

ANTIBODY-TARGETED RADIATION CANCER THERAPY

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Several monoclonal antibodies are now approved for cancer therapy, such as rituximab, an anti-CD20 monoclonal antibody for the treatment of B-cell non-Hodgkin's lymphoma. Such 'naked' antibodies can recruit the body's immune effector mechanisms to kill cells expressing the target of the antibody. In recent years, the linking of radionuclides to antibodies to either augment inherent activity or to exploit the specific targeting properties of monoclonal antibodies has been a major area of development. Two radionuclide-bearing monoclonal antibody therapies have recently been approved by the US FDA, and several more are in clinical trials. Here, we discuss the development and use of radiolabelled monoclonal antibody therapies, with a focus on radiolabelled monoclonal antibodies that have been evaluated in clinical trials.

The efficacy of traditional cytotoxic cancer therapies generally comes with the price of significant toxicity to normal cells, which can limit the success of therapy. But in the past two decades, greater understanding of the molecular differences between cancer cells and normal cells has led to the development of therapies that target cancer cells, including antibodies directed at tumour-associated antigens. The targeted nature of such therapies offers the promise of greater efficacy and less toxicity, and potentially greater treatment success.

A key step in the emergence of antibody-targeted cancer therapy was the development of hybridoma/monoclonal antibody (mAb) technology by Kohler and Milstein, whose seminal publication in 1975 described the fusion of a plasma cytoma with spleen cells and subsequent isolation of hybrids that secreted antibody with a predefined specificity¹. This breakthrough resurrected the concept put forth by Ehrlich a century ago that antibodies might serve as 'magic bullets'².

During the 1980s, the generation of murine mAbs against tumour-associated antigens became a focal point of research. Multiple preclinical studies provided proof-of-concept for the potential of mAbs in therapeutic applications for cancer treatment. Preclinical and clinical investigations with murine mAbs also highlighted several issues that required attention before success could be achieved in cancer management. Foremost of these

was the seemingly inevitable production of human anti-murine immunoglobulin antibodies (HAMA) after one to three treatments in immunocompetent patients³. Other factors limiting treatment included inadequate therapeutic dose delivered to tumour lesions; insufficient activation of effector function(s); slow blood compartment clearance; low mAb affinity and avidity; transport to, or targeting of, normal organs; heterogeneous antigen distribution on tumour cells; and insufficient tumour penetration³. Some of these limitations were addressed by chemical modification of the mAb, but most of these challenges have been addressed with genetic engineering techniques⁴. This effort has primarily been applied to eliminating HAMA through the production of chimeric mAbs, grafting of complementarity-determining region (CDR) or complete humanization of the protein⁴. Even so, some of these obstacles — such as tumour heterogeneity and penetration — are unresolved and research continues in these areas.

In 1997, rituximab (Rituxan; Genentech/Biogen Idec) became the first antibody to be approved by the FDA for cancer therapy. Rituximab, which is an anti-CD20 antibody for the treatment of **non-Hodgkin's lymphoma** (NHL), has been followed by several other antibody-based cancer therapies, such as trastuzumab (Herceptin; Genentech/Roche), an anti-HER2 antibody for the treatment of HER2-receptor-positive **breast cancer**.

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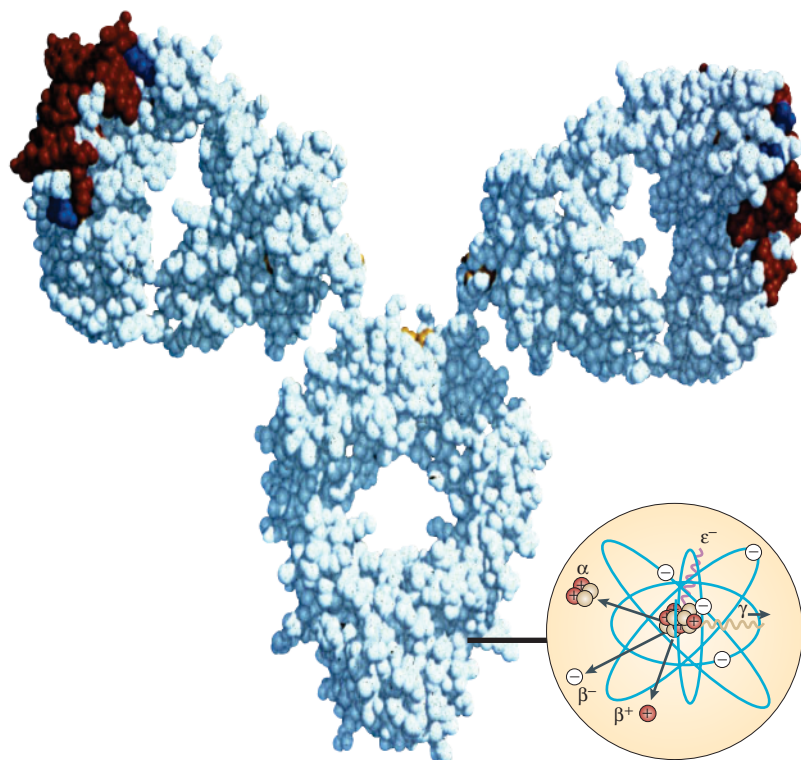


Figure 1 | A monoclonal antibody linked to a radionuclide. Monoclonal antibodies are linked to various radionuclides through several methods that are primarily based on the chemical characteristics of the specific radionuclide. Halogens, such as ^{131}I , are routinely introduced by direct halogenation of tyrosine residues of the protein. Metallic radionuclides, such as ^{111}In or ^{90}Y , require chelation of the metal through a suitable ligand. This chelating agent is routinely introduced through a reactive functional group that targets *N*-terminal and ϵ -amines of lysine residues. Such linking moieties include isothiocyanates, bromoacetamides, maleimides (post-thiolation of the protein) and active esters. Variations of these linking chemistries with proteins are also used for the indirect introduction of radio-halogens, including, but not limited to, ^{211}At .

Although such antibody therapies have shown significant success in cancer treatment, strategies to increase their efficacy are urgently needed.

