times more radiation per mCi to a tumor than does ¹³¹I, however, ⁹⁰Y also has a greater emission range in tissue. Therefore, the majority of the energy from ⁹⁰Y decay would be deposited in tumors only if their diameter is 1 cm or greater. ⁹⁰Y, unlike ¹³¹I, lacks an imageable emission thereby requiring a dosimetry / imaging phase that has usually been performed using ¹¹¹In. Thus, as with all aspects of this therapy, there are negative aspects. That same lengthy emission range can equally target the normal tissue surrounding the lesion. Additionally, myelosuppression tends to be the dose limiting toxicity and again the lengthy emissions occurring during circulation and through the marrow is a consistent limitation to the use of β-emitters.²⁷ ¹⁷⁷Lu

and ⁶⁷Cu have been evaluated in clinical trials for their therapeutic efficacy.^{28–30} With lower energy β-emissions and thus decreased range, toxicity originating from normal tissue exposure was proposed to be decreased. Additionally, these β-emitters also possess imageable γ-emissions allowing investigators to determine the extent of disease, and calculate the dosimetry of the radioimmunoconjugate (RIC), as well as monitor therapeutic efficacy. Again, while gaining this positive aspect one notes that this same γ-emission also contributes to normal tissue toxicity. Despite these concerns and limitations, β-emitters continue to be the dominant class of emitters investigated in preclinical and clinical trials due to convenience, availability, and familiarity to both researchers and regulatory agencies. Thus, there is no surprise that Zevalin and Bexxar, both recently approved, are each armed with a β-emitter. Limitations to the use of β-emitters clearly exclude treatment of

single cell, metastatic diseases, leukemias, and disseminated diseases such as acute myelocytic leukemia (AML) and adult T-cell leukemia (ATL).²⁵

The α-emitters have relatively short (40–100 μm), yet dense emission path lengths of high LET, ~ 400 times greater than that of β-emitters (80 versus 0.2 keV/μm), with energy deposition taking place immediately from the actual decay event.²⁵ Alpha-particle radiation is cytotoxic at a dose rate of 1 cGy/hr.³¹ Thus, there are again optimal ranges for therapy and therefore a complementary size of disease most appropriate for use with an α-emitter as a therapeutic. Most, if not all of the therapy that is generated results from the direct emission of the α-particle. The cell actually targeted with the radionuclide attached either to the surface or internalized is the effective target of that specific decay event. It is generally accepted that a very low number of traversals (1–3) are required to kill a cell.²⁵ Limitations inherent to the list of potentially useful α-emitters include possession of a short physical half-life. This taken in conjunction with emission path length defines usage to generally be limited to leukemias, highly vascularized tumors, and metastatic disease where there is adequate targeting time and appropriate disease size. Targeted radiation bone marrow purging for transplant conditioning seems potentially attractive using these emitters, while the selective targeting of tumor vasculature with a goal of tumor eradication through elimination of the nutrient flow would also seem an obvious application.^{32,33} Limitations towards the treatment of larger, bulky disease seem evident, yet adjuvant therapies in this same arena may be equally possible.

Thus it becomes highly important that one chooses the most appropriate radionuclide for treating specific diseases based upon size and presentation of the disease thereby matching those variables as closely as reasonably possible to the radiological properties of the available isotopes. While this seems obvious, a survey of the literature (vide infra) will indicate that this has not always been the case. One of the curious aspects of the use of targeted radiation therapy has been what seems an undue focus on use of single radionuclides, i.e., the “best radio-isotope” if that can be said, given as a single dose, while in fact this makes little rational sense as disease does not present itself in a sole form, particularly so in patients in Phase I/II trials, and that fractionation of both chemotherapeutics and external

beam are routine regimens. One would project that as the reality of limitations in both targeting vehicles and radionuclides become better understood and accepted that more rational application of both will occur in support of future clinical trials.

One of the convenience factors that has supported the use of the radio-iodine isotopes and ^{211}At is the chemistry for their radiolabeling of proteins has been reasonably well established. Direct radio-halogenation, that is to say radio-iodination occurring with iodination of the extant tyrosine moieties of the protein, has dominated the field. While such chemistry was initially applied for ^{211}At , this has been supplanted with linking reagents that address the inherent instability of direct tyrosine protein labelings. Contrary to this situation, all metallic radionuclides of interest require chelation chemistry for attachment to mAb. Bifunctional chelating agents (BCA), i.e., chelates to which reactive groups have been added to permit conjugation to proteins and also form a stable complex with select metallic radionuclides are required.

