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New insights into the mechanisms of action of radioimmunotherapy in lymphoma

Andrei Ivanov, Ruth Swann and Tim Illidge

Abstract

The exquisite sensitivity of haematological malignancies to targeted radiation alongside the impressive results achieved by the pioneers in this field suggests that radioimmunotherapy is likely to be a productive area for future clinical research. Recent experimental work has demonstrated that the combination of targeted radiation and antibody effector mechanisms are critical to long-term clearance of tumour. This review provides the background of clinical and biological insights into the mechanisms of action of radioimmunotherapy.

Introduction—The principles of radioimmunotherapy

The use of monoclonal antibodies (mAbs) in routine clinical practice is now well established and has led to impressive improvements in outcome for patients with a range of human cancers (Robak 2004; Adams & Weiner 2005). Although the single-agent activity of most mAbs has been modest, when used in combination with both chemotherapy and radiotherapy highly impressive increases in clinical responses and improvement in survival have been seen (Coiffier et al 2002, 2003; Robak 2004). Radioimmunotherapy (RIT) is, in contrast, the administration of mAb or mAb-derived constructs, which are chemically conjugated to therapeutic radioisotopes targeted to tumour. In this context, mAbs were initially regarded simply as direct carriers for the radioisotope, which deliver systemically targeted cytotoxic radiation to areas of disease, with relative sparing of normal tissue. It is, however, becoming clearer that the mAb effector mechanisms may also play an important additional role in killing lymphoma cells. The nature of RIT determines that its efficacy depends on a number of factors, including the properties of the targeted antigen (specificity, density, availability, shedding and heterogeneity of expression), the tumour (degree of vascularization, blood flow and permeability), the mAb (specificity, immunoreactivity, stability and affinity) and the properties of the chosen radioisotope (emission characteristics, half-life and bioavailability) (Knox & Meredith 2000).

A wide variety of different mAbs, delivery schedules, radioisotopes and doses of radioactivity have been used in RIT and have resulted in impressive durable partial and complete responses in the treatment of non-Hodgkin's lymphoma (NHL) (Park & Press 2007). In this review the principles of radioimmunotherapy will be discussed along with some recent data that provide new insights into the mechanism of action of RIT. Finally, the clinical data that led to the two radioimmunconjugates, ^{131}I-tositumomab and ^{90}Y-ibritumomab, being approved by the US FDA (US Food and Drug Administration) and ^{90}Y-ibritumomab tiuxetan within the EU, will be discussed.

School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Manchester, UK

Andrei Ivanov, Ruth Swann, Tim Illidge

Correspondence: T. Illidge, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Manchester, UK. E-mail: tmi@manchester.ac.uk

Antigen targeting

The use of radiotherapy in the treatment of haematological malignancies is well established and for localized disease it is a highly effective treatment modality, given the exquisite sensitivity of lymphomas and leukaemias to radiation-induced cell death. The systemic nature of the majority of lymphomas and leukaemias, however, makes localized irradiation inappropriate for most patients. Therefore the systemic delivery of targeted radiation in the form of RIT is a logical strategy given for disseminated tumours, especially those known to be so radiosensitive. The effective delivery of RIT requires the selection of a suitable tumour antigen target.

Table 1 Summary of characteristics considered ideal in an antigen target for RIT

Tumour cell specific
Highly expressed on tumour cells
No tendency to mutation
Not secreted or shed
Not rapidly modulated on antibody binding
Critical for target cell survival
Not expressed on critical or non-renewable host cells

Tumour specific antigens would be the ideal targets for RIT, but such a degree of specificity is unusual. In practice tumour-associated antigens expressed abundantly on tumour cells as well as some normal tissues represent the majority of potential targets. As most NHLs are of B-cell origin the pan-B-cell antigens, such as human leukocyte antigen DR (HLA-DR), CD19, CD20, CD22, CD37, CD52 and MHC II have been extensively evaluated as targets for RIT (Press et al 1989; DeNardo et al 1998; Illidge et al 1999; Kaminski et al 2001; Robak 2004; Adams & Weiner 2005). Table 1 shows the antigen characteristics that are considered ideal for RIT.

From these initial investigations, the CD20 antigen emerged as having many of the characteristics thought to be important for a good tumour target. Targeting this antigen has thus dominated clinical RIT of lymphoma (Grossbard et al 1992). The CD20 antigen is a transmembrane phosphoprotein that is expressed on mature B cells and at a higher density on pre-B cells. The antigen is also expressed on greater than 90% of B-cell NHLs. The CD20 complex does not internalize or shed from the cell surface and initiates signal transduction that triggers cell death through a caspase-independent pathway (Chan et al 2003). CD20 is highly expressed on the majority of B-cell lymphomas but is not expressed on stem cells or plasma cells. Following radiolabelled anti-CD20 mAb RIT, the B-cell pool of both malignant and normal B cells is substantially depleted, with the normal B cells recovering within 6 months (Witzig et al 2002b). Other B-cell antigens that are currently being investigated include the CD22 antigen (Sharkey et al 1997).

Radioisotopes used in RIT

The physical characteristics considered important for a radioisotope in RIT include half-life, type of radioactive emissions (α-, β- or γ-) and ionization path length. Particle energy and mean path length in tissue are also important determinants of therapeutic efficacy. The emission profile of the radioisotope not only determines its suitability for therapy, but also the toxicological profile of the radiopharmaceutical.