One such strategy is to link antibodies against tumour-associated antigens to highly toxic radioisotopes, which brings to bear the killing power of these isotopes on tumour cells. Recent advances have brought us to the stage where investigators are finally able to fully explore the real therapeutic potential of radiolabelled mAbs (FIG. 1). With the elimination of many obstacles and a better understanding of the inherent limitations of mAbs, coupled with interest and support from industry, several radiolabelled mAbs have been, or are now being evaluated in Phase III clinical trials (TABLE 1). In the past two years, the US FDA has approved the use of two anti-CD20 mAb regimens involving radionuclides for the treatment of NHL: Zevalin (Biogen Idec) and Bexxar (Corixa/ GlaxoSmithKline), which are based on the radiolabelled mAbs ^{90}Y ibritumomab and ^{131}I tositumomab, respectively, making further targeted radiation therapy products probable⁵. This review describes radiolabelled-mAb-directed approaches, with an emphasis on the components (protein, radionuclide and chemistry), and discusses clinical trials of radiolabelled mAbs in haematological cancers.

The radionuclide

Choosing the most appropriate radionuclide for treatment on the basis of the size and presentation of the disease is crucial. Although this seems obvious, a survey of the literature indicates that this paradigm has not been broadly observed. As fractionation of both chemotherapeutics and external beam radiation are routine regimens, it is curious that treatment with a single dose containing the ‘best radioisotope’ has become such an undue focus. No single radionuclide is likely to address every therapeutic need, as disease does not exclusively present in a sole form, particularly in Phase I/II trials. Unfortunately, isotope selection is also often driven by economic rather than medical or scientific considerations, which could negatively affect both pre-clinical and clinical trials. Ultimately, the limitations of the targeting vehicles and radionuclides will be more clearly defined and a rational plan for future clinical trials will follow.

Some of the crucial considerations for successful targeted radiation are those variables pertaining to the radiation; that is, emission type, energy/range of emission and the half-life. A sampling of available isotopes (TABLE 2) results in several alternatives comprising three types of emission: β^- -particles, α -particles and Auger electrons⁵ (FIG. 2). Historically, β^- -emitters have received the greatest focus. The emission path lengths of β^- -emitters are relatively long, yet sparse (mean range of 275 μm , maximum range of 500–600 μm for ^{90}Y), with low linear energy transfer (LET) (FIG. 3). Energy deposition takes place at some distance from the actual decay event⁶. Therapeutic benefit results from ‘crossfire’; that is, the cell targeted with the radionuclide — which can be targeted to the cell surface or designed to be internalized within the cell — is not necessarily the effective target of the decay event. Given this, some of the limitations of β^- -emitters become clearly evident: treatment of single-cell metastatic diseases, leukaemias and disseminated diseases cannot be adequately addressed with β^- -emitters⁶.

Advantages of β^- -emitters include their ability to bypass tumour antigen heterogeneity and differential penetration of the mAb. The capacity to uniformly target the entire lesion becomes possible when the emission range exceeds the radius of the targeted lesion⁷. Convenience, availability and familiarity with radiolabelling chemistry has traditionally supported the use of the iodine isotopes — for example, ^{131}I . Other clinically relevant β^- -emitters include ^{90}Y , ^{67}Cu , ^{186}Re and, more recently, ^{177}Lu , with others having either been investigated or proposed (see below). Discussion of these will be limited here, except to state that emission energy and half-life requirements can be met with a small cross-section of isotopes that are already available⁵. Direct radio-iodination occurring with extant tyrosine moieties of the protein has dominated this field. The convenience of this method, however, is compromised by potentially rapid de-iodination of the protein post-internalization, a characteristic that is lacking when radiometals are used^{8,9}. ^{90}Y has a pure β^- -emission that delivers ~ 4.5 times more radiation per mCi to a tumour than does ^{131}I . The greater emission range of ^{90}Y means that most of the decay energy is deposited in tumours

Table 1 | **Representative monoclonal antibodies in advanced radioimmunotherapy clinical trials**

Antibody	Antibody form	Radionuclide	Antigen	Disease	Clinical trial status
Tositumomab (Bexxar)	mulgG2a	¹³¹ I	CD20	NHL	Approved by FDA
Epratuzumab (Lymphocide)	hulgG1 (LL2)	⁹⁰ Y	CD22	NHL	Phase III
Labetuzumab (CEA-Cide)	hulgG1	⁹⁰ Y	CEA	Colorectal, breast, lung, pancreatic, stomach carcinoma	Pending Phase III
chTNT-1/B (Cotara)	chlgG1	¹³¹ I	DNA	Glioblastoma multiforme, anaplastic astrocytoma	Phase III
¹³¹ I Lym-1 (Oncolym)	hulgG1	¹³¹ I	HLA-DR10	NHL, CLL	Phase II/III
Pemtumomab (Theragyn)	mulgG1	⁹⁰ Y	PEM	Ovarian, gastric carcinoma	Phase III
Ibritumomab tiuxetan (Zevalin)	mulgG1	⁹⁰ Y	CD20	NHL	Approved by FDA; Phase IV

CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukaemia; hulg, human immunoglobulin; mulg, murine immunoglobulin; NHL, non-Hodgkin's lymphoma; PEM, polymorphic epithelial mucin.

only if their diameter is 1 cm or more. Unlike ¹³¹I, ⁹⁰Y lacks an imageable emission, thereby requiring dosimetry using ¹¹¹In for γ -SCINTIGRAPHY and SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING; and although similar, these metals have different chemistries, and so such an approach might not be entirely accurate. Furthermore, the longer emission range that is associated with ⁹⁰Y might be disadvantageous, in that irradiation of normal tissue surrounding the lesion might result. In general, the longer-range β^- -emissions that occur during circulation and subsequent myelosuppression is a consistent limitation¹⁰; exceptions might be realized with ¹⁷⁷Lu and ⁶⁷Cu, owing to their decreased β^- -emission energy and range. Both have been evaluated in clinical trials for therapeutic efficacy^{11–13}, and possess imageable γ -emissions that allow determination of disease extent, calculation/prediction of dosimetry and monitoring of therapeutic efficacy. It should be noted that this same γ -emission contributes to both efficacy and normal tissue toxicity, illustrating the choices and compromises that must be balanced in radionuclide selection. Despite these concerns and the limitations of β^- -emitters, their advantages mean that their use continues to dominate preclinical and clinical trials, and indeed both of the radiolabelled mAbs approved by the FDA are armed with β^- -emitters.