The metallic radionuclides of choice are somewhat diverse in their coordination chemistry properties and unfortunately, there is no one BCA that is suitable for all. Numerous chemical criteria should be considered in the choice of chelating agent, e.g., its design and its actual use. In brief, coordination number of the metal ion, the ionic radius of the ion, its proclivity for metal binding character (hard vs. soft), and reactivity (hydrolysis vs. complexation) all have been influencing factors on chelate design.³⁴ Lastly, the overall goal of “instant” radionuclide complex formation with “infinite stability”, or zero dissociation, while laudable is also fundamentally in conflict. A BCA might form and maintain adequately stable complexes, but the formation kinetics might also render the BCA impractical for the intended radionuclide. Specifically, ^{212}Bi and ^{213}Bi , form highly stable and kinetically inert complexes with the macrocyclic BCA, DOTA (1,4,7,10-tetra-azacyclododecane- $\text{N},\text{N}',\text{N}'',\text{N}'''$ -tetraacetic acid). However, the kinetics of bismuth complexation with DOTA requires 15–45 min for maximal formation while the half-life of the radionuclides are 46 or 60 min, thereby costing half of the patient dose just in the preparation time.³⁵ The acyclic BCA, cyclohexyl-DTPA (diethylene triaminepentaacetic acid), has been shown to be a viable alternative to DOTA for labeling of mAb with Bi(III) isotopes.^{36,37} This BCA forms complexes with bismuth “instantaneously” ($t_{1/2} = 0.27\text{ s}$). In another example, an N-functionalized DOTA, PA-DOTA, had been the BCA of choice for labeling mAb with ^{177}Lu and was also employed in clinical trials.^{28,29,38} The acyclic CHX-A” DTPA was recently shown to behave no differently in vivo as compared to PA-DOTA. The CHX-A” ligand might provide considerable versatility for radiolabeling mAbs with not only the α -emitters ^{213}Bi and ^{212}Bi , but also then the β -emitters, ^{90}Y and ^{177}Lu , to provide a wider range of radionuclides for clinical applications.^{37,39,40} Thus, there is a clear balancing of properties that has to be struck to achieve a reasonable radiolabeled final product that can be prepared within an acceptable time frame yet also possess adequate stability of the metal complexes within the boundary conditions set by half-life of the isotope and the biological milieu. Several Cu(II) isotopes have been investigated with ^{67}Cu for targeted radiation therapy being foremost.⁴¹ The choice of BCA for Cu(II) isotopes remains an open and unresolved topic despite use of several different macrocyclic chelating agents being touted as stable and inert yet then with issues arising from transchelation of the isotope to superoxide dismutase and the isotope also being detected in patients’ ceruloplasmin.^{42–44} ^{67}Cu remains an interesting isotope for therapy in regards to emission energy, half-life, and possessing imagable emissions, yet development of the chemistry remains necessarily active.⁴¹

This aspect of targeted radiation therapy has been one of considerable activity for many years. However, as the field has matured the choice of realistic isotopes has narrowed and focused while the choices of chelating agents have become equally narrowed and defined. Despite this maturation, refinement of BCAs for the conjugation of radionuclides with mAb for RIT applications remains an active area of endeavor.³⁴

RADIOIMMUNOCONJUGATES: CLINICAL TRIALS

The majority of cytotoxic agents, albeit chemotherapeutic or radiation in origin, have a low benefit versus risk (therapeutic) index. In other words, the dose required for maximal tumor cell killing may also damage normal tissues. Conceptually, mAbs might serve as “magic bullets” to selectively localize cytotoxic agents and thus enhance the therapeutic index. This section will describe some of the strategies with immunoconjugates that have been investigated in the clinic over the past two decades for targeting radiation. The scope of this review precludes the coverage of the plethora of preclinical studies and forces a select sampling of results obtained in human trials with full intact radiolabeled mAbs. While there has been considerable effort expended in the development and evaluation of mAb fragments and variants for targeting radiation, it has also been evident that normal tissue toxicity and renal dose limitations also then relegate these RICs primarily to imaging applications at this time. This limitation also applies in part to peptides as well which usually suffer from high renal retention and low tumor localization. Considerable effort continues in this arena with careful attention to variables such as rapid targeting with rapid internalization in conjunction with rapid reexpression of the target antigen to maximize uptake and retention while minimizing normal tissue exposure.

Numerous RIT clinical trials have been conducted that demonstrate the specificity for a variety of TAA.²⁷ To date, the majority of RIT trials have employed intact murine IgG mAb. Advances in molecular biology techniques have led to the genetic engineering of mAb and the development of forms such as chimeric and humanized (CDR-grafted) mAb.⁴ Many of these mAb are currently under evaluation in clinical trials. Phase I and II RIT clinical trials have been and are being conducted for the management of a variety of solid tumors including carcinoma of the colon, breast, prostate, kidney, pancreas, ovary, lung, testes and thyroid.²⁷ Other trials include therapy of hepatoma, melanoma, neuroblastoma, glioma and cholangiocarcinomas.⁴⁵ A wide range of mAb doses, albeit protein and/or radioactivity, have been administered to patients via different routes in combination with a variety of radionuclides. Thus, despite decades of research in the field of targeted radiation therapy, considerable variation in trial design and execution complicates the ability to directly compare the results of many trials. Thus, rather than attempt a comprehensive coverage of those efforts, we chose to present several capsules relating series of experiences to address developments in both the science and knowledge gained from these trials. RIT has been the most successful in the treatment of the hematologic malignancies. The accessibility of the tumor cells, the ability to characterize the tumor phenotype, to determine the stage of differentiation and their intrinsic radiosensitivity render the leukemias and lymphomas excellent targets for RIT.

