Radioimmunotherapy of human tumours

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Abstract | The eradication of cancer remains a vexing problem despite recent advances in our understanding of the molecular basis of neoplasia. One therapeutic approach that has demonstrated potential involves the selective targeting of radionuclides to cancer-associated cell surface antigens using monoclonal antibodies. Such radioimmunotherapy (RIT) permits the delivery of a high dose of therapeutic radiation to cancer cells, while minimizing the exposure of normal cells. Although this approach has been investigated for several decades, the cumulative advances in cancer biology, antibody engineering and radiochemistry in the past decade have markedly enhanced the ability of RIT to produce durable remissions of multiple cancer types.

Theranostics
A chemical moiety that can be used for both therapy and diagnostic purposes; for example, radioisotopes of iodine, ^{131}I and [124]I, can be used for both quantitative nuclear imaging and therapy.

Radioimmunotherapy (RIT) exploits the immune protein as a carrier for radioactivity, as a tracer or as a targeted therapeutic. The radioantibody is formulated as a drug in sterile and pyrogen-free form and is intravenously injected directly into the tumour or compartmentally into a body cavity such as the peritoneum, pleura or intrathecal space. Once injected, the radioantibody is distributed by blood flow, diffusion or convection to its natural target: an antigen-binding site on tumour cells. The radioactive cargo, in the form of a radionuclide that emits therapeutic quantities of particulate radiation, delivers the tumoricidal dose to the tumour mass. The radiation effects are due to the enormous energy release that occurs during radioactive decay, and is one of the most energy-efficient processes. For example, a tumoricidal radiation dose of 10,000 cGy requires ~6 picomoles per gram of the high-energy β-emitter yttrium-90.

Clinically, RIT is most widely applied to the most radiosensitive tumours, namely, leukaemias and lymphomas. Solid tumours are more radioresistant and require around fivefold to tenfold the deposited radiation doses for objective tumour response compared with leukaemias and lymphomas. Relative radiosensitivity or radioresistance is an intrinsic property of the cancer cell and correlates best with the cell of origin of the tumour. More radiosensitive normal tissues, such as those of the haematological system, give rise to tumours that tend to be considerably more radiosensitive; conversely, more radiation-resistant tissues, such as brain tissues or the bronchial epithelium, give rise to

more radioresistant tumours. Additional factors that increase radiation resistance include hypoxia and the ability to rapidly repair radiation-induced damage[1].

Regardless of intrinsic radiosensitivity, the goal of RIT is to safely deliver a high radiation dose to a tumour. One way to achieve this is by using RIT for situations in which the tumour is confined to an accessible body cavity or space, resulting in less dilution of the radioantibody as it homes in on its cancer-associated antigen target. Paediatric solid tumours such as central nervous system (CNS) metastases of neuroblastoma have shown excellent responses after intrathecal administration of therapeutic amounts of a radioantibody. For solid tumours, such as those in the pancreas, melanoma, prostate and colon, direct intravenous injection of a radioantibody has been relatively unsuccessful.

A more recent advance in RIT has been the development of quantitative methods for estimating the radiation-absorbed dose for human use, both for tumour tissue and for normal tissue, as a basis for individualizing patient treatment and for avoiding the toxicity that is associated with excessive radiation exposure. This fundamental concept is an example of a theranostics approach, in which the same reagent serves both a diagnostic and a therapeutic purpose; for example, the same radioisotope used in tracer quantities for diagnosis is followed by simple scale-up to larger quantities to achieve a therapeutic effect. Although in principle any nuclear imaging method may be used in theranostic approaches for RIT, the use of quantitative high-resolution positron emission tomography (PET)/computed tomography (CT)

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imaging of antibodies provides precise dosimetry to refine staging information that will improve patient selection and treatment planning as a prelude to effective treatment (BOX 1).

Features of the RIT approach

The therapeutic principle of RIT is based on the selective targeting of tumours relative to normal tissues, creating a therapeutic index. Ideally, this index would be infinite; that is, the radiation would be deposited only in the tumour. In practice, this ideal is never achieved because irradiation of normal radiosensitive tissues occurs during the process of targeting itself (that is, the bystander effect). The delivery of RIT is simple from the patient's perspective and may be more convenient than conventional chemotherapy. RIT is administered over a matter of minutes and it delivers the radiation payload over a timescale of days, during which the patient does not need to return for additional injections.

Tumour antigen targets. The selection of the optimal cell surface antigen and targeting antibody is crucial to the success of a therapeutic programme. An ideal antigen for RIT is highly expressed at a uniform density on the surface of all tumour cells, is not expressed on normal cells and is not 'shed' into the bloodstream.

A detailed list of antigen targets for clinically useful antibodies has been summarized in a recent review[2]. Antigenic targets are usually tumour cell surface-expressed macromolecules, which are easily accessible from the blood and the extracellular fluid, and include the haematopoietic cluster of differentiation (CD) antigens that are expressed during haematopoietic maturation of distinct cell lineages. These antigenic targets also include cell surface glycoproteins (for example, mucins); enzymes, such as prostate-specific membrane antigen (PSMA) and carbonic anhydrase IX (CAIX); glycolipids, such as GD2; carbohydrates, such as Lewis[y]; stromal components (for example, fibroblast activation protein-α (FAPα)); components of blood vessels (for example, integrins, vascular endothelial growth factor receptor (VEGFR) and the amino domain of fibronectin B); and signal transduction molecules (for example, growth factor receptors, epidermal growth factor receptor (EGFR) and HER2 (also known as ERBB2)).

Although no perfect antigen–antibody pair for RIT exists, several excellent targets have been identified for lymphoma, including CD20, CD22 and human leukocyte antigen-DR (HLA-DR) for B cell non-Hodgkin lymphoma (B-NHL); CD33 and CD45 for acute myeloid leukaemia (AML) (FIG. 1); and PSMA and the extra domain B (ED-B) of fibronectin for solid tumours[3–11]. In particular, CD20 is a successful target in B cell malignancies. CD20 is a 35,000 Da non-glycosylated phosphoprotein that is expressed on the surface of nearly all mature B lymphoid cells and in 95% of B cell lymphomas[12,13].

The metabolism of the antibody–antigen complex is an important consideration in the choice of the optimal radionuclide for use in RIT. Metabolism of the radionuclide by the cell may either enhance the anticancer effects by retaining the radionuclide within lysosomes or storage proteins, or reduce the radiation effects by expelling the radioactivity from the cell. Some antigens, such as CD5 or PSMA, are rapidly internalized by the cancer cell with resulting catabolism, including disconnecting the radiolabel from the antibody. Non-residualizing radiolabels such as radioactive iodides are rapidly released as a result of catabolism after internalization, whereas radiometal labels tend to be sequestered within the cell and retained (residualized). Other types of antigens such as GPA33 and CD20 are turned over much more slowly, and non-residualizing isotopes may be retained on the tumour cell membrane with relatively slow release.

Residualizing or radiometal labels are conducive to RIT because they are likely to be highly concentrated in neoplastic tissue, owing to progressive antigen–antibody complex internalization. However, a residualizing label may also be retained in normal tissue, such as liver or kidney tissue, leading to concerns about radiation damage (BOX 2).


Box 1 | **Dosimetry: estimating radiation deposited in tumours and normal tissue from RIT**

Radiation effects on biological tissues are caused by the energy emitted by radioactive decay that is deposited in tissues. For radioimmunotherapy (RIT) we are most concerned with radioisotopes, which decay with particulate and non-penetrating radiations, such as α-particles, β-particles, auger emission or low-energy X-rays. As not all components of tissues and cells are equally sensitive, the site of deposition of the radiation energy is also important, as is the distance over which the radiation energy is deposited at the tissue level, which is often referred to as linear energy transfer.

Internal radiation doses are computed by an established set of equations that convert the energy deposited in tissue into units of radiation-absorbed dose (rad) or centigray (cGy). The Committee on Medical Internal Radiation Dosimetry (MIRD) has developed a phantom validated approach that is most applicable to normal organs. This has been adopted by the US Food and Drug Administration (FDA) as a basis for estimating radiation doses to whole normal organs.