The majority of clinical trials to date have used either iodine-131 or yttrium-90 because of their favourable emission characteristics, widespread availability and well-documented radiochemistry that permit reliable and stable attachment to mAbs. Iodine-131 has the advantage of a long history of successful use in the management of thyroid cancer and a well-documented safety profile. It is readily available, inexpensive, easily conjugated and emits both β-particles with a path length of 0.8 mm and penetrating γ-emissions. The γ-photons enable uncomplicated imaging using a gamma camera for dosimetry purposes, but also result in a significant non-targeted normal tissue radiation dose, as well as radiation protection issues for visitors and medical/nursing staff.

Yttrium-90 offers a number of theoretical advantages over iodine-131, although the radioisotopes have not been directly compared by labelling the same mAb and performing a comparative study. Yttrium-90 is a pure β-emitter that produces higher energy radiation (2.3 MeV vs 0.6 MeV) at a longer path length than iodine-131 (5.3 mm vs 0.8 mm). Radiolysis induces cellular damage in both the targeted lymphoma cells and neighbouring cells. The increased path length would be expected to enhance the 'cross fire' effect and could therefore be potentially advantageous in treating bulky, poorly vascularized tumours with heterogeneous antigen expression (Knox & Meredith 2000). This longer path length is likely, however, to increase the normal tissue dose when targeting microscopic disease for which the shorter β-particle path length of iodine-131 may be preferable. The physical half-life of yttrium-90 is 64 h and it decays to a stable (non-radioactive) form of zirconium (^{90}Zr). The physical half-life of 64 h approximates to the biological half-life of murine mAbs and the absence of penetrating γ-emissions enables delivery as an outpatient (Press & Rasey 2000). Additionally, if a cell internalizes yttrium-90, it is likely to be retained within the cell (Sharkey et al 1997). In contrast, once iodine-131 conjugates are internalized by a cell there is rapid dehalogenation of the free iodide and subsequent excretion of the iodinated products out of the cell, reducing the desired tumour absorbed radiation dose as well as increasing normal tissue radiation exposure (Press et al 1996). The major disadvantages of yttrium-90 relate to its greater expense, relatively limited availability and requirement for chelation radiochemistry making radiolabelling a more complex procedure. Yttrium-90 does not emit γ-photons and therefore indium-111 is used as a surrogate to obtain images for biodistribution studies. Rhenium-186 and copper-67 are both β-emitters and have physical and chemical properties that make them attractive alternatives to either iodine-131 or yttrium-90. Nevertheless, their current limited availability has meant that these radioisotopes have received limited clinical use (DeNardo et al 1999).

α-Emitters produce a helium nucleus particle of very high energy but with a very short path length. The high Linear Energy Transfer (LET) radiation of α-emitters may be lethal to cells with a single collision, although the very short path length means that the isotope must be adjacent to, or internalized by, the cell to be effective and is likely to have little or no 'cross fire' effect. The suitability of α-emitters therefore appears limited to readily accessible tumours such as leukaemia cells confined to the blood or bone marrow. The short half-life of α-emitters (e.g. ^{211}At 7 h or ^{213}Bi 45 min) complicates the radiopharmaceutical preparation, meaning that such radioisotopes are likely to require generation on the same site as delivery in the clinic. Despite this logistical hurdle, early clinical data in the treatment of leukaemia appear extremely promising (McDevitt et al 1998; Jurcic et al 2002).

In practice, the choice of the optimal radioisotope for RIT remains controversial, with proponents advocating the relative merits of iodine-131, yttrium-90, rhenium-186, copper-67, and α-emitters such as astatine-211 (Press & Rasey 2000). Comparative studies are difficult to conduct and scientifically robust randomized human trials have not been performed. The ideal properties of a radioisotope for RIT remain unclear and it is likely that the optimal radioisotope for a particular situation will depend upon the bulk and type of tumour being targeted. An important area of potential future research will be to define the optimal radioisotope, or potentially a cocktail of different radioisotopes used in combination that may have separate benefits for tumour of varying sizes.

Factors affecting the therapeutic efficacy of radioimmunotherapy in lymphomas

Although RIT has emerged as a highly effective treatment for NHL, the mechanisms underlying the high response rates and in particular the interaction of tumour irradiation and mAb signalling in RIT are still poorly understood (Illidge & Johnson 2000; Press & Rasey 2000). A number of important translation research questions remain to be answered and to optimize RIT delivery, including: the optimal pre-dose of cold mAb; the relative contribution of targeted radiation and mAb effector mechanisms to the high response rates and the mechanisms involved in the durable responses seen in some patients; defining whether a tumour radiation dose–response exists in RIT for lymphomas and leukaemias.

Pre-dosing of mAb in radioimmunotherapy

There are several factors that may theoretically limit lymphoma targeting of radiolabelled pan-B-cell mAbs in RIT. These include the complex formation of administered mAb with free circulating target antigen, the cross-reactivity with antigen-positive circulating lymphoma cells, normal B-cells in the blood or spleen or non-lymphoid tissues and finally the non-antigenic binding of mAb, via the Fc region of a mAb. Poor tumour targeting of a radioimmunoconjugate leads to lower tumour-to-normal-tissue radiation dose ratios resulting in potentially reduced therapeutic efficacy.