Investigators at the University of California, Davis Medical Center have performed extensive clinical trials in patients with a particular focus on evaluating the therapeutic effectiveness of RIT with ^{67}Cu . Early studies with ^{131}I -labeled Lym-1, a mAb with high

affinity for the B chain of the HLA-DR10 antigen on malignant B-lymphocytes, demonstrated effectiveness in patients with nonHodgkin's lymphoma (NHL). A maximum tolerated dose (MTD) was of 100 mCi/m² with 4 patients showing a response to the RIT.⁴⁶ As with conventional radiotherapy, this trial demonstrated that fractionated systemic RIT was more effective. Subsequently, NHL patients were treated with a maximum of four doses of ¹³¹I-Lym-1 at 4-week intervals with the objective of defining the MTD (100 mCi/m² each) for the first two doses. Remissions (71%) were obtained with a mean response duration of 14 months with acceptable hematologic toxicity.⁴⁷ To improve the therapeutic index of this mAb, ⁶⁷Cu-Lym-1 was then assessed. A Phase I/II trial with 12 patients established the MTD of a single dose of ⁶⁷Cu-Lym-1 to be 50–60 mCi/m² (platelet count dependent).³⁰ A dose fractionation scheme based on the ¹³¹I clinical trials followed.⁴² Prior to a therapeutic dose, dosimetry and pharmacokinetics were determined with an imaging dose of ⁶⁷Cu-Lym-1. With this study design, and based on the single dose MTD, 3 patients were sufficient to establish 60 mCi/m² (2.22 GBq/m²) as the MTD for each of the first two doses with thrombocytopenia and neutropenia as the dose-limiting toxicities (DLT). Tumor regression was noted and one patient had a complete response (CR) that lasted 12 months. Investigators were able to demonstrate good imaging, a high therapeutic index, and favorable dosimetry with a ⁶⁷Cu-labeled mAb. However, one patient that received 5 doses (total of 524 mCi) required transfusions after the final dose. Of the normal organs, the liver had the highest radiation absorbed dose, however, no hepatotoxicity was evident, an important point, as uncomplexed Cu radionuclides localize in part to the liver.⁴⁸ Comparison of the ⁶⁷Cu results to those obtained with ¹³¹I indicated that the ⁶⁷Cu achieved a higher peak concentration in tumors in conjunction with a longer biological half-life. This resulted in the ⁶⁷Cu-labeled mAb delivering a mean radiation absorbed dose that was twice that of the same antibody labeled with ¹³¹I thereby demonstrating another important point of radio-metals being retained within cells versus de-halogenation and loss of effective dose.⁴⁹

In parallel, clinical trials have been conducted to evaluate the safety and MTD of ⁹⁰Y-Lym-1 in NHL patients using a modification of DOTA, a macrocyclic BCA. Thrombocytopenia was the DLT, with the MTD determined to be 0.37 GBq/m². Therapy with ⁹⁰Y-Lym-1 resulted in disease stabilization and partial responses (PR) in 5 of 8 patients. This trial also marked an observation concerning high absorbed radiation dose delivered to the liver which then led to development of a BCAs with a catabolizable peptide linker.⁵⁰ The peptide sequence was chosen to be susceptible to cathepsin and thus hypothesized to facilitate hepatocyte cleavage and excretion of the radiochelate during metabolic processing of the conjugate in the liver.⁵¹ Clinical trials with breast and prostate cancer patients have demonstrated that greater therapeutic indices were obtainable with this new BCA design. Tumor targeting was not significantly altered and no significant toxicities were incurred while the absorbed radiation dose to the liver was reduced.^{50,52}

Zevalin (ibritumomab tiuxetan), for the treatment of patients with NHL, is the first radiolabeled mAb approved by the FDA (2003) for the purposes of therapy. Zevalin is a murine anti-CD20 mAb labeled with ⁹⁰Y using the acyclic DTPA class BCA (tiuxetan). Early Phase I/II studies with ibritumomab labeled with ⁹⁰Y demonstrated the anti-tumor efficacy and safety of this mAb in patients with recurrent B-cell lymphoma.⁵³ This study also demonstrated the advantage of “pretreating” patients with unlabeled

ibritumomab as part of the targeted radiation therapy treatment scheme.⁵³ Targeted radiation therapy with doses ≤ 50 mCi resulted in minimal nonhematological toxicity and durable clinical responses; doses ≤ 40 mCi were nonmyeloablative. As with other mAb such as B1 that also targets CD20, pretreatment with unlabeled mAb decreased splenic uptake and urinary excretion while increasing the relative uptake at disease sites. Targeted radiation therapy provided both CR and PR as a result of this therapy with a progression-free survival (PFS) of 72% 3–29+ months and a PFS of 78% with doses ≤ 40 mCi. Other trials have since established doses administered to patients and compared to rituximab (chimerized ibritumomab) immunotherapy resulted in an improved overall response rate (ORR) (80% versus 56% respectively) and a greater number of CRs (30% versus 16%). Furthermore, in another trial, a 74% ORR was achieved with ⁹⁰Y-ibritumomab tiuxetan targeted radiation therapy in patients in whom rituximab failed. There has been a lack of correlation between dosimetric/pharmacokinetic parameters and hematologic toxicity suggesting that the toxicity is more dependent on the bone marrow reserve in what is a heavily pretreated patient population. When these various trials were integrated and analyzed, it was determined that a single-dose of ⁹⁰Y-ibritumomab RIT could be safely administered in patients with <25% bone marrow involvement by NHL, adequate bone marrow reserves, platelets >100,000 cells/μl and with neutrophils >1,500 cells/μl.⁵⁴