Because organs comprise multiple types of tissues and cells, microdosimetry is currently the subject of considerable development. Organ microdose and tumour dose must take into account the size of the irradiated tissues and the linear energy transfer path length to accurately estimate the true radiation-absorbed dose.

As a rule of thumb, tumour response will depend on the amount of tissue being irradiated, as well as the radiation sensitivity of the tissue. Radiosensitive tumours such as lymphomas may have complete responses with radiation doses in the range of 1,500–2,000 cGy, and solid tumours typically require 3,500–10,000 cGy for a meaningful response[122,123]. Normal tissue radiosensitivity also varies from the bone marrow (typically >150 cGy) to the lung and kidney (1,500–2,000 cGy). Owing to its quantitative nature, positron emission tomography (PET) imaging has been introduced as an optimal tool for theranostic imaging to determine radiation-absorbed doses to tumour and normal tissues[124].
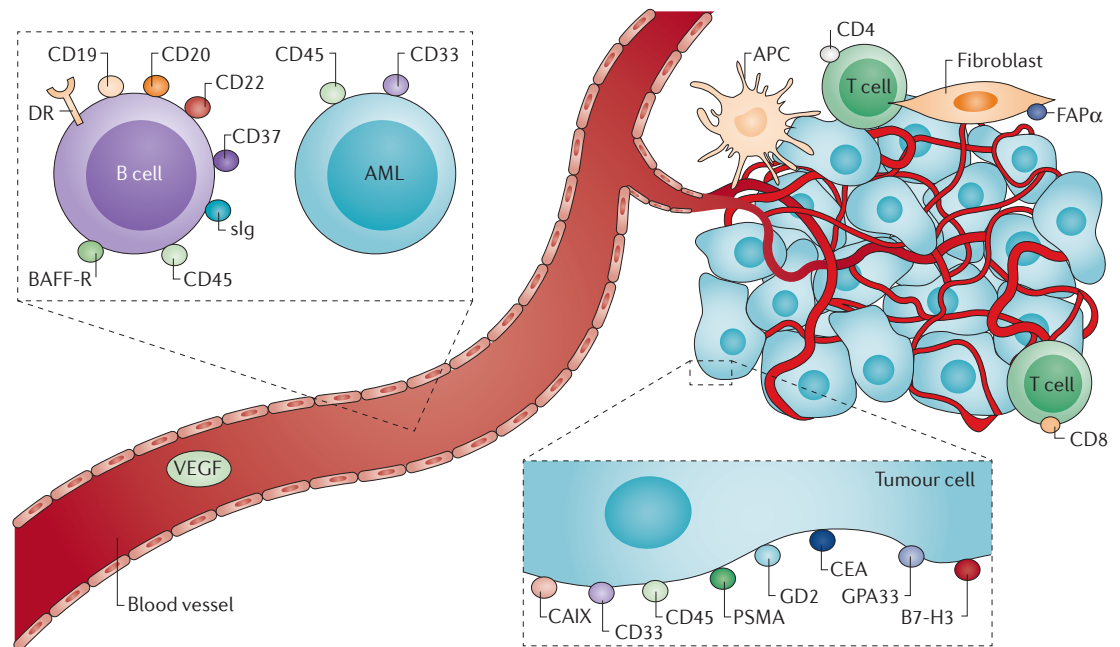
Figure 1 | Target antigens for RIT. Target antigens for radioimmunotherapy (RIT) are most commonly accessible antigens on the cell surface of the neoplastic or stromal cell, and are less commonly molecules that may be abundantly produced in the tumour environment. The simple case is to target antigens on myeloid cells, and to a certain extent lymphomas, which are freely circulating in the blood and bone marrow. Radiolabelled antibody therapy of B cell lymphoma includes the receptor for B cell activating factor (BAFF-R), class II histocompatibility antigens (HLA-DR), surface immunoglobulin (sIg), and cluster of differentiation antigens CD19, CD20, CD22, CD37 and CD45. Target surface antigens for RIT of myeloid leukaemias include CD33 and CD45. For solid tumours there is also induction of stroma, vascular and inflammatory cell components; in principle, RIT can be applied to any one of these components (for example, to fibroblast activation protein-α (FAPα)), and commonly used cancer cell membrane targets include carcinoembryonic antigen (CEA), prostate-specific membrane antigen (PSMA), GD2 ganglioside antigen (GD2) and carbonic anhydrase IX (CAIX). AML, acute myeloid leukaemia; APC, antigen-presenting cell; VEGF, vascular endothelial growth factor.

β-particles
Electron-like negative particles emitted from the nuclei of β-emitting radionuclides.

α-particles
Particles the size of a helium nucleus made up of two protons and two neutrons, produced by α-emitting radionuclides (for example, ^{225}Ac).

Bremsstrahlung
A type of electromagnetic radiation produced when a high-energy charged particle is decelerated or deflected by another charged particle.

Myelosuppression
A condition in which bone marrow activity is decreased, resulting in fewer red blood cells, white blood cells and platelets.

Cardiopulmonary toxicities
Adverse effects on the blood systems, heart or lungs, resulting from exposure to toxic chemicals, for example, cardiac ischaemia, pulmonary inflammation and an increased level of toxins in the blood.

Linear energy transfer
(LET). The action of radiation on matter that describes how much energy an ionizing particle transfers to the material transversed per unit distance.

Radionuclide selection. Radiation from RIT kills cells when their DNA is damaged beyond the capacity of the cancer cell to repair. Multiple therapeutic radionuclides that are tightly attached to antibodies by selective chemistries are available and may be chosen either alone or in combination to suit a specific treatment purpose. Radionuclides available for therapy emit particulate radiation (β-particles, α-particles or auger emission) that deposits a considerable amount of their radiation energy in the tumour mass (see Supplementary information S1 (table)).

The choice of the optimal radionuclide for RIT depends on both its intended use and the practical considerations governing its specific application. ^{131}I and ^{90}Y, both β-particle-emitting isotopes, have been used in >95% of clinical RIT trials and represent the current standard to which all other radionuclides are compared[14–17]. ^{131}I and ^{90}Y qualify for RIT because of their favourable emission characteristics, availability and tractable radiochemistry, which permits their reliable and stable attachment to antibodies. Furthermore, hundreds of published clinical trials attest to their efficacy for the treatment of both haematological and solid malignancies. Both isotopes have certain advantages: ^{131}I is relatively inexpensive, can be used for both imaging and therapy, and has a long successful history of treating several malignancies, including thyroid cancer, NHL and AML.

However, ^{131}I-labelled proteins degrade rapidly if endocytosed into tumour cells, resulting in the release of ^{131}I-tyrosine and free ^{131}I into the bloodstream[18–20]. In addition, the γ-rays emitted by ^{131}I may pose a radiation risk to family members and healthcare personnel, and patient hospitalization for radiation isolation may be required if large doses are injected.

^{90}Y is a reasonable alternative β-emitter for therapeutic studies, and it emits β-particles almost exclusively. As this form of radiation does not leave the patient's body, caregivers and family members are exposed to lower levels of radiation. ^{90}Y emits β-particles that are fivefold more energetic than those of ^{131}I, emits relatively weak electromagnetic radiation (Bremsstrahlung), is easily administered to outpatients and is stably retained by tumour cells even after endocytosis. For both ^{131}I and ^{90}Y, dose-limiting myelosuppression at conventional doses[21,22] and cardiopulmonary toxicities at myeloablative doses used in the setting of stem cell transplantation may be observed[4,23].

α-particle-emitting radionuclides have very high potency, making them attractive alternatives, or adjuncts, to β-emitting radionuclides in RIT[24,25]. This higher potency is due to the fact that the emission of an α-particle releases a large amount of energy in a linear manner within a few cell diameters (50–90 μm). The high linear energy transfer (LET) of α-emitters (~100 keV per μm)

confers a high relative biological effectiveness (RBE) for cell killing[26–28]. High RBE derives from the fact that the extent of damage (for example, DNA double-strand breaks) to the cell from α-particle exposure is so great that cell repair mechanisms are not effective and the cell undergoes apoptosis or necrosis. Furthermore, unlike β-emitting radionuclides, the effect of oxygen on cell killing is minimal, and thus effective cell killing can be expected even in areas of the tumour that are hypoxic[26,28]. Owing to availability and decay properties, only a few α-emitting radionuclides are considered suitable for *in vivo* applications, including [213]Bi (biological half-life ($t_{1/2}$) = 45.6 min), [211]At ($t_{1/2}$ = 7.2 h), [225]Ac ($t_{1/2}$ = 10 d)[27–34], [223]Ra ($t_{1/2}$ = 11.4 d) and [212]Pb ($t_{1/2}$ = 10.6 h).