To improve the biodistribution of radiolabelled mAb in RIT, it has become the established practice to give a pre-dose of 'cold' or unlabelled anti-CD20 mAb before the therapeutic dose of the anti-CD20 radioimmunoconjugate (Wahl 2003). The pre-dose is considered to prolong the circulating half-life of the radiolabelled mAb, block 'non-specific' binding sites (e.g. circulating and splenic B cells) and result in increased tumour retention of the labelled mAb. Buchsbaum et al (1992) investigated whether a pre-dose of anti-B1 improved the delivery of a subsequent radiolabelled mAb to tumour using in-vivo pre-clinical human xenograft models. The anti-B1 (anti-CD20) pan-B-cell mAb, reactive with human B-cell lymphomas but not with host mouse B cells, was used. A pre-dose of unlabelled anti-B1 was found to significantly increase the tumour uptake of the subsequent radiolabelled anti-B1 by blocking the B1-specific Fc receptor sites, although this improvement in tumour targeting appeared to plateau at the highest pre-doses of unlabelled anti-B1.

Relative contribution of antibody effector mechanisms and targeted radiation to therapy

Although RIT has emerged as an effective treatment for lymphoma, the relative contributions of antibody effector mechanisms and targeted radiation to tumour cell death remain poorly understood. By using different syngeneic murine B-cell lymphoma models the relative contributions of mAb and targeted radiation to the clearance of tumour in-vivo have been investigated (Du et al 2004). There is now substantial evidence that mAbs can form an active component of RIT and that mAb effector mechanisms may be important in the clearance of tumour in-vivo.

With regard to mAb-induced tumour cell killing, four potential mechanisms are thought to play a role: complement-dependent cytotoxicity (CDC); antibody-dependent cellular cytotoxicty (ADCC) via the recruitment of immune effector cells; direct induction of growth inhibition or cell death; and stimulation of host-adaptive immunity (Cragg et al 2005). Large amounts of experimental data support the first three of the aforementioned mechanisms in anti-CD20 mAb immunotherapy, although robust evidence for the fourth is currently lacking. In particular, several lines of evidence indicate that rituximab operates through conventional effector mechanisms involving complement and immune effector cells (Johnson & Glennie 2003). The most conclusive initial evidence for the role of immune effector cell recruitment by antibody Fc receptors comes from primate experiments where an IgG4 variant of rituximab was shown to be unable to deplete normal B cells (Anderson et al 1997). More recently, data has emerged demonstrating that type I anti-CD20 mAbs, such as rituximab and 1F5, require the presence of their Fc domains for optimal therapeutic activity in human lymphoma xenograft models (Cragg & Glennie 2004).

Despite impressive clinical effectiveness, however, the biological function of CD20 has remained obscure. This is at least in part due to the lack, until recently, of murine anti-CD20 mAb (Uchida et al 2004) to probe in-vivo the mechanisms of anti-CD20 mAb. Furthermore the enigma of the precise role of CD20 was compounded by the discovery that the CD20 knockout mouse surprisingly had a normal phenotype (O'Keefe et al 1998). Therefore, much of our understanding has been based upon experiments using mAb to ligate CD20 on human B cells and B-cell lymphoma cell lines. Engagement of CD20 with mAbs results in several measurable biologic events, such as enhanced survival (Holder et al 1995), activation and proliferation (Clark & Shu 1987; Smeland et al 1987) and adhesion (Kansas & Tedder 1991; Leveille et al 1999) as well as growth inhibition (Tedder et al 1986) and death (Shan et al 1998; Hofmeiseter et al 2000; Chan et al 2003). The exact in-vivo mechanisms of tumour killing by anti-CD20 mAb remain incompletely understood. Pre-clinical data have suggested that the action of rituximab may include mAb-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and the direct induction of caspase-independent cell death through cell-surface-mediated downstream signal transduction (Cragg et al 2005). Cragg et al (2005) have previously shown that

anti-CD20 mAbs may be sub-divided as either rituximab-like (type I) or B1-like (type II) according to their linked activity in a number of in-vitro assays. For example, rituximab and other type I mAbs redistribute CD20 into Triton X-100 insoluble membrane rafts, correlating with their ability to engage complement effectively and cause target cell lysis (Cragg et al 2005). In contrast type II mAbs such as B1 do not redistribute CD20 membrane rafts, but are generally potent at inducing cell death in target cells (Chan et al 2003). Importantly, these differences appear to translate to the in-vivo mechanisms employed by these mAbs, at least in xenograft tumour models (Cragg & Glennie 2004).

Binding of anti-CD20 mAb to lymphoma cells in-vitro has been shown to induce modest levels of cell death presumably via signalling through the CD20 molecule (Shan et al 1998). A range of signalling events may be induced following ligation of CD20 (reviewed by Cragg et al 2005), including activation of the mitogen-activated protein kinase (MAPK) cascade (Mathas et al 2000). The Ras–Raf–MEK–ERK1/2 pathway is an evolutionarily conserved pathway that is involved in the control of many fundamental cellular processes that include cell proliferation, survival, differentiation, cell death, motility and metabolism (reviewed by Kolch et al 2005). Although commonly thought of as a component of proliferation and survival pathways, ERK1/2 signalling has been associated with apoptotic signalling in immature B-cell lymphoma (Lee & Koretzky 1998) and diffuse large B-cell lymphoma cells (Hollmann et al 2006). Similarly, it has been suggested that the overall balance of MAPK activity may determine B-cell fate depending on the kinetics of activation and maturation state of the cell (Sutherland et al 1996; Gauld et al 2002).