Another anti-CD20 mAb that has also recently received FDA approval is Bexxar (mAb B1, or tositumomab, labeled with ¹³¹I). A Phase II clinical multi-center trial confirmed the efficacy and safety of this anti-CD20 mAb for RIT in patients with relapsed or refractory low-grade or transformed low-grade NHL.⁵⁵ Subsequently, a study was designed to assess the benefit of ¹³¹I-tositumomab RIT in patients with poorer prognostic outcome, i.e., those who had failed chemotherapy. Interestingly, patients in this trial served as their own controls. In brief, 65% of these patients experienced PRs or CRs as compared to 17% responding to their chemotherapy regimen. The duration of those responses was 6.5 months versus 3.4 months. The median duration of the response was 47+ months versus 6.1 months, respectively, for those receiving targeted radiation therapy versus chemotherapy only with a corresponding CR of 20% and 3% respectively.⁵⁶

ATL is an aggressive disease with a median survival of 9 months. It is a malignancy in which there is an overexpression of the high-affinity IL-2 receptor by mature lymphocytes but not in resting cells. Taking advantage of this differential, a Phase I/II clinical trial was conducted to establish the dose and assess the efficacy of ⁹⁰Y-Anti-Tac for targeted radiotherapy of ATL.⁵⁷ Nine patients (3 per cohort) were treated with 5, 10 and 15 mCi, with an additional nine patients treated at 10 mCi. Patients were given additional cycles of RIT as they responded, which ranged from 1 to 9: the mean number of cycles was 3. Nine of the 16 evaluable patients responded to the ⁹⁰Y-Anti-Tac RIT (PR = 7, CR = 2). At 20 months following the RIT, 35% of the patients were event-free. One patient remained in complete remission for >3 yr. Six patients developed an immune response to the murine Anti-Tac after one or two cycles which precluded continuing therapy. At present there is an ongoing Phase I/II trial with the humanized form of the anti-Tac mAb.

The CD33 antigen expressed on early myeloid progenitor cells and myeloid leukemia cells serves as a target molecule for targeted radiation therapy in leukemia patients. The anti-CD33 murine mAb M195 was shown to specifically target foci of leukemia in patients in a Phase I trial followed by a RIT trial in which ¹³¹I-M195 eliminated

large tumor burdens in patients with myeloid leukemias.⁵⁸ Myelosuppression was observed at doses of 135 mCi/m² or greater and enabled those patients to undergo bone marrow transplantation (BMT). To improve on the effectiveness of the BMT, ¹³¹I-M195 (120–230 mCi/m²) was combined with busulfan and cyclophosphamide to intensify therapy prior to a first or second BMT.⁵⁸ The data indicated that M195 could be effective in reducing tumor burden and that RIT had potential as part of a BMT regimen.

¹³¹I-M195 has also been shown effective in the reduction of minimal residual disease in patients with relapsed AML. Patients in second remission following retinoic acid therapy received ¹³¹I-M195 and an MTD of 50 mCi/m² was found. Two of the seven patients who were positive for retinoic acid receptor prior to RIT (determined by RT-PCR) transiently converted to negative status 5 and 13 weeks post-therapy. The median disease free survival (DFS) was 8 months versus 3 months without RIT. Myelosuppression and the development of neutralizing HAMA permitted only a single dose of ¹³¹I-M195 to be administered to patients and thus reinforces two major obstacles to the further development of murine mAbs for targeted radiation therapy.⁵⁸ A humanized M195 (HuM195) has since been developed and found to be as effective as the parental murine mAb.

In order to deliver sufficient radiation doses to the marrow, myeloablative therapy with a ¹³¹I-HuM195 required multiple infusions. The long half-life of ¹³¹I impeded the reinfusion of the patient with stem cells and required the patient to remain hospitalized in isolation. Hence, efficacy of HuM195 labeled with ⁹⁰Y was evaluated in a Phase I trial. Patients with relapsed or refractory AML received 0.1–0.3 mCi/kg of ⁹⁰Y-HuM195. Patients experienced a reduction in bone marrow blasts, some showed no evidence of disease for 2–4 weeks; 1 patient achieved complete remission. Conclusions drawn from this clinical trial included that ⁹⁰Y-HuM195 had potential as a component of a pretransplantation regimen.⁵⁹ The elimination of minimal residual disease in this patient population is viewed as the means of achieving long-term remission with prolonged DFS. To this end, ²¹³Bi-HuM195 has been administered to 18 leukemia patients to estimate the pharmacokinetics and dosimetry using direct real time imaging of the therapeutic dose necessitated by the half-life of the ²¹³Bi.⁶⁰ This is the first mAb targeted radiation therapy trial in humans using a radionuclide with α -particle emissions. The investigators determined that the absorbed dose ratio between the bone marrow, liver and spleen (sites of disease in leukemia patients) and the whole body for the ²¹³Bi-labeled mAb was 1,000–10,000 times greater than those determined for ¹³¹I and ⁹⁰Y.⁶⁰ Fourteen of 15 evaluable patients had reductions in their peripheral blood leukemia cells; 14 of 18 patients had a decrease in the percentage of bone marrow blasts. Myelosuppression lasted 12–42 days (median of 22) with transient, low-grade liver function abnormalities seen in 6 patients. The MTD was not reached in this trial in which patients were treated with up to 95 mCi in up to 7 dose fractions.⁶⁰