Pharmacology of antibody–antigen targeting

The molecular pharmacology of antigen targeting by an antibody takes into account time-dependent biodistribution in physiological spaces after parenteral injection. A major goal is to describe the immunokinetics of radioactive antibody targeting in a mathematical model, which comprehensively characterizes the factors that determine the therapeutic index and the radiation-absorbed dose for tumour tissue. A practical purpose for this modelling strategy is to identify approaches that will lead to optimized RIT under the proposed conditions of use.

Such a model has been developed for fairly straightforward two-compartment situations in which [131]I–3F8 or [131]I-8H9 monoclonal antibodies are administered intrathecally for the treatment of recurrent neuroblastoma that is metastatic to the leptomeninges. These radioantibodies are injected into the intraventricular space in the brain, followed by subsequent distribution through the cerebrospinal fluid (CSF; approximately 150 ml of volume). A simplified assumption for this model, which is reasonable for leptomeningeal metastasis, was that the distribution of the tumour was only one cell thick, such that the issue of diffusion of the antibody through the tumour mass could be ignored[35]. A series of differential equations was used to provide a mathematical description of the compartments, including rates of exchange through both bulk flow and diffusion of the antibody, half-life of the isotope, specific activity of the antibody, percentage of the radioantibody that was immunoreactive and antigen density on the tumour. Both antigen-specific binding to the tumour and nonspecific binding to normal tissues was also considered.

The results showed a high correlation between the observed clearance and distribution within the CSF. The model made a number of useful predictions, which were subsequently implemented into clinical practice, including increasing immunoreactivity from 10% to 80%, which improved the therapeutic index by 7.4-fold; dividing the single therapy dose into four doses with a mass of around 1.4 mg each for a radioantibody with an affinity of 10^{-9} litres per mole (l m^{-1}); and immunoreactivity of 50% was predicted to be sufficient to deliver more than 100 Gy to the tumour with less than 10 Gy to normal tissues. The radioantibody distribution is documented in FIG. 2, which shows a quantitative PET image of [124]I-8H9 obtained at 2 hours, 24 hours and 48 hours after intrathecal injection of the radioantibody. Major responses and improvement in long-term survival after these CNS events have been achieved using this compartmental RIT (cRIT) approach[36,37] (see Supplementary information S2 (figure)). The published survival curve for the first 21 patients with neuroblastoma treated with an intra-Ommaya cRIT-based regimen, compared with patients with CNS neuroblastoma who were treated with conventional regimens without cRIT, is shown in Supplementary information S3 (figure). In a recent update presented at the Advances in Neuroblastoma Research conference (Cologne, Germany, 2014), survival data on 43 patients with CNS neuroblastoma who were treated with cRIT-based therapy demonstrated an overall survival of 62%, with a median survival of 5.3 years (1.3–10.8 years)[38].

For the intravenous administration of RIT, the situation is considerably more complex, and mathematical models that are intended to quantitatively describe the

Half-life
The characteristic period of decay during which half of the population of radioactive atoms will undergo spontaneous radioactive decay.

Leptomeninges
The two innermost layers of tissue (arachnoid mater and pia mater) that cover the brain and spinal cord.

process of antibody–antigen targeting must take a number of additional factors into account, such as the large volume of distribution of antibody *in vivo* (up to 15 litres for the extracellular space in adults), and must include specific uptake in the actively competing non-tumour tissues that may contain the antigen, as well as catabolism and clearance in the liver, kidney and gut. At the tumour site, there is evidence that the binding of antigen and antibody drives uptake after intravenous administration, such as occurs after the intrathecal administration of cRIT. For example, in a recent study using intravenously administered [124]I–A33 antibody for patients with colorectal cancer, the uptake was demonstrated to be linearly related to the expression of GPA33 antigen on the cell surface[39,40]. This finding is consistent with the chemical laws of mass action, and indicates that a complete model that accurately describes *in vivo* targeting must also include nonlinear or saturation kinetics (see Supplementary information S4 (figure)).

Wittrup *et al.*[41] have developed a practical guide for selecting targeting agents for optimal uptake in to the tumour mass itself after intravenous administration, using a kinetic model that is based on chemical engineering principles. Their guide includes a set of design features for tumour targeting agents with respect to agent size, binding affinity and target antigens. Examples have principally been provided from mouse studies, which offer partial validation of the model predictions.

This theoretical analysis suggests that tumour targeting agents the size of whole IgG — that is, 20 nm or so in diameter — strike the right balance between diffusion rates into the tumour mass and renal clearance to allow for optimal tumour uptake. By contrast, agents that are much smaller (that is, 6–8 nm in diameter), such as Fv fragments, are excreted through the kidney too rapidly

to diffuse into the centre of the tumour mass. The model also takes into account pharmacological dose, binding affinity of the agent for its cognate antigen and antigen expression level. This model predicts that for binding affinities of less than 10 nM or so, molecules the size of IgG are not greatly affected, as affinity increases with regard to uptake, but also predicts that smaller molecules (less than 5 nm) benefit greatly from high affinity with increased uptake. Emphasis is also placed on a balance between mass delivered to the tumour and binding affinity. Weinstein *et al.*[42] introduced the term 'binding site barrier', and proposed that the uptake of very high affinity antibodies could be limited to the periphery of tumours, unless the appropriate pharmacological dose was used to allow the saturation of outer binding sites, so that diffusion into the centre of the tumour mass could occur. An additional contribution provided by Wittrup *et al.* is the important role that the endocytosis of the antigen–antibody complex may have in limiting diffusion throughout the tumour mass. Thus, there is a balance needed among diffusion, antigen–antibody binding and internalization with respect to *in vivo* targeting.

RIT of haematological malignancies

RIT is a particularly attractive approach for haematological malignancies for a number of reasons, including the fact that many lineage-specific cell surface antigens that are not expressed on other tissues have been identified; a multitude of high-quality antibodies that target haematological malignancies are available; leukaemias and lymphomas are exquisitely sensitive to radiation therapy; and human anti-mouse antibodies (HAMAs) are less likely to form in patients with haematological malignancies than in patients with solid tumours owing to the inherent immunosuppressive nature of haematopoietic malignancies. In addition, the widespread availability of haematopoietic cell transplantation makes myeloablative RIT an attractive option to increase the radiation dose that is delivered to malignant cells, while sparing patients from unacceptable extramedullary toxicities[4,10,23,43–46]. This is particularly true when the patient's own stem cells can be harvested before receiving the high dose of RIT; this type of autologous transplant (also known as stem cell rescue) has become routine in many oncology centres, and is also used in the context of high-dose chemotherapy.

The initial studies[2] that were carried out using high-dose RIT in myeloablative doses followed by bone marrow transplant set a high standard for the magnitude of response (>80% complete remissions), as well as duration of response (median >5 years), and a number of patients with advanced B cell malignancies were permanently cured (TABLE 1). However, the technical challenge of bone marrow transplantation and high-dose ^{131}I labelling discouraged widespread application. The development of outpatient RIT regimens using smaller doses of radiation, along with tailored doses based on individualized patient clearance and the metabolism of a diagnostic level pre-dose, gave the entire effort a greater impetus, and yielded two US Food and Drug Administration (FDA)-approved drugs: one labelled

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Ommaya reservoir
A device surgically placed under the scalp and used to deliver anticancer drugs to the cerebrospinal fluid.