Intriguingly, the MAPK cascade is also triggered by radiation and other DNA-damaging agents (Hagan et al 2000; Lyng et al 2006) where it may be necessary for DNA double-strand break repair by homologous recombination (Golding et al 2007). Given the observation that both anti-CD20 mAb and radiation can trigger MAPK activation and that MAPK activation can result in diverse biological outcomes, we have therefore investigated the impact of combining radiation and anti-CD20 mAb on this signalling pathway in an attempt to further elucidate the potential underlying mechanisms of cell death seen when anti-CD20 mAbs are combined with radiation in RIT. Specifically, we have attempted to investigate the two RIT modalities currently approved by the US FDA, ^{90}Y-ibritumomab tiuxetan (Zevalin) and ^{131}I-tositumomab (Bexxar) by combining rituximab (derived from ibritumomab) and tositumomab with irradiation (IRR).

We have specifically investigated the mechanisms underlying cell death in B-cell lymphoma cells treated with irradiation (IRR) and either type I (rituximab) or type II (B1/tositumomab) anti-CD20 mAb. Increased cell death was observed with B1 mAb combined with IRR but not with rituximab. This additive cell death was found to be MAPK/ERK kinase signalling dependent and could be reversed with pharmacological MEK inhibitors as well as siRNA targeting MEK1/2. Furthermore we found that this increased death was associated with ERK1/2 nuclear accumulation following B1 mAb treatment, which was greatly enhanced in combination with IRR (Figure 1). Importantly, although Bcl-2 overexpression resulted in resistance to IRR-induced apoptosis, it had no impact on the cell death induced by B1 plus IRR, suggesting a non-apoptotic cytoplasmic form of cell death that was confirmed by ultrastructural and TUNEL analysis. Taken together our data provide new clinically relevant insights into how radioimmunotherapy with B1 mAb causes additive cell death that can overcome apoptosis resistance (Ivanov et al 2008).

The relative importance of mAb effector mechanisms and targeted radiation is difficult to quantify and it is not possible to dissect the action of the two components, using in-vitro assays, as an intact host immune system to augment the antibody effector functions. It is also challenging to answer this type of question in the clinic. We have therefore turned to preclinical studies using well-defined syngeneic animal models to further clarify the relative contributions of mAb effector mechanisms and targeted radiation. Du et al (2004) investigated the relative contributions of antibody and targeted radiation to the clearance of tumour in-vivo using two different syngeneic murine B-cell lymphoma models. We used the highly expressed non-modulating major histocompatibility complex class II (MHCII) as a target antigen. This target was initially pioneered in the clinic by DeNardo et al (1998), see

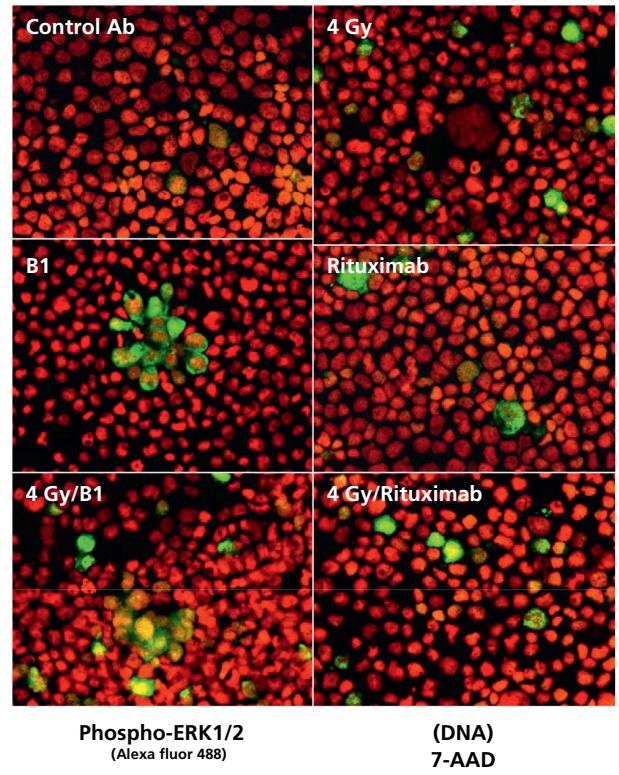

Figure 1 ERK1/2 activation following treatment with anti-CD20 antibodies and radiation. The effect of different anti-CD20 antibodies on the intracellular localization of activated ERK1/2 at the 24-h time point. SU-DHL4 cells were treated with anti-CD20 mAb (5 μg mL^{-1}) in the presence or absence of IRR (4 Gy). Cells were then harvested 24 h after treatment, carefully sedimented onto PLL-coated microscope slides and stained for phosphorylated ERK (green) and DNA (7-AAD, red).