The CR rate, duration of remission and the overall response to targeted radiation therapy in either single or fractionated doses of B-cell malignancies has been more impressive in clinical trials administering high myeloablative doses in conjunction with either autologous bone marrow support (ABMT) or stem cell transplantation (SCT).^{61–63} One such trial treated 24 patients with relapsed B-cell lymphoma with ¹³¹I-labeled anti-CD20 mAb, including B1 now known as tositumomab or BexxarTM.⁶¹ Bone marrow transplantation was performed in 15 of the patients due to myelosuppression following therapy. Complete remission was noted in 16 patients with 9 patients remaining in remission from 3–53 months. A subsequent

study with ablative ¹³¹I-B1 therapy and autologous SCT in 21 patients resulted in objective responses noted in 18 patients; 16 were complete remissions. The PFS of these patients was 62% and the overall survival (OS) was 93% with a median follow-up of 2 years.⁶² Direct comparison between high dose targeted radiation therapy (HD-RIT) and conventional high dose radiotherapy (HD-XRT) has been conducted in patients with follicular NHL. A PFS of 48% and 29% with an OS of 67% and 53% for HD-RIT and HD-XRT, respectively, was reported.⁶³ Disappointingly, the majority of the patients receiving HD-RIT did eventually relapse, leading to a proposal to include chemotherapy in the RIT regimen.^{64,65}

The most recent report describes a Phase II trial in which CHOP chemotherapy was followed by tositumomab/¹³¹I-tositumomab for untreated follicular NHL.⁶⁵ Patients received CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) therapy and after 4–8 weeks, 450 mg of tositumomab was injected followed by a low dose of ¹³¹I-tositumomab for imaging and dosimetric calculations. After 1–2 weeks, patients received another infusion of tositumomab which was then followed by a therapeutic dose of ¹³¹I-tositumomab. This treatment scheme resulted in 67% complete remissions and 23% partial remissions. The 2 year PFS was estimated at 81% and the 2 year OS was 97% with a median follow-up of 2.3 years. A Phase III clinical trial with CHOP in combination with Rituximab is currently underway. A treatment plan that combines chemotherapy, total body irradiation and RIT for the treatment of advanced acute lymphocytic leukemia (ALL) and myelodysplastic syndrome (MDS) has also shown potential in a Phase I trial.⁶⁶ MDS Patients treated with ¹³¹I-labeled anti-CD45 mAb had a median survival of 65 months (15–89 months) while the three ALL patients had a disease free survival of 19, 54 and 66 months.

Success in a general sense has been more limited in the treatment of solid tumors by targeted radiation therapy as demonstrated in part by the successes incurred in the lymphoma and leukemia arena of clinical trials. This is in part due to antigenic heterogeneity and lack of tumor penetration of the antibody being a major impediment to effective RIT.

One of the first trials on assessing RIT dose fractionation in the treatment of a solid tumor was with an ¹³¹I-labeled chimeric mAb (B72.3) that targeted TAG-72 in metastatic colon carcinoma patients.⁶⁷ The MTD of a single dose of this mAb was found to be 36 mCi/m². Despite a notable immune response against the chimeric antibody that altered pharmacokinetics and biodistribution of the radiolabeled antibody upon retreatment of patients, a study assessing the efficacy of dose fractionation was executed. Patients received 2 or 3 doses at 1 week intervals for a total of 28 or 36 mCi/m². A minor response (25–50% tumor regression) was observed in 1 of 12 patients. Five of the patients repeated their course of RIT and in four of these patients their whole-body dose was appreciably lower than what was incurred during the first round. Presumably this resulted as a consequence of the development of an immune response against the infused mAb.⁶⁸

The mAb CC49, another anti-TAG-72 mAb with higher affinity than B72.3, has been evaluated in a range of patient populations with three different radionuclides. This provides a rare opportunity by which direct comparison of radionuclide versus efficacy might actually be assessed. A Phase I trial with colon carcinoma patients established that a dose of 75 mCi/m² of ¹³¹I-CC49 was tolerated with 50% of the patients experiencing Grade III or IV marrow suppression.⁶⁹ A Phase II trial was then conducted evaluating ¹³¹I-CC49 in patients with hormonally unresponsive metastatic

prostate cancer. Bone pain relief was realized in 6 of 10 symptomatic patients, but no objective responses were noted. Tumor targeting of the mAb was affirmed in 13 of 15 patients receiving the targeted radiation therapy. Fourteen of the patients developed antibodies against the mAb.⁷⁰ High-dose (up to 300 mCi/m²) targeted radiation therapy with ¹³¹I-CC49 and autologous SCT in patients with gastrointestinal cancers led to a conclusion that this specific mAb antibody was unable to deliver sufficient radiation doses to tumor lesions.⁷¹ The obstacles of tumor antigen heterogeneity and lack of tumor penetration may also be compounded by dehalogenation of the mAb, however, this usually is associated with significant internalization, a property not generally noted for this mAb.