Human anti-mouse antibodies
(HAMAs). Antibodies found in humans that react to immunoglobins found in mice.
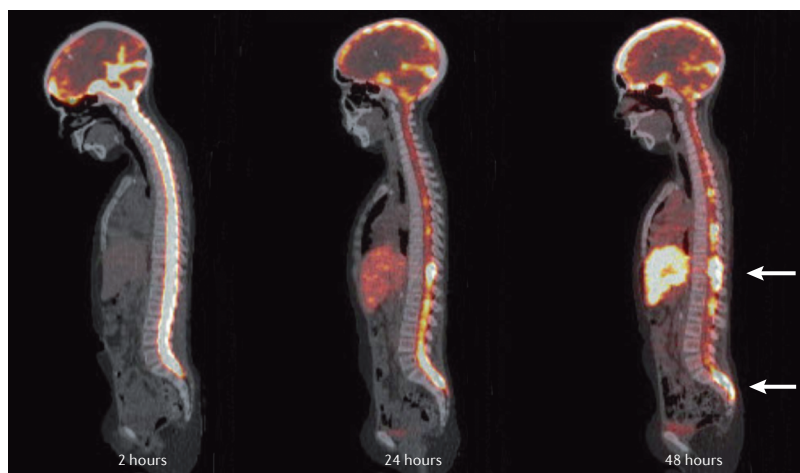
Figure 2 | **Intrathecal RIT imaged quantitatively with PET imaging using [124]I–8H9 antibody.** The images illustrate the localization of radioactivity to leptomeningeal tumours over the course of 72 hours. All images are sagittal images through the intrathecal space. Immediately after intrathecal injection via an Ommaya reservoir, a 2-hour image shows complete filling of the intrathecal space, with distribution throughout the cerebrospinal fluid (CSF) and progressive clearance at 24 hours and 48 hours, except at the tumour site. At 48 hours, there is focal uptake at tumour sites, which is evident in the thoracic and lumbar spine (arrows). PET, positron emission tomography; RIT, radioimmunotherapy.

Table 1 | **Clinical experience with RIT in lymphomas and leukaemias**

Treatment approach	Therapy antibody	Antigen target	Study population	Special feature	Main findings	Refs
Lymphomas						
Non-myeloablative doses	^{131}I-Lym 1	HLA-DR	Relapsed B cell malignancies	First-in-human RIT in lymphoma	• 52% ORR • 33% CRs • Main toxicity: thrombocytopenia	6
	^{131}I-tositumomab	CD20	Relapsed B cell malignancies	FDA approval after pivotal trials	• 60–80% ORR • 15–40% CRs	21,47, 48
	^{90}Y-ibritumomab tiuxetan	CD20	Relapsed B cell malignancies	FDA approval after pivotal trials	• 60–89% ORR • 15–40% CRs	5,22
	^{90}Y-epratuzumab	CD22	Relapsed B cell malignancies	Fractionated doses used	• 61% ORR • 48% CRs	8
	[131]I-, [90]Y- various radioantibodies	CD20	Newly diagnosed: with or without chemotherapy	Phase II	• 90–100% ORR • 60–100% CRs	3, 60–67
	^{131}I-tositumomab	CD20	Newly diagnosed: CHOP versus consolidation	Phase III randomized	• 2-year survival 97% • 2-year PFS 80%	69
	^{90}Y-ibritumomab tiuxetan	CD20	Newly diagnosed: observation versus consolidation with RIT	Phase III randomized	PFS 37 months versus 13.5 months favouring RIT consolidation	68, 131
	• B9E9FP -streptavidin fusion protein • ^{90}Y-DOTA	CD20	Relapsed B cell malignancies	Phase I: pre-targeted radioimmunotherapy	• Average tumour-to-whole body radiation dose ratio of 49:1 • 2 of 15 CRs • Minimal haematoxicity	113
Myeloablative doses with haematopoietic stem cell transplant	• ^{131}I-B-1 and 1F5 • ^{131}I-MB-1	• CD20 • CD37	Relapsed or refractory B cell malignancies	Autologous stem cell rescue	• ORR 85–90% • CRs 75–80% • PFS 10–20 years	4,23
	^{90}Y-ibritumomab tiuxetan	CD20	Relapsed or refractory B cell malignancies	• Combined with high-dose BEAM • Dosimetry with ^{111}In-ibritumomab	• PFS after 3 years: 43% • 15 Gy to critical organs (liver, lungs and renal) is MTD	44
	^{90}Y-ibritumomab tiuxetan	CD20	Relapsed or refractory B cell malignancies, post-chemotherapy	High-dose 1.2 mCi per kg, with autologous rescue	• PFS after 30 months: 69% • OS after 30 months: 89%	45
	^{90}Y-ibritumomab tiuxetan	CD20	Relapsed or refractory B cell malignancies, post-chemotherapy	Etoposide and cyclophosphamide combination therapy plus autologous stem cell rescue	• 2-year PFS: 78% • 2-year OS: 92% • Low toxicity	46
	^{131}I-tositumomab	CD20	Relapsed or refractory B cell malignancies, post-chemotherapy	Etoposide and cyclophosphamide combination therapy plus autologous stem cell rescue	• 2-year PFS: 68% • OS after 24 months 83%	58
Leukaemias						
Non-myeloablative doses	^{131}I-M195	CD33	Acute myeloid leukaemia	Biodistribution with γ-camera	Retention in bone marrow, liver and spleen	132
	[213]Bi-M195	CD33	Acute myeloid leukaemia	First antibody trial using an α-emitting radionuclide in humans; with or without cytarabine	• Reduced blasts in 14 of 18 patients • Some CRs • Reversible blood cell suppression	73
	[225]Ac-M195	CD33	Acute myeloid leukaemia	Phase I trial underway in combination with cytarabine (NCT01756677); nanogenerator concept	• Reduced blasts • Reversible blood cell suppression	27,73
Myeloablative doses	^{90}Y-M195	CD33	Acute myeloid leukaemia	Bone marrow ablation (NCT00006040)	Bone marrow transplant preparative regimen	–
	^{131}I-BC8	CD45	Acute myeloid leukaemia; myelodysplasia	Preparation for bone marrow transplant by RIT to bone marrow	• Effective pre-transplant regimen • Lower relapse rates at higher bone marrow doses	10,43, 72

CD, cluster of differentiation; CR, complete response; FDA, US Food and Drug Administration; HLA-DR, human leukocyte antigen-DR; MTD, maximum tolerated dose; ORR, overall response rate; PFS, progression-free survival; RIT, radioimmunotherapy.

with ^{131}I (^{131}I-tositumomab) and another with ^{90}Y (^{90}Y-ibritumomab tiuxetan). Both agents target CD20 as a part of regimens that treat B cell lymphomas and show considerable activity in non-myeloablative regimens of modest toxicity.
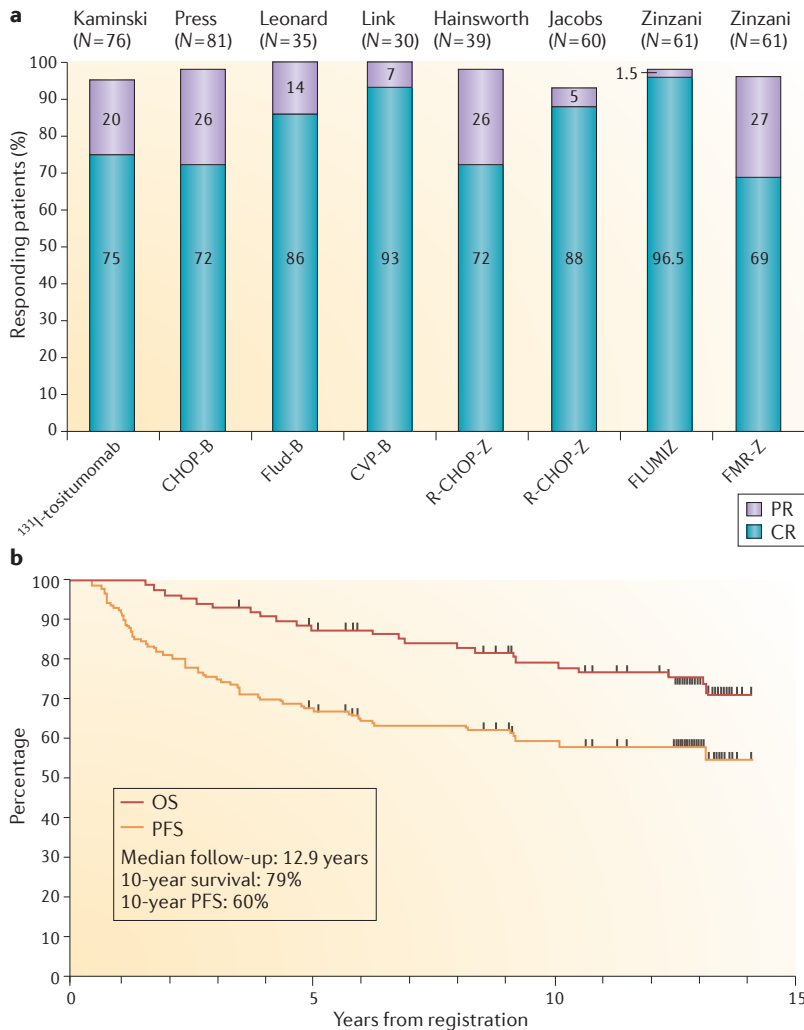
The majority of RIT clinical trials for haematopoietic tumours have focused on radiolabelled CD20 antibodies (TABLE 1). CD20 antibodies conjugated to ^{131}I or ^{90}Y produce higher overall response rates (ORRs) and complete response rates (CRs) — 60–80% ORR and 15–40% CRs — in relapsed NHL than unlabelled antibodies, such as rituximab[3–5,23,47–49], as demonstrated in two randomized