below. We had previously shown that this target was the best of a number of different antigen targets in delivering the highest dose of radiation to the tumour (Illidge et al 1999). Although RIT with ^{131}I-anti-MHCII was effective in targeting radiation to tumour, no improvement in survival was seen by escalating the radiation dose alone and there were no long-term survivors (Du et al 2004). In contrast, using the combination of ^{131}I-anti-MHCII in the presence of unlabelled anti-idiotype (Id) 100% prolonged disease free survival was seen in both B-cell lymphoma models at the higher radiation dose. Using in-vivo tracking we showed that treatment with radiation plus anti-Id mAb results in a substantially greater reduction of splenic tumour cells than with either treatment alone. Prolonged survival could also be achieved using ^{131}I-anti-MHCII plus the signalling anti-CD19 mAb. Furthermore, the ability of some anti-B-cell mAbs to improve survival with targeted radiotherapy appeared to correlate with their ability to initiate intracellular signal transduction. Together these data illustrate that using one mAb to target radiation to tumour and a second to induce cell signalling may be an effective new treatment strategy in RIT.

We next went on to investigate how the microdistribution of the targeted radiation component of this combination impacts on the long-term clearance of lymphoma. ^{131}I-labelled mAbs targeting CD45 and MHCII antigens were found to deliver similar doses of radiation to tumour-bearing organ using conventional dosimetry (approximately 1.0 Gy per MBq when ^{131}I was labelled to 500 μg mAb and given intravenously per mouse), but when used as radiation vectors in combination therapy only ^{131}I-anti-MHCII plus anti-Id produced long-term survival. The profound differences in therapy did not appear to be dependent on levels of ^{131}I-mAb tumour binding or antibody-dependent cytotoxicity. Instead the microscopic intratumoral dosimetry appeared to be critical with the ^{131}I-anti-MHCII delivering more concentrated and therefore substantially higher radiation dose to tumour cells. When the administered activity of ^{131}I-anti-CD45 was increased, a radiation dose response was demonstrated in the presence of anti-Id and long-term survival seen. We believe that these new insights should influence the selection of new antigen targets and the design of dosimetric methods in RIT of lymphoma.

Defining whether a tumour radiation dose response exists in radioimmunotherapy

There is currently little preclinical data that demonstrates whether a radiation dose response exists. Du et al (2004) have demonstrated that there does appear to be a radiation dose response in syngeneic B-cell murine lymphoma models and that this radiation dose response becomes more significant in the presence of an additional 'cold' antibody that provides cell death signalling (Du et al 2004).

To date, despite the high response rates seen in lymphoma RIT, clinical dosimetry studies have thus far failed to show a consistent dose–response relationship (Knox & Meredith 2000). More recently, the Michigan group has concluded that there could be a radiation dose response at least for ^{131}I-tositumomab (Koral et al 2003a,b; Wahl 2003), although their conclusions differ to others (Postema 2004; Goldenberg & Sharkey 2005). Postema (2004) argues that none of the RIT dosing methods use tumour dosimetry to determine the dose administered to patients because the myelotoxicity of radiolabelled mAb will limit the increments of radioactivity dose, but not the tumour-absorbed dose. More recently, Goldenberg and colleagues commented that because RIT has two potentially therapeutic arms, namely radiation and mAb mechanisms, poor radiation targeting does not exclude a good therapeutic response from the mAb (Goldenberg & Sharkey 2005).

Clinical non-myeloablative radioimmunotherapy in lymphomas

Clinical RIT trials in NHL differ in terms of eligibility criteria, mAb and radioisotope used, dose, number of treatments, dose of unlabelled mAb pre-infused or co-infused and the biodistribution or dosimetry estimation required for administration of a therapeutic dose of radiolabelled mAb. Nevertheless, virtually all clinical studies performed to date have shown high response rates for RIT in NHL and have been well reviewed (Wilder et al 1996; Knox & Meredith 2000; Davis et al 2004; Kaminski et al 2005; Sharkey & Goldenberg 2005; Park & Press 2007).

DeNardo et al (1998) initially pioneered RIT for NHL with ^{131}I-anti-HLA-DR mAb (Lym-1). The efficacy of escalating fractionated doses of ^{131}I-Lym-1 in the range 1480–3700 MBq m^{-2} (40–100 mCi m^{-2}) resulted in an overall response rate of 52% in 21 treatment courses administered to 20 patients, with seven patients (33%) achieving complete response and four patients (19%) achieving partial response (DeNardo et al 1998). Goldenberg et al (1991) used an ^{131}I-LL2 (anti-CD22) mAb to treat a variety of B-cell lymphomas. In one of their trials, 4 out of 17 patients achieved objective remission, including one complete response. In another trial, ^{90}Y-LL2 was administered to seven patients with B-cell lymphomas, two of whom achieved partial response (Goldenberg et al 1991). Table 2 summarizes the results of randomized controlled trial of Zevalin versus rituximab in refractory low-grade, or transformed follicular B-cell lymphoma.

Impressive responses have been observed in all of the clinical trials using ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab in relapsed B-cell lymphomas. Although both ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan bind to the same CD20 antigen, tositumomab binds to a unique epitope of CD20 (Tedder & Engel 1994). The radioisotopes also have important differences in their emission characteristics. Table 3 compares the main characteristics of ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan.