The mAb CC49 has also been radiolabeled with ¹⁷⁷Lu and been administered as a therapeutic to patients with ovarian and colorectal cancer. The Phase I trial with colorectal patients determined an MTD of ¹⁷⁷Lu-CC49 administered intravenously to be 15 mCi/m² with bone marrow uptake of the ¹⁷⁷Lu being the limitation as determined by scintigraphy and measurement of radioactivity in bone marrow biopsies.²⁸ In contrast, when ¹⁷⁷Lu-CC49 was given to chemotherapy-resistant ovarian cancer patients via intraperitoneal injection the MTD was determined to be 45 mCi/m² with no observed bone marrow uptake. Anti-tumor activity was noted along with a prolonged DFS of most of the patients with microscopic disease. As a means of enhancing the therapeutic index of ¹⁷⁷Lu-CC49, a clinical trial was conducted in which patients were treated with IFN α 2b and Taxol.⁷² Patients received 4 treatments of IFN α 2b, 3 prior to and 1 after targeted radiation therapy, and 1 dose of Taxol prior to targeted radiation therapy. The addition of IFN increased hematologic toxicity, effectively decreasing the MTD to 40 mCi/m². Taxol was found to not have an effect on the pharmacokinetic or dosimetry parameters of the ¹⁷⁷Lu-CC49. In contrast, IFN increased the whole-body half-life of radioactivity and thus the whole body radiation dose. Partial responses were seen in 4 of 17 patients; 4 of 27 patients with nonmeasurable disease had prolonged PFS intervals.⁷²

The same study design was employed to assess the feasibility of and to determine the MTD for targeted radiation therapy of ovarian cancer employing ⁹⁰Y-CC49.⁷³ This was the first trial assessing ⁹⁰Y-CC49, administered by intraperitoneal injection, for efficacy in ovarian patients. Partial responses were obtained in patients with measurable disease while patients with nonmeasurable disease experienced a prolongation of disease free-survival. No evidence of disease occurred in 4 patients at 9+, 18+, 19+ and 23+ months. The MTD was reported at $\leq$ 24.2 mCi/m².

Intralesional administration of targeted radiation therapy has been another strategy explored in clinical trials to increase therapeutic efficacy while minimizing normal organ toxicity. A trial was conducted to identify objective responses to intrathecally administered ¹³¹I-81C6, an anti-tenascin mAb, in patients with leptomeningeal malignancies or those with brain tumor resection cavities.⁷⁴ Adult patients tolerated doses up to 80 mCi while no MTD was determined in pediatric patients (up to 40 mCi); toxicities remained hematological in nature. Stable disease occurred in 42% of the patients with one partial response noted; the median follow-up was >320 days. In patients with recurrent glioma, DLT of 120 mCi manifested as acute neurologic with reversible hematologic toxicity.⁷⁵ These clinical trials were extended to determine efficacy and MTD of ¹³¹I-81C6 in patients with newly diagnosed glioma following surgical resection of the tumor. In a patient population with no previous treatment, the MTD was 120 mCi, although 180 mCi could be reached with

a manifestation of delayed neurotoxicity. None the patients developed hematological toxicities. The median survival was 79 weeks.⁷⁶

A Phase II has since been conducted in the same patient population. In this trial patients received targeted radiation therapy post-surgical removal of the tumor. Patients then received conventional external beam radiotherapy followed by chemotherapy.⁷⁷ A median survival of 86.7 weeks was found to exceed historical controls that had received radiotherapy and chemotherapy. They also found that resection of the tumor site due to radionecrosis was significantly lower.

Future clinical trials will address increasing the absorbed radiation doses to the tumor/cavity margins by adjusting delivery volumes on an individual patient basis. Phase III clinical trials to validate the locoregional administration of RIT for the treatment of brain tumors have been proposed.⁷⁷ A Phase I trial evaluating ⁸¹C6 radiolabeled with ²¹¹At provided the second targeted α -emitter therapy trial for treating recurrent glioma patients with the agent being injected into surgically resected cavities. Seventeen patients were treated with a neurotoxicity limiting dosage to a routine 6.7 mCi. Median survival was reported as 53.6 weeks for all patients, and noteworthy, 2 patients remained alive greater than 150 weeks thereby justifying optimism for treating newly diagnosed high-grade glioma patients.⁷⁸

Dose-limiting toxicity from mAb targeted radiation therapy routinely is myelosuppression. In many instances, therapeutic doses of radioactivity delivered by mAb cannot be administered without autologous bone marrow or stem cell transplantation support. Multi-step protocols have been designed to pretarget tumor with a mAb followed by the administration of the radionuclide. This strategy disconnects the radionuclide from the targeting vector. One of the first pretargeting systems to be evaluated and is still under evaluation uses bispecific mAbs and bivalent haptens.⁷⁹ Another pretargeting design exploits the affinity of biotin for streptavidin (or avidin) to maintain specificity. In a two-step protocol, the patient is injected with a streptavidin (avidin) mAb conjugate. Sufficient time to target tumor and clear from the circulation is allowed and then radiolabeled biotin is administered. The streptavidin (SA)-mAb localized at the tumor captures the radiolabeled biotin, while the unbound radiolabeled biotin is excreted via the urine thereby enhancing tumor uptake versus background. The corollary to this strategy is that the vast majority of injected isotope is excreted thereby requiring injection of rather large doses to counter balance this clearance. Concerns about renal toxicity are thus evident in this arena.