Figure 3 | **Results of selected trials of RIT as part of front-line therapy for follicular lymphoma.** **a** | Overall response rates, partial remission (PR) rates (purple), and complete remission (CR) rates (blue) are indicated for eight studies that used ^{131}I-tositumomab alone[3]; cyclophosphamide, doxorubicin, vincristine and prednisone followed by ^{131}I-tositumomab (CHOP-B)[60]; fludarabine followed by ^{131}I-tositumomab (Flud-B)[61]; cyclophosphamide, vincristine and prednisone followed by ^{131}I-tositumomab (CVP-B)[67]; rituximab plus CHOP followed by ^{90}Y-ibritumomab tiuxetan (R-CHOP-Z)[129]; fludarabine plus mitoxantrone followed by ^{90}Y-ibritumomab tiuxetan (FLUMIZ)[64]; or fludarabine, mitoxantrone, rituximab and zevalin followed by ^{90}Y-ibritumomab tiuxetan (FMR-Z)[130]. Data are taken from the published studies. **b** | Progression-free survival (PFS) and overall survival (OS) of 90 eligible patients with advanced follicular non-Hodgkin lymphoma treated with six cycles of CHOP chemotherapy followed by tositumomab/ ^{131}I-tositumomab on SWOG protocol S9911 (O.W.P., unpublished observations).

studies[22,50]. The median remission duration with non-myeloablative RIT has been 1 or 2 years in most studies, with 15–20% of patients achieving sustained remissions, and in some cases, remission duration of 10 years or more[51].

RIT has been well tolerated, though myelosuppression, fatigue, thyroid dysfunction (with ^{131}I) and HAMA formation have been observed. Myelosuppression and secondary malignancies have been reported, but their incidence is not increased when compared with patients treated with chemotherapy[52]. Because of the higher risk of myelosuppression in patients with significant bone marrow involvement (>25%) or limited bone marrow reserve ^{131}I-tositumomab or ^{90}Y-ibritumomab tiuxetan are not recommended. Nonetheless, some studies suggest that lower adjusted activities may be administered[53]. The incidence of delayed second malignancies or myelodysplasia associated with RIT has been reported for patients with haematological malignancy treated with ^{131}I-tositumomab as 3.5%[52] and treated with ^{90}Y-ibritumomab tiuxetan as 2.5%[54].

Five strategies have been proposed to enhance the durability of responses: incorporation of RIT into front-line treatment for NHL; use of myeloablative doses of RIT with autologous haematopoietic stem cell transplant; multistep 'pre-targeting' protocols (discussed below); combining RIT with other monoclonal antibodies[55]; and simultaneous targeting of multiple B cell antigens[11] (see TABLE 1 for a summary of myeloablative approaches). With the advent of outpatient haematopoietic stem cell transplantation, RIT alone or in combination with other treatments is becoming increasingly practical.

In this regard, preclinical investigations suggest that treatment with the chemotherapy drugs fludarabine and cytosine arabinoside is synergistic with RIT, and that treatment with etoposide, doxorubicin and camptothecins can produce supra-additive benefits when combined with radiolabelled antibodies[56]. Clinical trials to assess the efficacy of treatment with concurrent fludarabine and high-dose RIT[57] or treatment with etoposide, cyclophosphamide and RIT[58] have validated the promise of these combinations. An alternative approach is to combine RIT targeting one antigen with unlabelled monoclonal antibodies targeting a different antigen, as has been done with ^{90}Y-epratuzumab tetraxetan (anti-CD22) combined with veltuzumab (anti-CD20)[59].

Incorporation of RIT into front-line therapy of NHL. Seven Phase II studies and two Phase III studies have tested RIT in patients with newly diagnosed NHL who received front-line therapy either alone or as consolidation following chemotherapy[3,60–67]. These studies have all demonstrated efficacy with ORRs of 90–100% and CRs of 60–100% (FIG. 3a). In addition, the CRs induced by this approach have been very durable, with median remission durations exceeding 6 years in many studies[3,60] (FIG. 3b). Upfront RIT converted many partial responses elicited with immunotherapy or chemotherapy to CRs, and thus many PCR-positive patients who expressed specific tumour cell-associated DNA became PCR-negative

patients[60,61,64]. The efficacy of this strategy has been validated in a Phase III randomized trial of ^{90}Y-ibritumomab tiuxetan consolidation after first remission in advanced stage follicular lymphoma[68]. These findings have led to the regulatory approval of ^{90}Y-ibritumomab tiuxetan RIT as first-line consolidation therapy in both Europe and the United States. A second Phase III study comparing a front-line CHOP chemotherapy regimen with ^{131}I-tositumomab consolidation with CHOP chemotherapy plus six doses of rituximab did not reach statistical significance[69,70].

Despite the safety and efficacy of RIT for lymphomas and the approval of two radioimmunoconjugates by the FDA, this therapeutic modality is less frequently used than chemotherapy regimens, and one of the approved agents, ^{131}I-tositumomab, is no longer marketed. The limited adoption of RIT by the medical community, despite its advantages, seems to have resulted from a combination of factors, including concerns about inducing myelodysplasia, the availability of multiple novel competing targeted agents (ibrutinib, idelalisib and brentuximab vedotin), and the inability of practicing haematologists and oncologists to administer the agents in their offices[71]. It remains to be seen whether future innovations (such as pre-targeting or α-emitters) will sufficiently enhance the efficacy of the approach to overcome these practical limitations, particularly with the probable emergence of additional competing treatments, including antibody–drug conjugates, which have shown considerable promise in early clinical trials and which present fewer logistical hurdles for practicing physicians.

RIT for other haematological malignancies. AML has also been effectively treated using RIT targeting CD33, CD45 or CD66 (REF. 72) (TABLE 1). Of particular promise in this setting are the α-emitters [212]Bi and [225]Ac. Radioimmunoconjugates of [225]Ac act as atomic nanogenerators, delivering cascades of α-particles to cancer cells, resulting in a potency estimated to be 1,000-fold greater than that of [213]Bi-conjugates, and perhaps 5,000–10,000 fold the potency of the β-emitters[27,73]. Promising preclinical and clinical studies of RIT have also been conducted targeting CD66 for AML, CD5 for

chronic lymphocytic leukaemia, CD30 and ferritin for Hodgkin lymphoma, CD25 for acute T cell leukaemia and lymphoma, and CD45 for peripheral T cell lymphomas[74–77]. These radioimmunoconjugates are likely to grow in importance in the future.