The list of α -emitting radionuclides qualified for targeted radiation therapy is admittedly short, largely owing to half-life constraints. At present, only ²¹²Bi, ²¹³Bi and ²¹¹At are being actively studied^{14,15}; additionally, ²²⁵Ac (TABLE 2) has shown promise despite concerns about the lengthy half-life and trafficking of decay products *in vivo*¹⁶. The bismuth radioisotopes ²¹²Bi and ²¹³Bi are available from generators based on ²²⁴Ra and ²²⁵Ac, respectively, and decay via branched pathways that result in both α - and β^- -emissions. ²¹²Bi possesses a high-energy γ -emission in 32% abundance that ²¹³Bi lacks, and so the latter is generally considered a more attractive candidate for radioimmunotherapy¹⁷. Protocols that are analogous to radio-iodination chemistry

were initially applied for ²¹¹At; however, this has been supplanted with linking reagents that address the inherent instability of direct tyrosine protein labelling with this isotope (see below)¹⁸.

As a group, the α -emitters produce high-energy particles (4–9 MeV) that travel relatively short distances (40–100 μ m). They are characterized by dense emission path lengths of high LET, ~400 times greater than that of β^- -emitters (80 versus 0.2 keV per μ m), with energy deposition taking place immediately at the decay site⁶ (FIG. 2). The emission of α -particles is highly cytotoxic at a dose rate of 1 cGy per hr¹⁹. The short range limits their use to a complementary scale of disease. Most, if not all, of the therapy results from the direct emission of the α -particle, making the cell targeted with the radionuclide and the immediate neighbouring cells the effective targets. In contrast to β^- -emitters, a very low number of nuclear traversals (one to three) are generally all that is required to kill a cell with an α -emitter⁶. An inherent limitation of these α -emitters is their relatively short physical half-life. This, coupled with their emission path length, has generally been thought to limit the use of α -emitters to leukaemias, highly vascularized tumours and metastatic/disseminated disease in which adequate access, targeting time and appropriate disease size converge. In addition, bone-marrow purging for transplant conditioning, as well as selective tumour vasculature targeting with the goal of tumour eradication, seem obvious avenues for future work^{20,21}. Adjuvant therapies might also be viable^{15,22}.

Auger emitters, such as ⁶⁷Ga, ^{195m}Pt, ¹²³I and ¹²⁵I (TABLE 2), have received the least attention. This is due to the accepted premise that their extreme cytotoxicity, and therefore efficacy, is limited by the prerequisite for emissions to occur within the cell nucleus²³. Despite this apparent limitation, studies have demonstrated that Auger emitters might have a significant role as therapeutics, even if their clinical use might be limited to irradiation of microscopic residual disease^{24,25}.

SCINTIGRAPHY
Imaging of γ -emissions.

SINGLE PHOTON EMISSION
COMPUTED TOMOGRAPHY
IMAGING
An imaging technique using
corrected photon emissions.

Table 2 | Therapeutic radionuclides

Radionuclide	Type	Half-life	E _{max} (MeV)	Mean range (mm)	Imageable
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 h	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 h	6.09	0.04–0.1	Yes
²¹¹ At	α	7.2 h	5.87	0.04–0.1	Yes
²¹² Pb	β	10.6 h	0.57	0.6	Yes
¹²⁵ I	Auger	60.1 d	0.35	0.001–0.02	Yes
¹²³ I	Auger	13.2 h	0.16	0.001–0.02	No
⁶⁷ Ga	Auger, β, γ	3.3 d	0.18	0.001–0.02	Yes
^{195m} Pt	Auger	4.0 d	0.13	0.001–0.02	No

Linking radionuclides to protein

This aspect of radioimmunotherapy has been one of considerable activity, as researchers have sought to balance the conditions required to achieve a radiolabelled product with adequate stability of the resulting complex, within the constraints imposed by isotope chemistry and half-life. As should be expected, the choices of realistic isotopes and chelating agents have narrowed and focused as this field has matured.

Direct radio-iodination (with ¹³¹I, ¹²⁵I or ¹²³I) is well established and will not be addressed here. All metallic radionuclides require chelation chemistry for attachment to a mAb (FIG. 4). Bifunctional chelating agents (BCAs) — CHELATES that possess specific functional groups that allow both conjugation to proteins and stable complex formation with metallic radionuclides — are required, and this is an active area of research²⁶. Similarly, ²¹¹At also requires a linking agent, as direct radio-halogenation is inappropriate. Development of BCAs exceeds the scope of this work and only a brief discussion follows.

The suitable radiometals are diverse in their properties and coordination chemistry, so, unfortunately, no single BCA is suitable for all of these metals²⁶. A selection of examples is provided in FIG. 5. The ultimate goal of ‘instant’ radionuclide complex formation with infinite stability, or zero dissociation, although laudable, has proven non-trivial. Numerous chemical criteria must be considered in the choice of chelating agent; for example, its design and its actual use. Characteristics of the metal, such as COORDINATION NUMBER, ionic radius, METAL-BINDING CHARACTER (hard versus soft) and reactivity (hydrolysis versus complexation) must also be considered with respect to chelate design²⁶. A BCA might form and maintain an adequately stable metal complex, but the formation kinetics might also render the BCA impractical for the intended radionuclide.