^{90}Y-ibritumomab tiuxetan consists of a monoclonal IgG1 kappa light chain anti-CD20 mAb, the murine parent immunoglobulin of rituximab, covalently attached to a metal chelator molecule (tiuxetan; an isothiocyanatobenzyl derivative of the polyaminocarboxylic acid DTPA), which stabilizes the mAb–isotope complex for delivery to the lymphoma site (Grillo-Lopez 2002). The biological elimination half-life of ^{90}Y-ibritumomab tiuxetan is 30 h. More than 90% of the β-radiation is absorbed within a 5-mm proximity (corresponding to a diameter of 100–200 cells) of the radiation source. This facilitates highly targeted delivery of radiation without the need for patient isolation or shielding (Press & Rasey 2000).

Table 2 Updated duration of response data in two phase II and the single phase III 'pivotal' trials of ^{90}Y-ibritumomab tiuxetan versus rituximab in relapsed or refractory low-grade or transformed follicular B-cell NHL

	Phase I/II (n = 51) (Gordon et al 2004)	Phase II (n = 30) (Witzig et al 2002a)	Phase III (n = 73) (Witzig et al 2002b)
Overall response (%)	73	83	80
Median DR (months)	11.7	11.5	13.9
CR, CRu (%)	29*	47	34
Median DR (months)	28*	23	23
Ongoing CR, CRu (%)	19	14	32
Median DR (months)	62.1	41.2	42.2
Range	60+ to 66+	40+ to 42+	33+ to 48+

DR, duration of response; CR, complete response; CRu, unconfirmed complete response.
*Patients with CR only.

Table 3 Characteristics of ^{131}I-tositumomab (Bexxar) and ^{90}Y-ibritumomab tiuxetan (Zevalin)

	^{131}I-tositumomab	^{90}Y-ibritumomab tiuxetan
US Trade name	Bexxar	Zevalin
Monoclonal antibody	Tositumomab (anti-B1)-murine	Ibritumomab (2B8)-murine
Chelation	Simple	More complex
Isotope	^{131}I	^{90}Y
Isotope emissions	γ and β	β only
β-energy	0.606 MeV	2.293 MeV
β-particle path length	0.8 mm	5.3 mm
Isotope half-life	8 days	2.6 days
γ-energy	0.364 MeV	None
Radiation protection measures	4–6 day inpatient stay in shielded room. Outpatient in USA	Outpatient
Isotope excretion	Renal (variable)	Limited
Normal tissue uptake	Bone marrow. Thyroid (pre-blocked with KI)	Bone
Pre-dose (unlabelled mAb)	Tositumomab (450 mg/patient)	Rituximab (250 mg m^{-2}) × 2
Dose	75 cGy whole body dose	14.8 MBq kg^{-1} (0.4 mCi kg^{-1})
	Dosimetric dose obligatory	Dosimetric dose not required
	Dose reduction for thrombocytopenia	Dose reduction for thrombocytopenia

The tiuxetan chelator molecule provides a stable link between the mAb and the radioisotope, and therefore free isotope clearance rates are minimal and predictable with 7.3 ± 3.2% of the radiolabelled activity being excreted in the urine over 7 days (Witzig et al 1999). Consequently ^{90}Y-ibritumomab tiuxetan may be administered on an outpatient basis. Figure 2 outlines the ^{90}Y-ibritumomab tiuxetan therapeutic regimen.

Four clinical trials, including three phase I/II and one randomized study, formed the basis of the FDA submission for ^{90}Y-ibritumomab tiuxetan. The initial phase I/II study demonstrated that the dose-limiting factor was myelotoxicity (Witzig et al 1999). The maximum tolerated dose was identified as 14.8 MBq kg^{-1} (0.4 mCi kg^{-1}) to a maximum of 1184 MBq (32 mCi) for patients with a baseline platelet count of $\geq 150 \times 10^9$ L^{-1} and 11.1 MBq kg^{-1} (0.3 mCi kg^{-1}) for patients with baseline platelet counts of $< 150 \times 10^9$ L^{-1} but $\geq 100 \times 10^9$ L^{-1}. In this study a high overall response rate for the intent-to-treat population (n = 51) was seen at 67% (26% complete response; 41% partial response); for low-grade disease (n = 34), 82% (26% complete response; 56% partial response); for intermediate-grade disease (n = 14), 43%.

A phase II study of patients with mild thrombocytopenia (baseline platelet count of 100×10^9 L^{-1} to 150×10^9 L^{-1}) was conducted using the reduced dose of 11.1 MBq kg^{-1} (0.3 mCi kg^{-1}). The overall response rate was 83% (37% complete response, 6.7% complete response unconfirmed, and 40% partial response). Kaplan–Meier estimated median time to progression (TTP) was 9.4 months (range, 1.7–24.6). In responders, Kaplan–Meier estimated median TTP was 12.6 months (range, 4.9–24.6). Toxicity was primarily haematological, transient and reversible. The incidence of grade 4 neutropenia, thrombocytopenia and anaemia was 33%, 13% and 3%, respectively. The conclusion from this study was that reduced-dose ibritumomab tiuxetan is safe and well tolerated and has significant clinical activity in this patient population (Wiseman et al 2002).