RIT of solid tumours

A large number of clinical trials with intravenously administered RIT have been reported over more than three decades, with modest clinical results (TABLE 2; see Supplementary information S5–S7 (tables)). The extensive experience with IgG-based RIT comprising various radionuclides leads to the conclusion that the therapeutic index for the antibody–antigen systems tested thus far is insufficient. This is because target-to-background ratios for tumour-to-normal tissue are inadequate owing to the tendency of the IgG molecule to distribute to blood and other organs, as well as normal tissues of the liver and especially bone marrow. Dose-limiting toxicity is almost exclusively haematopoietic. Observed responses include generally stable disease or a reduction in tumour biomarkers (TABLE 2). In a few cases, there is a suggestion of enhanced survival, but actual shrinkage of the tumour and well-documented RECIST (Response Evaluation Criteria in Solid Tumours) responses[70] are few and far between. An approach to increase radiosensitivity by combining ^{90}Y-clivatuzumab tetraxetan (hPAM4), an antibody that recognizes pancreatic cancer, with low-dose gemcitabine has shown objective tumour responses by RECIST, with 16% partial remission[78]. These data have led to an ongoing Phase III double-blind, randomized trial (ClinicalTrials.gov identifier: NCT01510561; see Further information).

However, when the radioimmunoconjugate is injected directly into the body compartment in which the tumours are confined, tumour shrinkage and long-term impact on survival has been observed (further discussed below). Of course, these situations require special circumstances — the tumour must be accessible either for direct injection or within a compartment that can facilitate targeting. In preclinical studies, pre-targeted RIT (PRIT; also known as multistep targeting) enhances tumour uptake relative to normal tissues. The advantage of a multistep targeting approach is the high

Table 2 | α-particle emitters in solid tumours

Therapy antibody	Antigen target	Study population	Special feature	Main findings	Refs
[213]Bi-9.2.27	Glial antigen 2 (NG2)	Stage IV or in transit melanoma	Long-term evaluation of response?	10% PR, 8% SD, no MTD	133
[213]Bi-9.2.27	Glial antigen 2 (NG2)	Stage IV or in transit melanoma	First-in-human direct injection	Antitumour effect at 600 µCi. Safe, no MTD, activity administered 150 to 1350 mCi	134
[211]At-ch81C6	Tenascin	Primary brain tumours	18 patients 71–347 MBq post-resection, delivery into surgically created resection cavity	No MTD achieved, no DLT. No haematological >grade 2. Limited neurotoxicity. Determined biodistribution. Median survival 54 weeks for GBM and 52 weeks for AA and 116 weeks for OD, 2 of 14 GBM survived ~3 years. Proof-of-concept regional targeted radiotherapy with [211]At	81
[213]Bi 9.2.27	Glial antigen 2 (NG2)	22 patients with stage IV melanoma	Phase I dose escalation. 1.5 to 25.6 mCi	Well tolerated; no DLT. 14% PR, 50% SD	135

AA, anaplastic astrocytoma; DLT, dose-limiting toxicity; GBM, glioblastoma; MTD, maximum tolerated dose; OD, oligodendroglioma; PR, partial remission; SD, standard deviation.

therapeutic ratio, even when administered intravenously. Finally, a combination of chemotherapy and RIT, along with the use of special radionuclides such as α-emitters, is favourable in certain circumstances.

Intra-compartmental therapy. Intrathecal and intraventricular administration of [131]I-81C6 (a tenascin monoclonal antibody) for the treatment of leptomeningeal carcinomatosis and intra-tumoural therapy of malignant brain tumours have produced objective responses and prolonged patient survival[79,80]. [131]I-81C6 is an example of α-particle therapy for RIT of malignant glioma and it is likely to be used more extensively if problems of radionuclide supply can be overcome[81].

Intraventricular [131]I-3F8 (anti-GD2; NCT00445965)[36] and [131]I-8H9 (anti-B7-H3; NCT00089245)[37,82] are also being tested in leptomeningeal cancers in both children and adults, with highly favourable CSF-to-blood radiation dose ratios. Children with recurrent neuroblastoma with CNS metastases have achieved long-term remissions[37]. Intra-compartmental injections seem to be more effective than systemic injections because there is more effective targeting to the tumour, with typical radiation-absorbed doses in the 5,000–10,000 cGy range, which is approximately tenfold the dose that is typically achieved with intravenous injection. In all probability this is due to simple binding kinetics because the total RIT is distributed in no more than 150 ml of CSF within the intrathecal compartment after direct intrathecal injection.

Furthermore, the CSF is devoid of white cells or proteins that can interfere with antibody binding in comparison to a much larger dilution when the dose is distributed systemically within 5 l of blood volume. In addition, CSF flows in one direction and renews every 7–8 hours, providing a built-in washout step for unbound RIT. The apparent absence of an anatomical barrier could also facilitate the movement of antibodies between the CSF and the extracellular space of the brain[83,84], especially if there is damage to the meninges either by tumour or by surgery. As there is no B7-H3 expression in normal brain tissue, [124]I-8H9 (a β-emitter that targets B7-H3) is being successfully tested as a theranostic agent by convention-enhanced delivery into brainstem gliomas (NCT01502917).

Tumour targeting with intravenous injection of RIT. The slow clearance of unbound RIT from the blood circulation and the resulting high levels of background radioactivity are pharmacokinetic features that limit the tumour-to-normal organ ratios of absorbed radiation that can be achieved[85–87]. One approach to reduce the toxicity of RIT is to use smaller antibody moieties to decrease the circulating half-life of the RIT[88–91]. Although opinions vary on the clinical potential of antibody fragments for RIT, most experts have concluded that the smaller molecules penetrate solid tumours faster, more deeply and more homogeneously than intact antibodies, but that they achieve lower intra-tumoural concentrations, exhibit shorter tumour retention times than intact antibodies and may demonstrate undesirable renal accumulation[92].

Approaches to increase therapeutic efficacy have included dose fractionation with the expectation of bone marrow recovery in between doses, leading to higher administered doses. This dose fractionation approach has been shown to be feasible in lymphoma[6,93] and in solid tumours[78].

In particular, to enhance the therapeutic index in solid tumours relative to normal tissues, three avenues are being followed that seem to show some promise, particularly in preclinical studies: PRIT, the addition of chemotherapy to RIT and the application of radionuclides with favourable emissions, especially α-emitters.

PRIT. PRIT uses multistep pre-targeting to dissociate the slow distribution phase of the antibody molecule from the administration of the therapeutic radionuclide. These strategies administer tumour-reactive antibody in a non-radioactive form, allowing it to localize to solid tumour sites and to accumulate without subjecting the rest of the body to nonspecific irradiation from circulating RIT[85,90,94–99]. After maximal accumulation of antibody in the tumour, a low molecular weight radioactive moiety with a high affinity for the tumour-reactive antibody is administered. Because of its small size, this second reagent rapidly penetrates solid tumours, where the pre-targeted antibody traps it. Furthermore, unbound molecules of the second (radioactive) reagent are small enough to be rapidly cleared from the blood and excreted in the urine. In some pre-targeting approaches, a clearing agent can be injected shortly before the radiolabelled small molecule to remove the unbound antibody from the bloodstream and to prevent it from complexing with the radiolabelled small molecule in circulation[95,96,100,101]. Several strategies have been proposed and implemented preclinically to accomplish this binding, but one of the most promising strategies exploits the extraordinarily high affinity of avidin (or streptavidin) for biotin (FIG. 4).

Bispecific antibodies. Goldenberg *et al.*[90] have developed bivalent haptens that permit cooperative binding, thereby linking two bispecific antibodies together on the tumour cell surface using the bivalent hapten (for example, histamine-succinyl-glycine (HSG)) as a bridge. Their affinity enhancement system uses fragment antigen-binding fragments (Fab fragments) of tumour antibodies with Fab fragments of hapten antibodies (FIG. 4). Spontaneous cyclization of the bivalent hapten with two molecules of bispecific Fab binding to two antigen molecules on the tumour cell surface stabilizes the radiolabelled ligand on the cell surface through cooperative binding. HSG–hapten-containing peptides have been synthesized with chelates for either radiometals (^{111}In, ^{90}Y or ^{177}Lu) or a technetium–rhenium chelate. They can be radiolabelled to a highly specific activity that avoids the need for purification[102]. In preclinical studies, this approach has yielded impressive results in both imaging and therapeutic applications[103,104].

A clever modification of the bispecific antibody targeting approach uses molecularly engineered dimerization and docking domains that contain self-assembling protein kinase A motifs with engineered cysteine residues[105,106] (FIG. 4). Another novel approach

Convention-enhanced delivery
A therapy in which therapeutic compounds are forced directly into the region of interest through a needle or cannula by applying a low-pressure gradient.