For example, DOTA (1,4,7,10-tetra-azacyclododecane-*N,N',N'',N'''*-tetraacetic acid) forms highly stable and kinetically inert complexes with ²¹²Bi and ²¹³Bi (REF. 25). However, Bi(III) complexation kinetics with DOTA require 15–45 minutes for reaction completion. The half-lives of the radionuclides are 60 and 46 min, respectively, making this particular combination wasteful²⁷. Higher temperatures traverse this in part, but are limited owing to the inherent characteristics of the protein targeting vehicle. In contrast to macrocyclic BCAs, acyclic BCAs tend to show far faster complex-formation rates, but these are not as stable, representing another forced compromise. The acyclic CHX-A'', a cyclohexyl-DTPA (diethylenetriamine pentaacetic acid) (FIG. 5), has been shown to be a viable alternative to DOTA for the labelling of mAbs with Bi(III) isotopes^{28,29}. This BCA complexes with bismuth ‘instantaneously’ (*t*_{1/2} = 0.27 s) and is sufficiently stable for clinical trials³⁰. In addition, it was reported to have similar stability with the β⁻-emitter ¹⁷⁷Lu versus DOTA and PA-DOTA, the latter of which is used in clinical trials in combination with ¹⁷⁷Lu (REFS 11,12,31). In summary, this ligand not only provides considerable versatility for radiolabelling mAbs with the α-emitters ²¹³Bi and ²¹²Bi, but also with β⁻-emitters such as ⁹⁰Y and ¹⁷⁷Lu, allowing a wider range of clinical applications^{29,32,33}. ⁶⁷Cu remains an interesting candidate for therapy with regards to emission energy, half-life and imageable emissions; as such, development of the chemistry remains active³⁴. The choice of BCA for ⁶⁷Cu remains an open and unresolved topic; several different macrocyclic chelating agents have been touted as stable and inert with ⁶⁷Cu, despite reports of transchelation to superoxide dismutase and detection in patients’ ceruloplasmin^{35–37}. Production and availability questions currently compromise ⁶⁷Cu, and so ⁶⁴Cu might eventually be judged to be more viable³⁸.

The chemistry for linking ²¹¹At to proteins has been reviewed and has been dominated by aryl active ester reagents that have advanced to clinical trials^{18,39}. Issues of inadequate *in vivo* stability for general application in clinical settings are unresolved, which might be addressed with a better understanding of the chemistry of the element itself.

The protein

Although only present at a low concentration in most targeted radiation therapies, the protein and its contribution to therapeutic action should not be discounted. Direct tumour-cell killing can be achieved by two separate pathways: antibody-dependent cell cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC)⁴⁰. ADCC is triggered when the Fcγ receptor on effector cells engage the Fc region of an antibody bound to a tumour cell. CDC is induced when complement component C1q binds to the Fc region of antibody bound to the tumour cell surface. In either situation, cell killing can proceed through a cell-dependent (phagocytosis) or a cell-independent mechanism (lysis). The Fc portion of the mAb can also be engineered to enhance CDC activity. Cell killing by apoptosis has also been attributed to binding CD20 as applied to B lymphocytes⁴¹.

CHELATES
A ligand–metal complex.

COORDINATION NUMBER
The number of metal acceptor sites occupied by ligand donors.

METAL-BINDING CHARACTER
Acids (metals) and bases (ligands) can be categorized on the basis of polarizability: ‘hard’ (less polarizable) metals tend to form stronger complexes with ‘hard’ ligands than with ‘soft’ ligands; the converse is also applicable.

		<table border="1"> <tr> <td>γ-emitters</td> <td>β^+-emitters</td> </tr> <tr> <td>β^--emitters</td> <td>Auger electrons</td> </tr> <tr> <td>α-emitters</td> <td></td> </tr> </table>										γ -emitters	β^+ -emitters	β^- -emitters	Auger electrons	α -emitters																													
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Figure 2 | **A representation of the periodic table, highlighting those elements that are of interest for nuclear medicine and radiation oncology applications.** The elements are colour coded by emission type. With the exception of the halogens, most of the medically relevant radionuclides require chelating chemistry for attachment to proteins or other targeting vehicles. Specific radionuclides from cyclotrons or reactors can be obtained in pure form for longer-lived isotopes, or as the products of generator elutions for short-lived isotopes.

Receptors are integral to all aspects of cellular function, such as proliferation, migration and communication, and triggering or blocking receptors with a mAb can induce either cell growth or death^{42,43}. Preclinical experiments have demonstrated a reduced therapeutic effect against breast cancer and lymphoma, respectively, by rituximab and trastuzumab when the Fc receptor activation is absent^{44,45}. Rituximab binding to CD20 seems to invoke a series of signalling events, including increased phosphorylation, phospholipase C γ activation, c-Myc upregulation and induction of apoptosis in B lymphocytes^{46–48}. Trastuzumab, which binds to HER2, has been postulated to have a direct antiproliferative signalling effect and blocks receptor–ligand interactions, causing downregulation of the receptor^{49,50}. In addition, the mAb anti-Tac (daclizumab), which recognizes the 55-kDa α -chain of the interleukin-2 (IL-2) receptor (CD25), achieves therapy through interference of cellular signalling and functioning by blocking IL-2 binding, thereby inhibiting tumour-cell proliferation⁵¹.

An mAb that modifies cell signalling might result in synergistic effects when used in conjunction with chemo- or conventional radiotherapy. Studies in animal models have served to confirm this, as anti-HER2 mAbs in combination with external beam radiation have resulted in antitumour effects in systems in which radiation or mAbs alone had minimal effect on tumour xenografts⁵². A number of ongoing clinical trials are investigating the effects of combined drug/targeted radiation therapy to integrate radioimmunotherapy into mainstream clinical cancer therapy. As an example, trastuzumab combined with paclitaxel or doxorubicin enhanced both the rates of response and the duration of response in patients with metastatic breast cancer⁵³.

Radioimmunoconjugates: clinical trials

Radioimmunotherapy has been evaluated in clinical trials across the full spectrum of malignancies^{10,54}. Despite decades of research in the field, dissimilarities in experimental design and execution have complicated the direct comparison of the data obtained from separate sources. Comparisons are further complicated by a host of parameters, including dosing (protein and radioactivity), administrative methodology, radionuclide, targeting vehicle, and selection of both end point and the manner of its determination. Rather than an exhaustive coverage of the subject area, which is beyond the scope of this review, a selection of radio-labelled monoclonal antibodies in clinical development for the treatment of lymphohaematopoietic diseases are presented to shed light on the information gained. General reviews and those of a more specific nature, for example, on radiobiology, are available^{10,54–60}. Considerable effort has been spent developing and evaluating peptides and mAb fragments and their variants, with respect to normal tissue toxicity and renal dose limitations in preclinical settings; these studies are also precluded from this review, as most of these studies are presently focused on imaging applications.