A further single-arm phase II study of ^{90}Y-ibritumomab tiuxetan was undertaken to examine the efficacy of ^{90}Y-ibritumomab tiuxetan in a group with rituximab-refractory disease (Witzig et al 2002a). Fifty-four heavily pre-treated patients with follicular lymphoma were recruited who were refractory to, or progressed after, rituximab. The trial showed an overall response rate of 74% and a complete response rate

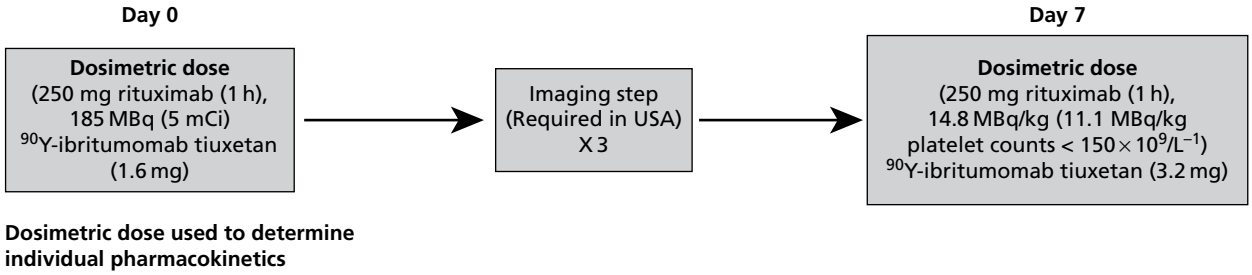

Figure 2 Treatment regimen for ^{90}Y-ibritumomab tiuxetan.

of 15%, despite a median of 4 previous therapies and 73% of patients having bulky disease (≥5 cm diameter). Kaplan–Meier-estimated duration of response was 6.4 months, with a time to progression of 6.8 months in all patients and 8.7 months in responders. The randomized controlled trial has been described earlier and compared ^{90}Y-ibritumomab tiuxetan with rituximab in relapsed or refractory low-grade B-cell NHL. This confirmed that ^{90}Y-ibritumomab tiuxetan results in superior overall and complete response rates to those seen with the 'naked' mAb, rituximab.

A randomized phase III trial including 143 patients with relapsed or refractory low-grade, follicular or transformed NHL compared the efficacy of a single dose of 14.8 MBq kg^{-1} (0.4 mCi kg^{-1}) ^{90}Y-ibritumomab tiuxetan with rituximab (375 mg m^{-2} once weekly for 4 weeks) (Witzig et al 2002b). Response rates were significantly higher in the ^{90}Y-ibritumomab tiuxetan arm with an overall response rate of 80% vs 56% ($P = 0.002$) and a complete response rate of 30% vs 16% ($P = 0.004$). Subgroup analysis revealed a superior benefit for patients with follicular histology with an overall response rate of 86% vs 55% ($P < 0.001$) and a significantly increased ($P < 0.04$) time to treatment progression for this subgroup. The overall time to progression was, however, no different between both treatment groups, but patients treated with ^{90}Y-ibritumomab tiuxetan showed a trend towards longer median duration of response (14.2 months vs 12.1 months) and more often achieved responses lasting longer than 6 months (64% vs 47%).

A recent retrospective analysis suggests that treatment with ^{90}Y-ibritumomab tiuxetan is associated with higher response rates and longer duration of response when used earlier in the therapy schedule (Gordon et al 2004). An integrated analysis of 211 patients treated in clinical trials compared efficacy and safety of ^{90}Y-ibritumomab tiuxetan in patients who had received one previous therapy (n = 63) with patients who received two or more previous therapies (n = 148). Patients receiving ^{90}Y-ibritumomab tiuxetan as second-line therapy had a greater overall response rate (86% vs 72%; $P = 0.051$) and confirmed or unconfirmed complete response rate (CR/CRu; 49% vs 28%; $P = 0.004$) and a significantly longer median time to disease progression (TTP) (12.6 months v 7.9 months; $P = 0.038$). In CR/CRu patients, the median TTP (23.9 months v 15.6 months; $P = 0.0442$) and median duration of response (22.8 months vs 14.6 months; $P = 0.0429$) were both significantly increased in patients with only one previous therapy (n = 53).

The results from a large phase III randomized European and Canadian intergroup study (n = 414) in previously untreated follicular NHL were presented at the American Society of Hematology (ASH) in 2007 (Hagenbeek et al 2007). This study included patients with follicular (grade 1 or 2) lymphoma, advanced stage (III or IV) at time of diagnosis. The initial chemotherapy used was left to the discretion of the individual clinician between single-agent oral chlorambucil and anthracycline-based combination chemotherapy. For patients who responded to first-line chemotherapy (i.e. partial response or CR/CRu), ^{90}Y-ibritumomab tiuxetan (Zevalin) was administered 6–12 weeks after the last doses of chemotherapy. For all patients the complete response rate in the control group was 53.3% compared with 87.4% in those that received ^{90}Y-ibritumomab tiuxetan (Zevalin). This high complete response rate was almost identical in all subgroups of pre-treatment chemotherapy despite the difference in complete response rate between the initial regimens, such as single-agent chlorambucil (complete response rate 31%) and CHOP (complete response rate 56%). In summary it appears that ^{90}Y-ibritumomab tiuxetan (Zevalin) was the 'equaliser' for less active chemotherapy and as a consolidation therapy improved the response quality in 77% from partial response to CR/Cru in all patients, regardless of the type of initial chemotherapy given. ^{90}Y-Ibritumomab tiuxetan (Zevalin) consolidation significantly ($P < 0.0001$) prolonged the median progression-free survival by 2 years compared with no further treatment in patients responsive to first-line induction treatment. For the patients receiving Zevalin the median progression-free survival was 37 months compared with 13.5 months in the control group (Hagenbeek et al 2007).