Haptens
Small molecules that, when combined with larger carriers such as a protein, can elicit the production of antibodies that bind specifically to them (in the free or combined states).

Bispecific antibodies
Artificial proteins composed of fragments of two different monoclonal antibodies, which consequently bind to two different types of antigen.
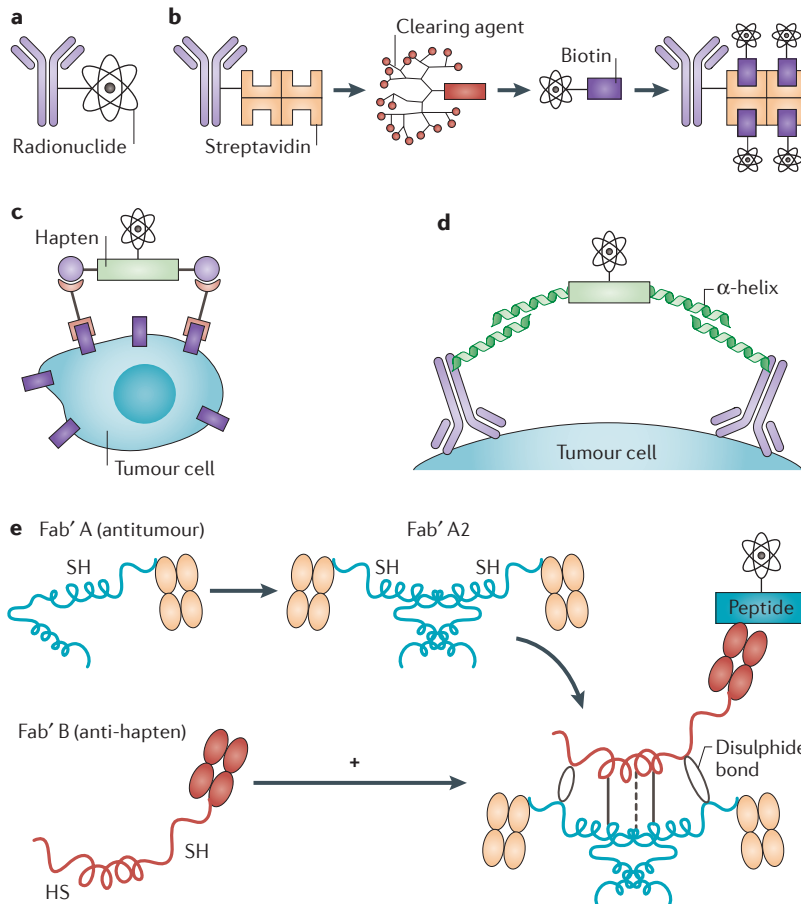
Fragment antigen-binding fragments
(Fab fragments). Regions on an antibody that bind to antigens and that are composed of one constant and one variable domain of each of the heavy and the light chains.

to bispecific antibody pre-targeting has been suggested by Chmura et al.[107], who have developed molecularly engineered bispecific antibodies that incorporate complementary reactive groups in the antibody-binding pocket, which covalently and irreversibly bind to radio-labelled electrophilic ligands[107,108]. Potential advantages of this approach compared with the streptavidin–biotin approach are less immunogenicity and faster and more

Figure 4 | **Schemas for conventional and pre-targeted RIT.** **a** | Conventional single-step radioimmunotherapy (RIT), with monoclonal antibody conjugated directly to radionuclide. **b** | Multi-step pre-targeted RIT (PRIT) using antibody-streptavidin (Ab-SA) conjugates, followed by radiolabelled DOTA-biotin. The tetrameric streptavidin molecule can bind four radio-DOTA-biotin moieties, amplifying tumour-targeted radioactivity. The 16-merous, N-acetylgalactose-containing clearing agent removes excess Ab-SA conjugate from circulation via hepatic asialoglycoprotein receptors before systemic delivery of radio-DOTA-biotin, improving tumour-to-normal organ ratios of absorbed radioactivity. **c** | Bispecific antibody pre-targeting for RIT using radiolabelled bivalent haptens and affinity enhancement system. Another PRIT strategy uses bispecific antibodies recognizing both tumour-associated cell surface antigen and radiolabelled bivalent hapten (for example, histamine-succinyl-glycine (HSG)), which facilitates cooperative binding by linking two bispecific antibodies together on the cell surface. **d** | Pre-targeting using antibodies conjugated to an antisense phosphorodiamidate morpholino oligomer (MORF) oligonucleotide followed by administration of a radiolabelled complementary c-MORF oligomer. **e** | Bispecific antibody pre-targeting using dock and lock technology with self-assembling protein kinase A domains[105,106]. A clever modification of bispecific antibody targeting approach uses molecularly engineered dimerization and docking domains containing self-assembling protein kinase A motifs with engineered cysteine residues. Upon mixing, two antitumour Fab fragments and one anti-hapten Fab fragment spontaneously associate, leading to locking of the fragments into a covalent trivalent complex.

homogeneous penetration into tumour sites, owing to the smaller size of a radiometal DOTA. Multistep targeting has been limited so far by immunogenicity with certain high-affinity reagent combinations (for example, immune responses to streptavidins or unusual antibody forms), the absence of a clinical clearing agent, difficulty in manufacture and purification, and interfering substances in human blood.

A proposed novel solution to many of these problems is the use of a modular (IgG–single-chain variable fragment (scFv)) antibody developed by Wittrup et al.[109] with an IgG portion that is specific for the tumour and the high-affinity scFv specific for DOTA-metal. The bispecific bivalent constructs have high avidity for both the tumour and radiolabelled DOTA, and the high molecular weight (~200 kDa) ensures a long plasma half-life for optimal tumour targeting. More importantly, because the scFv affinity for DOTA depends on the chelated metal, ranging from 8 pM–50 nM affinity, dextrans or dendrimers carrying DOTA-metal of low affinity for scFv can be exploited as clearing agents. Besides targeting ^{90}Y (15 pM affinity for scFv) or ^{177}Lu (11 pM affinity for scFv) in RIT, DOTA-metal provides a convenient method to target nanoparticles. Another novel pre-targeting approach uses complementary hybridization (Watson–Crick pairing) of DNA and other oligomers, particularly phosphorodiamidate morpholino oligomers (MORFs), as a recognition system[110,111] (FIG. 4).

Regardless of the PRIT approach used, all investigators who have conducted comparative tumour targeting studies in animals have concluded that pre-targeting is superior to conventional one-step RIT to improve tumour-to-normal organ ratios of absorbed radioactivity and tumour responses in preclinical models. For example, in a DOTA-PRIT approach using a bifunctional antibody with antigen reactivity to GD2 ganglioside in neuroblastoma xenografts, the therapeutic index for tumour to bone marrow was 50:1, and the therapeutic index for kidney was 7, and CRs were observed with no detectable toxicity[112].

Pilot PRIT human studies

Pilot clinical trials of PRIT have been very encouraging in patients with both solid tumours and lymphoma[113–115]. In one pilot study investigating streptavidin–biotin PRIT for NHL, four of seven patients with advanced NHL (who had failed multiple prior therapies, including haematopoietic stem cell transplantation and prior conventional RIT) achieved objective responses, including two CRs[115]. Additional studies using streptavidin–biotin pre-targeting are currently underway for patients with AML, acute lymphoblastic leukaemia (ALL) and myelodysplastic syndrome (MDS) (J. M. Pagel, O.W.P., A.K. Gopal and J. Rajendran, unpublished observations). Pre-targeting using antibodies to carcinoembryonic antigen (CEA)[116] was tested in colorectal cancer (CRC)[117], small-cell lung cancer (SCLC)[118] and medullary thyroid carcinoma[116,119]. Pre-targeting using NR-LU-10 antibodies in CRC[115], pre-targeting using CC49 antibodies in gastrointestinal cancer[114] and pre-targeting using CD20 monoclonal antibodies in NHL[113,115] were also tested,

Glossary sidebar

DOTA
1,4,7,10-tetraazacyclodode-cane-1,4,7,10-tetraacetic acid. DOTA functions as a chelating agent for the radioisotope $^{90}Y^{3+}$ or other radiometals. It can be conjugated to monoclonal antibodies by attachment of one of the four carboxyl groups as an amide.