Most cytotoxic agents, whether in the form of chemo- or radiation therapy, have a low therapeutic index (benefit versus risk); that is, the maximal tumour-cell killing dose can also damage normal tissues. The use of mAbs as ‘magic bullets’ might enhance the therapeutic index because they selectively localize the cytotoxic agent. Most radioimmunotherapy clinical trials have used intact murine IgG mAbs. However, genetic engineering has allowed the development of chimeric and humanized (CDR-grafted) mAbs that are now being

evaluated in clinical trials⁴. Substantial effort continues in this arena with careful attention to rapid targeting with internalization, maximized retention through target antigen re-expression and minimized exposure of normal tissue. In a general sense, success has been limited in the treatment of solid tumours, whereas the greatest accomplishments have been made with lymphohaematopoietic malignancies, despite toxicity issues originating primarily from myelosuppression. The accessibility of these tumours and our ability to characterize tumour phenotypes, coupled with the ability to determine the stage of differentiation and their intrinsic radiosensitivities, also suggests that these cancers present themselves optimally for treatment. Antigenic heterogeneity and lack of tumour penetration of the antibody are not major impediments to radioimmunotherapy of lymphohaematopoietic disease, whereas these variables, coupled with myelosuppression and other dose-limiting toxicities (for example, hepatic, renal and gastrointestinal), have limited therapeutic doses from being actively delivered to solid tumours^{3,4}.

Ibritumomab tiuxetan (Zevalin; Biogen Idec) became the first therapeutic radiolabelled mAb to be approved by the FDA in February 2002. Zevalin is a murine anti-CD20 mAb labelled with ⁹⁰Y using an acyclic DTPA class BCA (tiuxetan) (FIG. 5), and is approved for the treatment of relapsed or refractory low-grade, follicular or transformed B-cell NHL. This indication includes patients with Rituxan (rituximab)-refractory follicular NHL. Zevalin has been approved as part of a therapeutic regimen involving Rituxan. Phase I/II studies with Zevalin demonstrated the antitumour efficacy and safety in patients with recurrent B-cell lymphoma, and also established 'pre-treating' patients with unlabelled mAb as part of the treatment protocol⁶¹. Targeted radiation therapy with doses up to 50 mCi resulted in minimal non-haematological toxicity and durable clinical responses. 'Pre-treatment' of patients decreased splenic uptake and urinary excretion, whereas it increased relative disease-site

uptake. Both complete and partial responses (CRs and PRs) resulted from this approach, with a progression-free survival (PFS) of 72% 3–29+ months and a PFS of 78% with doses up to 40 mCi. Other trials have since established patient doses, and comparison to Rituximab (chimerized ibritumomab) immunotherapy indicated an improved overall response rate (ORR) (80% versus 56%) and a greater number of CRs (30% versus 16%). Furthermore, a 74% ORR was achieved with Zevalin in patients who failed Rituxan therapy. Interestingly, there has been a lack of correlation between dosimetric/pharmacokinetic parameters and haematological toxicity, indicating that toxicity might be inversely correlated with bone-marrow reserves in this patient population. When these various trials were integrated and analysed, a single dose of Zevalin was determined to be safe in patients with <25% bone-marrow involvement by NHL, adequate bone-marrow reserves, platelets >100,000 cells per μ l and neutrophils >1,500 cells per μ l⁶².

A second anti-CD20 mAb regimen — tositumomab and ¹³¹I tositumomab (Bexxar; GlaxoSmithKline) — received FDA approval in June 2003 for the treatment of patients with CD20-positive, follicular NHL, with and without transformation, whose disease is refractory to Rituxan and who have relapsed following chemotherapy. A Phase II multi-centre trial confirmed efficacy and safety in patients with relapsed or refractory low-grade or transformed low-grade NHL⁶³. A study with Bexxar also demonstrated improved survival in patients with chemorefractory NHL. As compared with 17% responding to chemotherapy, 65% of the Bexxar-treated patients experienced PRs or CRs averaging 6.5 months versus 3.4 months. The median response duration was ~eightfold longer for those receiving targeted radiation therapy versus chemotherapy only, with a corresponding CR of 20% and 3%, respectively⁶⁴. A randomized study comparing tositumomab alone and with Bexxar has been presented with the goal of determining whether the ¹³¹I provided any additional benefit to the patients. In the

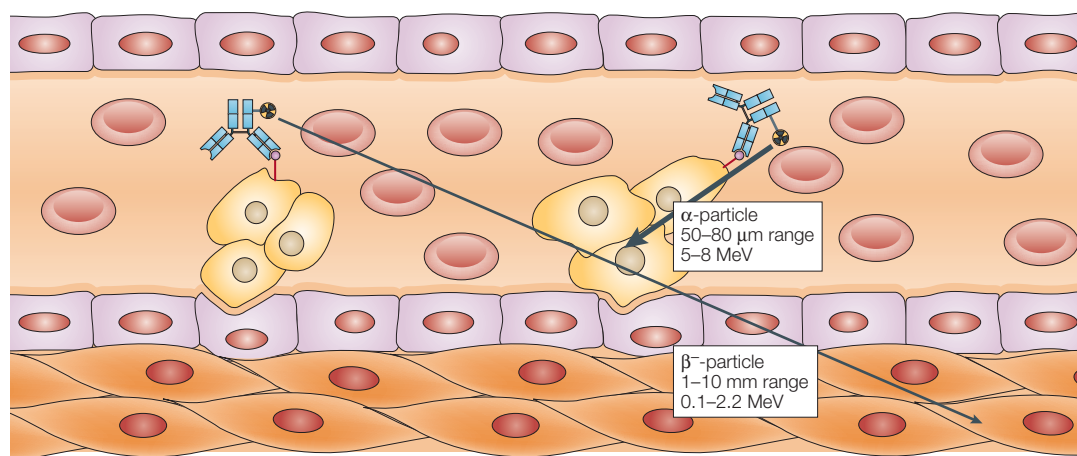


Figure 3 | Comparison of path lengths and emission tracks of α - and β -particle emissions used in antibody-targeted radiation therapy. The β -particle emissions occur in a spectrum of path lengths directly related to particle energy. The sparse energy track from these emissions is deposited over many cell diameters some distance from the decay event. The α -particle emissions occur at a discrete energy and path length, resulting in a high linear energy transfer. The dense energy track from these emissions is deposited directly from the decay event over only a few cell diameters, 50–80 μ m in tissues.