Kalofonos et al. first tested this strategy in patients with squamous cell carcinoma of the lung using mAb HMFG-1 coupled with streptavidin.⁸⁰ The SA-mAb was administered to patients and after a 24 hr interval, ¹¹¹In labeled biotin was injected. The investigators were able to demonstrate localization by scintigraphic images finding that ~80% of the ¹¹¹In-labeled biotin was excreted by 24 hr, thus minimizing the radiation exposure of normal tissues. The SA has a molecular weight of ~65 kD thus contributing significantly to the molecular weight of the SA-mAb conjugate and accessing tumor was challenging whereas biotin is a small molecule. This led to inverting the strategy conjugating biotin to the mAb and utilizing streptavidin as the radionuclide carrier. However, this strategy was not as successful.⁸¹ In a study utilizing an mAb (MOv18)-biotin conjugate, tumor targeting was achieved in ovarian carcinoma patients. However, at 24 to 48 hr, only 14% of the ¹¹¹In-streptavidin had been excreted and 14% remained in the blood resulting in exposure to normal tissue.⁸¹ This pretargeting scheme was further refined by the addition of a third step wherein unlabeled avidin was administered 24–36 hr after the

mAb-biotin as a “chase” to clear and/or fill the biotin sites of the circulating mAb-biotin conjugate. This was followed ~24 hr later with injection of radiolabeled biotin. Patients with residual or recurrent grade III or IV glioma following conventional therapies were treated with ^{90}Y -biotin pretargeted by BC4, an anti-tenascin mAb.⁸² The MTD was 80 mCi/m², 2–8 times higher than conventional RIT with ^{90}Y , with objective responses ranging from complete remission to stabilization of disease. The median survival of Grade IV glioblastoma patients was 11 months and 19 months for those with Grade III anaplastic glioma. In this study, 24 patients received 2 cycles of pretargeting therapy while 6 patients were given 3 cycles.

Pretargeting radioimmunotherapy (PRIT) has also been evaluated as adjuvant therapy in the treatment of glioma patients.⁸³ Based on the aforementioned study, the authors were interested in further improving the time to relapse and in improving the overall survival. Nineteen of thirty-seven patients received pretargeting RIT following the conventional regimen of surgery and radio-chemotherapy. Those with glioblastoma had a disease free interval of 28 months and a median survival of 33.5 months (vs. 8 months for those that did not receive the PRIT). For patients with Grade III glioma, the disease free survival was 56 months. The median survival of these patients was not reported since only 2 of 17 had died. PRIT was also found to be tolerated when administered as a locoregional injection to glioma patients.⁸⁴ The MTD of ^{90}Y -biotin pretargeting using an anti-tenascin mAb (BC4) was determined to be 30 mCi with neurological complications and no hematological toxicity was noted. Patients completed two cycles of PRIT that resulted in a median survival of 20 months for glioblastoma patients (vs. 6–12 months) and 52 months for those with anaplastic glioma (vs. 15–27 month).

There have also been clinical trials evaluating PRIT that employed the strategy of conjugating the mAb with avidin/streptavidin. A study was conducted with 43 patients with advanced epithelial malignancies to optimize each of the three steps of PRIT.⁸⁵ In this case, the pretargeting vector was mAb NR-LU-10 conjugated with streptavidin, the chase or clearing agent was a biotin-galactose-human serum albumin conjugate, and biotin was the vector for the radioactivity, ^{90}Y . This study was also designed to establish

- component safety,
- demonstration of targeting by the mAb-streptavidin,
- effectiveness of the clearing agent,
- pharmacokinetics of the DOTA-biotin,
- targeting of the ^{90}Y -biotin,
- dosimetry calculations and
- assessment of the immunogenicity of each of the components.⁸⁵

Tumor/bone marrow ratios of 63:1 with no gradable hematologic toxicity were reported. Nonhematological toxicities included increased liver function, nausea, fever, fatigue, anorexia, diarrhea and others. No objective tumor responses were detected. Despite that streptavidin possesses four binding sites for biotin, a commensurate increase in the absorbed radiation dose to the tumor was not noted. Speculation was that either the clearing agent was “filling” the biotin sites on the mAb-streptavidin conjugate at the tumor, or there was release of biotin from the clearing agent that then competed and blocked ^{90}Y -biotin. Further development of the clearing agent with a metabolically stable biotin link was necessitated. Most notable, however, is the confession that a major obstacle to this particular study is the inherent cross-reactivity of the mAb with normal tissues resulting in the noted nonhematological toxicities in conjunction with the lack of any therapeutic benefit. Nevertheless, a Phase II trial

was conducted. Twenty-five patients with metastatic colon cancer were treated with a single dose of 110 mCi/m² of ^{90}Y -DOTA-biotin using NR-LU-10-streptavidin as the targeting vector.⁸⁶ In this clinical trial hematological toxicities were also observed, but these were secondary to the toxicities caused by the cross-reactivity of NR-LU-10 with normal tissues, primarily the epithelium of the bowel and kidney tubules. Some objective responses were noted and some encouragement by achievement of the proof-of-principle could be taken as a positive result. However, the poor choice of the targeting vector was the Achilles heel to the study.