Single-chain variable fragment
(scFv). A fusion protein of the variable regions of the heavy (VH) and light (VL) chains of immunoglobulins, connected with a short linker peptide of ∼10–25 amino acids.

Phosphorodiamidate morpholino oligomers
(MORFs). A family of synthetic oligomers that are water soluble and reported to be stable both *in vitro* and *in vivo*.

Area under the curve
(AUC). The overall amount of drug in the bloodstream or other tissue after a dose.

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and met with variable success. A three-step approach using biotinylated monoclonal antibodies, followed by avidin–streptavidin and then biotinylated radiometal-chelate, was also applied to patients with glioma, with encouraging results[120,121].

The goal of these approaches is to improve the therapeutic index, and localization can be impressive in antigen-expressing tissues. A case in point is the study of NR-LU-10 in CRC, which has documented responses in patient tumours. However, studies in humans were suspended when gut toxicity developed. In retrospect, the targeting of an antigen expressed in normal gut was the probable cause of serious toxicity. PRIT studies in humans must be carried out cautiously, with attention to possible targeting to normal tissues, by concomitant imaging and normal tissue dosimetry estimates that are carried out in parallel with therapeutic regimen (theranostic approach) (BOX 3).

Conclusions

In principle, intravenous RIT could deliver curative radiation to widely disseminated tumours within the human body. In practice, the effectiveness of RIT depends on the complex interplay of the tumour radiosensitivity and the amount of radiation that can be safely administered and targeted to the tumour. RIT delivered systemically has been most effective in haematopoietic cancers, and has even resulted in long-term response and cures, especially when targeting CD20 — in this case both ^{131}I and ^{90}Y

radiation have prolonged patient survival for patients with tumours that are refractory to chemotherapy or unlabelled antibody.

Therapy with an α-emitter, ^{225}Ac, has been effective when carried by IgG antibodies targeting CD33 or CD45 on human leukaemia cells. In solid tumours, long-term remissions have been achieved using compartmental RIT injections especially via the intrathecal route, probably because of the better access of the antibody to tumour-associated antigens in these tissues. Intravenous RIT has been mostly ineffective in solid tumours. Novel methods to improve therapeutic index have greatly enhanced the prospect of the intravenous route to deliver sufficient radiation to kill more radioresistant solid tumour cells. One promising strategy is multistep targeting, which pre-targets the tumour with a bispecific antibody without its therapeutic payload, followed in sequence by the therapeutic ligand after the pre-targeted tumour antibody, which maximizes radiation in tumours compared with radiosensitive normal tissues. Through quantitative imaging methods such as PET, estimates of tumour dosimetry will become more precise in RIT, even at tumour sites deep within the body.

The major hurdle that needs to be overcome to achieve the full potential of RIT is delivering tumoricidal doses specifically to tumours, ranging from 3,000–5,000 cGy for more radiosensitive tumours such as haematopoietic tumours to up to 10,000 cGy for most radiation-resistant solid tumours, such as thyroid tumours. This must be accomplished while sparing normal radiosensitive tissues so that organs such as the kidney, lung, colonic mucosa and bone marrow receive less than 2,000 cGy, 1,500 cGy, 250 cGy and 100 cGy, respectively. These dose estimates come from a variety of sources, including the external beam normal dose tolerance projections by Emami *et al.*[122], estimates from Maxon[123], and thresholds for kidney-sparing dosing during peptide-targeted radiotherapy, which has emerged from recent large, but unpublished, experiences (R. Baum, personal communication). *In vivo* targeting approaches have already come close to this optimal radiation balance in some clinical scenarios, such as intrathecal injections for tumours invading the meninges and intravenous injections in lymphomas, especially in conjunction with bone marrow-sparing agents such as granulocyte colony-stimulating factor (G-CSF) and stem cell rescue.

To aid the further refinement and optimization of RIT that is needed for clinical use, more effort should be placed on developing better real-time dosimetry methods, especially those that use the intrinsic theranostic features of the therapeutic radionuclides themselves. From a laboratory perspective, methods with increasingly better therapeutic ratios for PRIT are being developed, and these should be encouraged. More effort to understand the radiobiology of targeted therapy is sorely needed, especially with respect to whether the emission properties of therapeutic radionuclides can be optimally used in specific clinical situations to improve selective tumour killing. For example, α-emitters and low-energy β-emitters and conversion electron emitters may have intrinsic advantages because

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Box 3 | Development of human immune targeting reagents

The molecular engineering of antibodies has resulted in the development of a wide range of potential antibody forms that can be radiolabelled and serve as a key component of the radioimmunotherapy (RIT) approach. An ideal antibody binds with high avidity to the target antigen, exhibits minimal binding to non-malignant tissues, penetrates rapidly and uniformly into tumour nodules, and clears from the blood circulation soon after maximal tumour binding is achieved to avoid nonspecific irradiation of normal tissues by circulating RITs.

GD2 example

Antibody 3F8 and its humanized form, hu3F8, bind to the cell surface tumour target disialoganglioside (GD2; Kd ~10 nM), a ceramide-anchored antigen that is highly restricted in its tissue distribution and is shielded from the GD2$^+$ central nervous system (CNS) owing to the blood–brain barrier[125]. GD2 is widely expressed among human tumours including neuroblastoma, osteosarcomas, soft-tissue sarcomas, small-cell lung cancer, retinoblastoma, brain tumours and tumour stem cells[126]. In neuroblastoma, this antigen is abundant (>10^6 per cell), relatively homogeneous within and between tumours and rarely lost following GD2-based immunotherapy[126]. Anti-GD2 antibodies for the treatment of metastatic neuroblastoma have proven to be safe even in young patients, with no late toxicities of the CNS or peripheral nervous system with up to 20 years of follow-up[127]. Although 3F8 targets tumours in patients unusually well by immunoscintigraphy, the area under the curve (AUC) of tumour-to-blood ratio for intact IgG was never more than 5:1 even in preclinical models. Pre-targeting strategies using biotin–streptavidin systems substantially improved the AUC ratios[128]. However, immunogenicity of streptavidin and the ubiquitous presence of biotin in tissue fluids will constrain clinical development until the advent of humanized pre-targeted RIT (PRIT) strategies using the benzyl (Bn)–DOTA–C825 system. C825 is an affinity-matured antibody that is specific for Bn-DOTA metal complex with differential affinities for radiometal-Bn-DOTA complexes. Using hu3F8-C825 to deliver β-emitters such as ^{177}Lu, radioactivity AUC ratios of >100:1 for blood and >10:1 for kidney are achieved, translating into complete tumour ablation with no dose-limiting toxicities in preclinical models[112]. The utility of this PRIT strategy has since been successfully applied to other human tumour targets.

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radiation is primarily delivered to the site of molecular targeting, rather than to bystander cells. In addition, a combination of radionuclides and antibody–antigen systems may overcome intrinsic heterogeneity and promote more effective targeting.

Finally, this Review has not placed much emphasis on the most common solid tumours such as those of the lung, colon, breast and pancreas. This is because limited clinical benefit for patients with these tumours has been observed with RIT, despite considerable effort. Intravenously injected radiolabelled antitumour antibody to target solid tumours has not been effective for solid tumour therapy. In Supplementary information S6 (table), we provide a brief overview of solid tumour RIT reports to support this conclusion. Instead, our deliberate emphasis in this Review has been on the more successful application of RIT for haematological tumours, and intracompartmental solid tumour RIT has resulted

in high response rates, often durable, and in some cases, long-term complete responses and cures. We focused on this aspect of RIT to highlight what has worked as a basis for improving RIT for broader applications across the oncology spectrum. We believe that the greatest single limitation encountered in the use of RIT so far is low therapeutic index for parenteral targeting in the setting of radioresistant solid tumours.

We are optimistic that a combination of advances, such as better dosimetry through quantitative imaging, radionuclides of higher potency, PRIT, as well as protein engineering of optimal antibody forms, will correct these problems and lead to future success in solid tumour RIT. In short, the field of RIT is still a challenging frontier, with many promising scientific opportunities waiting to be explored, particularly in the major solid tumours, where curative therapies are sorely needed.

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