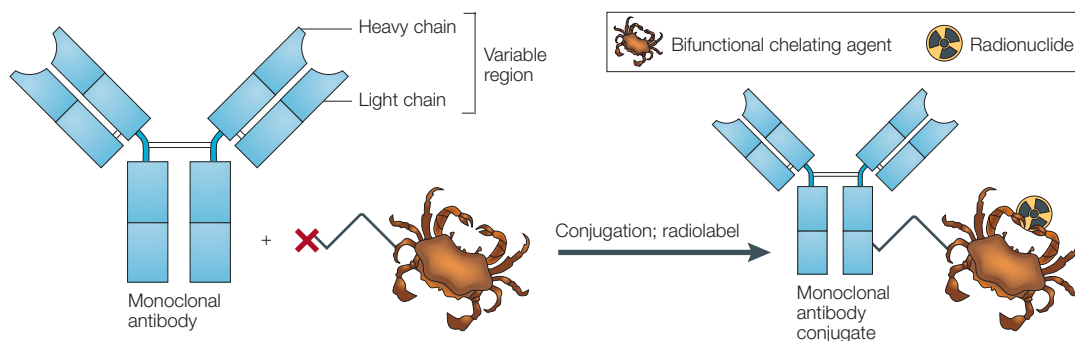


Figure 4 | A general view of the conjugation of a bifunctional chelating agent to a monoclonal antibody. Specifically, a bifunctional chelating agent possesses two functionalities. One portion binds (crab = chelos = chelate) metallic radionuclides, and the other portion bearing a reactive functional group (X) reacts and covalently binds to *N*-terminal and ϵ -amines from lysines on the antibody. In general, the metallic radionuclide is added last in this sequence, before purification of the final product; however, variations in which a pre-formed radiometal complex is conjugated to the monoclonal antibody are known.

words of the investigators, “all therapeutic outcome measures were significantly enhanced by the conjugation of ^{131}I to tositumomab.”⁶⁵ In a follow-up study of 1,071 Bexxar-treated patients, no increased incidence of treatment-related myelodysplastic disease or **acute myeloid leukaemia** was found when this was the initial therapy, which might be an expected outcome of conventional chemotherapeutic and radiotherapeutic regimens⁶⁶.

The CR rate, remission duration and the ORR from 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 transplantation (BMT) or stem-cell transplantation (SCT)^{60,67–70}. This is significant, as it is often found that therapeutic doses of radioactivity delivered by an mAb cannot be administered without BMT or SCT support. In one such trial, patients with relapsed B-cell lymphoma were treated with ^{131}I -labelled anti-CD20 mAbs, including Bexxar⁶⁷. Those patients developing myelosuppression underwent BMT. Complete remissions were noted, with several lasting up to 53 months. A later study with ablative Bexxar therapy and autologous SCT resulted in 86% ORs and 76% CRs. The PFS of these patients was 62% and the overall survival (OS) was 93%, with a median follow-up of two years⁶⁸. Direct comparison between high-dose targeted radioimmunotherapy (HD-RIT) and conventional high-dose radiotherapy (HD-XRT) has also been evaluated. A PFS of 48% and 29% with an OS of 67% and 53% for HD-RIT and HD-XRT, respectively, was reported⁶⁹. Disappointingly, most patients receiving HD-RIT eventually relapsed, leading to the proposal of including chemotherapy in the radioimmunotherapy regimen^{70,71}. A recent report describes a Phase II trial in which CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone) was followed by tositumomab/ ^{131}I -tositumomab for untreated follicular NHL⁷¹. Patients received CHOP therapy and, after four to eight weeks, a low dose of ^{131}I -tositumomab was administered for imaging and dosimetric calculations. Patients then received a therapeutic dose of ^{131}I -tositumomab. This treatment scheme resulted in 67% CRs and 23% PRs.

The two-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 Rituxan is ongoing. A treatment plan that combines chemotherapy, total body irradiation and radioimmunotherapy for the treatment of advanced **acute lymphocytic leukaemia** (ALL) and myelodysplastic syndrome (MDS) has also shown potential⁷². MDS patients treated with ^{131}I -labelled anti-CD45 mAbs had a median survival of 65 months (15–89 months), whereas the ALL patients had a disease-free survival (DFS) of 19, 54 and 66 months.

Issues pertaining to repeat treatments by either Bexxar or Zevalin potentially eliciting HAMA, although of concern owing to the murine nature of these mAbs, have been minimal. HAMA occurs <2% of the time with Zevalin, and there has been ~5–10% occurrence with Bexxar. Re-treatment has not been limited by this mechanism in immunocompetent patients.

Clinical trials at the University of California, Davis, USA, have focused on evaluating the therapeutic effectiveness of radioimmunotherapy using ^{67}Cu . Studies with ^{131}I -labelled Lym-1, a mAb with high affinity for the B chain of the HLA-DR10 antigen on malignant B lymphocytes, have demonstrated effectiveness in NHL patients. Patients who received up to 100 mCi per m^2 have responded to radioimmunotherapy⁷³. A fractionated radioimmunotherapy study treated patients with ^{131}I -Lym-1 at four-week intervals. Remissions (71%) averaged 14 months in duration, with acceptable haematological toxicity⁷⁴. A Phase I/II trial with ^{67}Cu -Lym-1 established the maximum tolerated dose (MTD) of a single dose at 50–60 mCi per m^2 . In a dose-fractionation study, scintigraphy was used to determine dosimetry and pharmacokinetics¹³. Tumour regression was noted, with one CR lasting 12 months, although one patient required transfusions after five doses totalling 524 mCi. Good imaging, a high therapeutic index and favourable dosimetry with a ^{67}Cu -labelled mAb was achieved. The liver received the highest radiation absorbed dose; however, no hepatotoxicity was evident, which is an important point, as uncomplexed