Utilizing the same motif just described, a Phase I/II trial was conducted treating ten NHL patients with PRIT.⁸⁷ Patients were injected with a Rituximab-streptavidin conjugate followed 34 hr by injection of a clearing agent (biotin-n-acetyl-glucosamine) and then finally injected with 30 or 50 mCi/m² of ^{90}Y -DOTA-biotin 18–24 hours later. Objective responses were noted in 6 of 7 patients with transient hematological toxicities and a tumor:whole body ratio of 38:1 was achieved. With an appropriate mAb as the targeting vector, pretargeting radioimmunotherapy protocols may have a place in the repertoire of cancer patient treatment regimens. However, the greatest caveat remains the immunogenicity of the streptavidin component limiting repeat therapies.

In conclusion, there are currently 13 monoclonal antibodies approved for use by the FDA of which there are 2 radiolabeled monoclonal antibodies approved for use, both for the treatment of CD20+ NHL. They are 1) Bexxar, a murine IgG2a anti-CD20 mAb directly radiolabeled with ^{131}I and 2) Zevalin, a murine IgG1 anti-CD20 mAb radiolabeled with ^{90}Y via a conjugated BCA.^{53,62} A 67% overall response rate has been achieved in NHL patients receiving Zevalin.⁵³ Experience with a single dose of Bexxar in 582 patients in 5 clinical trials has resulted in responses lasting from 3 months to 5.5 years. A total of 161 patients (28%) has a CR with the median duration of the response lasting 4.8 years.⁸⁸ These two demonstrate the effectiveness of not only enhanced efficacy due to being armed with a radionuclide, but also the rational approach to targeting a cell surface antigen that is not shed or modulated and also can also directly signal cell death through apoptosis unlike other B-cell targets such as CD19.¹² This inherent activity of the target provides a combination therapy. Thus, one can appreciate the choice of a carefully selected target that provides an additive efficacy and stable cellular site from which irradiation of disease can be effected. This is a key factor to the success of both of these agents and in particular for Bexxar in that internalization and concomitant de-halogenation of excretion of the ^{131}I is minimized. Considerable efforts continues to fully understand the mechanisms of action in such instances as well as in those regimens of combining various drugs (eg., Taxol, doxorubicin) and radiosensitizers (Gemzar).^{65,72,89} These may be acting in complete independence, however, some evidence exists that, particularly with the anti-CD20 mAbs, Herceptin, and Erbitux that additive or synergistic or complementary modes of action are in effect making cells more sensitive to radiation thereby enhances their therapeutic index.⁹⁰

CONCLUSION

This article has presented an overview of radiolabeled mAb directed cancer therapy approaches that have been evaluated with an emphasis on the components (protein, radionuclide, chemistry) and a selection of clinical trials. After twenty-five years, the promise of mAb-based therapies is generally recognized as being ready to make

a significant impact on the treatment of disease. The efficacy and recent approvals of Zevalin and Bexxar has fueled renewed enthusiasm by pharmaceutical companies for exploring development of such agents.

Complementary to that position is the probability that their full potential is only now just beginning to be appreciated and understood. However, one can also see that despite the wealth of knowledge and capability in antibody engineering, the first two approved radiolabeled mAbs were murine in nature and subject to all of the limitations therein, i.e., immunogenicity and biological half-lives. Knowledge of actual clinical use of radiolabeled mAbs remains in its infancy in many aspects, particularly so in regards to fractionated dosing and rational construction of drug combination cocktail therapies to integrate the modality of targeted radiation therapy with established chemotherapies and external beam therapies. Clear evidence exists that value added results are achieved by pursuit of such strategies.

The dominance of mAb therapies for the lymphohematopoietic malignancies and their success is a reflection of the accessibility of the tumor cells by the mAb. The consensus of the literature appears to be that the majority of mAb-based therapies for solid tumors will provide the greatest benefit to patients if applied in the treatment of minimal residual or micrometastatic disease and if they are a component of a multi-modality treatment regimen. However, these limitations have been arrived at through what may have been less than optimal targeting agents, sub-optimal chemistry, incorrect radionuclide choice, and a less than rational experimental design now in hindsight. Much of what has been achieved defines negative territory. Thus, there remains a continuing effort to refine and optimize each of the strategies that have been discussed. This effort continues to include goals of improving efficacy and minimizing toxicity. The next decade should prove exciting as rational exploration and investigation of the possibilities of using and understanding the application of targeted radiation therapy becomes a reality and mainstream aspect for the treatment and management of cancer patients.

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