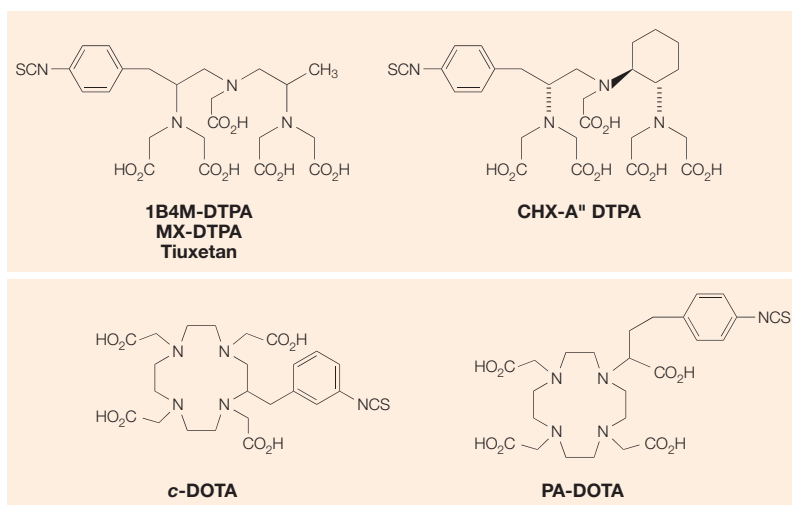


Figure 5 | Selected examples of bifunctional chelating agents that have been or are currently in antibody-targeted radiation therapy clinical trials. Conjugation occurs through the isothiocyanate of those agents depicted here. Metals/radiometals interact with the chelate via the amine and carboxylate groups.

Cu radionuclides localize in part to the liver⁷⁵. A higher peak concentration in tumours, as well as a longer biological half-life, was achieved with ⁶⁷Cu compared with ¹³¹I. The ⁶⁷Cu-labelled mAb delivered twice the mean radiation absorbed dose as ¹³¹I, thereby demonstrating the importance of radiometal being retained within cells, rather than the de-halogenation and loss of effective dose found with ¹³¹I (REF. 76).

This group has also evaluated Lym-1 radiolabelled with ⁹⁰Y. Therapy with ⁹⁰Y-Lym-1 (up to 0.37 GBq per m²) resulted in disease stabilization and PR. High absorbed radiation dose to the liver was noted and led to the modification of the BCA. A catabolizable peptide linker was introduced for cleavage by cathepsin in hepatocytes, thereby facilitating excretion of the radiochelate during metabolic processing of the conjugate^{77,78}. Clinical trials treating breast and prostate cancer using this linker as an element of the radioimmunoconjugate improved therapeutic indices. Tumour targeting was not significantly altered, no significant toxicities occurred and the absorbed radiation dose to the liver was reduced^{77,79}.

Adult T-cell leukaemia is an aggressive disease with a median survival of nine months. There is an overexpression of the IL-2 receptor by mature versus resting lymphocytes, thereby providing a selective target. A Phase I/II clinical trial was conducted to establish the dose and assess the efficacy of ⁹⁰Y-anti-Tac, which targets the IL-2 receptor⁸⁰. Patients were treated with 5, 10 or 15 mCi, with an additional cohort treated at 10 mCi. Additional cycles of radioimmunotherapy (average of three) were administered as patients responded to therapy. Nine of the sixteen evaluable patients responded to the ⁹⁰Y-anti-Tac radioimmunotherapy (PR = 7, CR = 2). At 20 months post-therapy, 35% of the patients were event-free and one patient remained in complete remission for more than three years. Six patients developed

HAMA after one or two cycles precluding continued therapy. A Phase I/II trial with the humanized form of anti-Tac is ongoing at present.

Expression of CD33 on early myeloid progenitor cells and myeloid leukaemia cells provides yet another molecular target. The murine anti-CD33 mAb M195 has been shown to target leukaemia in patients, and a trial applying ¹³¹I-M195 eliminated large tumour burdens⁸¹. Myelosuppression occurred at ≥ 135 mCi per m², allowing patients to undergo BMT. The effectiveness of the BMT was improved when ¹³¹I-M195 (120–230 mCi per m²) was combined with chemotherapy before a first or second BMT⁸¹. The data indicated that M195 could be effective in tumour reduction and that radioimmunotherapy had potential as part of a BMT regimen.

¹³¹I-M195 has also been shown to be effective in the reduction of minimal residual disease. Patients in remission following retinoic-acid therapy who were positive for retinoic-acid receptor before ¹³¹I-M195 therapy transiently converted to negative status following radioimmunotherapy. The median DFS was eight versus three months without radioimmunotherapy. Myelosuppression and the development of neutralizing HAMA permitted only a single dose of ¹³¹I-M195 to be administered to patients, thereby reinforcing two significant obstacles to the further development of murine mAbs⁸¹. A humanized M195 (HuM195) has since been developed and found to be effective.

Myeloablative therapy with a ¹³¹I-HuM195 requires several infusions to deliver sufficient radiation doses to the marrow. The half-life of ¹³¹I impedes re-infusion of the stem cells and requires the patient to remain hospitalized in isolation. Subsequently, efficacy of HuM195 labelled with ⁹⁰Y was evaluated in a Phase I trial. Patients with relapsed or refractory acute myelocytic leukaemia were treated with ⁹⁰Y-HuM195. A reduction in bone-marrow blasts was achieved and some patients showed no evidence of disease for two to four weeks; one CR was noted. Conclusions indicated that ⁹⁰Y-HuM195 had potential as a component of a transplantation regimen⁸².

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 patients, marking the first antibody-targeted radiation therapy trial in humans using an α -emitter. Real-time imaging, necessitated by isotope half-life, was used to estimate pharmacokinetics and dosimetry³⁰. The absorbed-dose ratio between the bone marrow, liver and spleen (sites of disease in leukaemia patients) and the whole body for the ²¹³Bi-labelled mAb was determined to be 1,000–10,000 times greater than ¹³¹I and ⁹⁰Y (REF. 30). Patients had reductions in their peripheral blood leukaemia cells and 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 some patients. Patients were treated with up to 95 mCi in up to seven dose fractions with no toxicities evident³⁰.

Multi-step 'pre-targeting' protocols have been designed to disconnect the radionuclide from the targeting vector to improve the therapeutic index^{83–85}. The goal

is to inject the mAb, allow the mAb sufficient time to target the tumour and to 'clear' remaining mAb from the circulation. One pre-targeting design exploits the affinity of biotin for streptavidin/avidin (SA) to maintain specificity. These protocols use a two- or three-step procedure to obtain adequate targeting, and then clearance, of circulating mAb. Clinical trials have been conducted in which the pre-targeting agent consisted of a mAb–biotin conjugate, while SA delivered the radionuclide⁸³. The reverse design has also been evaluated in clinical trials. In this case, the pre-targeting moiety consisted of a mAb conjugated with SA, followed by treatment with radiolabelled biotin^{84,85}. The SA–mAb localized at the tumour binds the radiolabelled biotin, whereas the unbound radiolabelled biotin is renally excreted, thereby enhancing tumour uptake versus background. The corollary to this strategy is that the vast majority of injected isotope is excreted, thereby requiring injection of large doses to counterbalance the clearance. Obviously, renal toxicity remains a concern when using this strategy. Using this latter motif, a Phase I/II trial was conducted that treated NHL patients with rituximab–SA conjugate injected sequentially, followed by a clearing agent (biotin-*N*-acetyl-glucosamine) and thereafter with 30 or 50 mCi per m² of ⁹⁰Y-DOTA-biotin⁸⁶. Patients responded favourably, with only transient haematological toxicities. Using an appropriate mAb as the targeting vector, pre-targeting radioimmunotherapy protocols might have a place in the repertoire of treatment regimens for cancer patients. However, the greatest caveat remains the immunogenicity of the streptavidin component, which limits repeat therapies. This aspect, in part, might be obviated by application of the mAb fragment–streptavidin fusion protein tetramer; however, use of these engineered proteins remains to be fully evaluated⁸⁷. One variation on pre-targeting has been the application of bispecific mAbs that bind to the cell and to a therapeutic element simultaneously⁸⁸. One limitation to this methodology is the elimination of bi-valency in mAb binding, which potentially limits or lowers cell-binding affinity. However, eliminating immunogenic streptavidin makes this an attractive technology. The creation of bispecific mAbs that initially bind a radiometal complex possessing a reactive group that then covalently and irreversibly binds to the mAb is truly innovative, as it creates an 'infinite affinity' for the complex⁸⁹. Evaluation in a clinical setting will provide a measure of the real value of this technology.

In summary, thirteen monoclonal antibodies have been approved by the FDA at present, and two of these are radiolabelled, both for the treatment of CD20⁺ NHL: Bexxar, a murine IgG2a anti-CD20 mAb labelled with ¹³¹I; and Zevalin, a murine IgG1 anti-CD20 mAb labelled with ⁹⁰Y via a BCA^{61,68}. A 67% overall response rate has been achieved in NHL patients receiving Zevalin⁶¹. Experience with a single dose of Bexxar in 582 patients in five clinical trials has resulted in responses lasting from three months to five and a half years. A total of 161 patients (28%) had a CR with the median duration of the response lasting 4.8 years. The results with these two agents show the effectiveness of enhanced efficacy from the targeted radionuclide. The results also illustrate the

rational approach of targeting a cell-surface antigen that is not shed or modulated, and which can also directly signal cell death through apoptosis, unlike other B-cell targets such as CD19 (REF. 46). This inherent activity of the target provides a true molecularly targeted combination therapy. 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 and excretion of the ¹³¹I is minimized. Considerable efforts continue towards fully understanding the mechanisms of action in such instances, as well as in those regimens that combine various drugs (for example, paclitaxel and doxorubicin) and radiosensitizers (Gemzar)^{71,90,91}. Although these might be acting in complete independence, some evidence exists — particularly with the anti-CD20 mAbs, trastuzumab and the anti-epidermal growth factor receptor antibody cetuximab (Erbix; ImClone/Bristol-Myers Squibb) — that additive, synergistic or complementary modes of action might make cells more sensitive to radiation, thereby enhancing the therapeutic index⁹².

Conclusion

After more than two decades, mAb-targeted therapies are generally recognized as making a significant impact on cancer therapy. The recent approvals of Zevalin and Bexxar have fuelled renewed enthusiasm for developing mAb-directed therapies. As such, their full potential is only now just beginning to be appreciated and understood.

However, despite the wealth of knowledge and capability in antibody engineering, the first two approved radiolabelled mAbs were murine in nature and subject to all of the resultant limitations; that is, immunogenicity and biological half-lives. Knowledge of actual clinical use of radiolabelled mAbs remains in its infancy in many respects, particularly with regard to therapies beyond lympho-haematological diseases, fractionated dosing and the rational construction of drug-combination cocktail therapies to functionally integrate targeted radiation therapy with established chemotherapies and external beam therapies. Clear evidence exists that value-added results are achieved by execution of these strategies.

Dominance of mAb therapies for the lympho-haematopoietic malignancies and their success in these cancers reflects their accessibility and radiosensitivity. Literature consensus seems to support mAb-based therapies of solid tumour applied in the treatment of minimal residual or micrometastatic disease and as a component of a multi-modality treatment regimen to provide the greatest benefit to patients. However, these limitations might have been arrived at through less than optimal targeting agents, sub-optimal chemistry, incorrect radionuclide choice and a less than rational experimental design, and so much of what has been achieved defines negative territory. There remains a continuing effort to refine and optimize all of the components to improve efficacy and minimize toxicity. The next decade should prove exciting as rational investigations applying the cumulative knowledge bring us towards making targeted radiation therapy a mainstream component for the treatment and management of cancer.

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Competing interests statement
The authors declare that they have no competing financial interests.

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