Interestingly a 2-year improvement in progression-free survival was seen in patients with CR/CRu after first-line treatment, which suggests that ^{90}Y-ibritumomab tiuxetan benefits those with minimal residual disease and the median progression-free survival was increased to 54.6 months compared with 29.9 months for the control patients ($P = 0.01$). For patients who only achieved a partial response after initial induction chemotherapy, ^{90}Y ibritumomab tiuxetan improved the progression-free survival from 6.3 months (control) to 29.7 months ($P < 0.0001$). The improvement in response quality with conversion from partial response to CR/CRu in 77% of patients after ^{90}Y-ibritumomab tiuxetan (Zevalin) is impressive, and ^{90}Y-ibritumomab tiuxetan consolidation was well tolerated with no unexpected toxicity and a low incidence of infectious events despite a high proportion of patients with grade 3/4 neutropenia (66%).

In summary, there is now good evidence to demonstrate that RIT is extremely effective at improving the quality of the response, from partial to complete in follicular lymphoma after primary chemotherapy, and an overall complete response rate of over 87% makes this a highly active approach that leads to a large improvement in progression-free survival. Using RIT in patients that fail to achieve a complete response after initial induction therapy may therefore be a useful strategy. A definitive role for RIT in the rituximab–chemotherapy (R-Chemo) era has not, however, been conclusively demonstrated and this question forms the basis of a European inter-group study whereby patients receive R-Chemo induction regimens and are then randomized to receive ^{90}Y-ibritumomab plus maintenance rituximab or maintenance rituximab alone.

Clinical responses have also been observed for transformed follicular and relapsed diffuse large B-cell lymphoma (DLBCL) when treated with ^{90}Y-ibritumomab tiuxetan. An initial phase I/II study reported a response rate of 58% with a 33% complete response rate in a group of just 12 patients that had relapsed following 2 previous chemotherapy regimens that included CHOP (Witzig et al 2002b). A prospective, single-arm, open-label, non-randomized, multi-centre phase II trial was therefore undertaken to evaluate the efficacy and safety of ^{90}Y-ibritumomab tiuxetan in patients over 60 years of age with relapsed or primary refractory DLBCL not suitable for autologous stem cell transplantation (ASCT). Patients were divided into two groups—those previously treated with chemotherapy alone (Group A, n = 76) and those previously treated with chemotherapy and rituximab but who had a short duration of response (Group B, n = 28) (Morschhauser et al 2007). All patients received a single dose of 14.8 MBq kg^{-1} (0.4 mCi kg^{-1}) of ^{90}Y-ibritumomab tiuxetan up to a maximum dose of 1184 MBq kg^{-1} (32 mCi). In total, 103 patients were able to be evaluated for treatment efficacy, and 104 for safety. An overall response rate of 44% was observed in the entire study population. In Group A, the overall response rate was > 50%. In Group B, where 37% of patients were refractory to rituximab–CHOP, the overall response rate was 19%. Adverse events, with the exception of haematological side-effects, were generally mild (grade 1/2) and the incidence of severe infection was low, with only 7% of patients hospitalized for infection during the study. The results of this study were encouraging and clinical trials are now underway in the US, or at an advanced stage of development in the EU, to integrate ^{90}Y-ibritumomab tiuxetan into a front-line treatment for DLBC alongside R-chemo schedules.

Tositumomab was the first mAb to be produced against a B-cell antigen (Nadler et al 1981). The ^{131}I-tositumomab regimen is completed within 1–2 weeks and consists of a tracer dose of the radioimmunoconjugate followed by the therapeutic dose 7–14 days later. Each infusion of ^{131}I-tositumomab is preceded by an infusion of a pre-dose of 450 mg 'cold' or unlabelled tositumomab and the therapeutic regimen is outlined in Figure 3. Whole-body gamma camera imaging is performed three times over the week following the trace-labelled infusion to calculate the whole-body half-time and the dose required for the therapeutic infusion to deliver a 65–75 cGy whole-body dose (WBD) (usually 3700–5550 MBq (100–150 mCi). Dose adjustments to 65 cGy were made for a baseline platelet count of 100 000 mm^{-3} to < 150 000 mm^{-3} and for obesity.

Kaminski and colleagues initially conducted a series of trials at the University of Michigan using ^{131}I-tositumomab, for the treatment of relapsed follicular lymphoma (Kaminski et al 1993, 1996). In a pivotal study, 60 extensively pre-treated patients were given a single administration of ^{131}I-tositumomab (Kaminski et al 2001). Disease responses were compared with the patients' previous responses to chemotherapy for follicular or transformed follicular NHL. A partial or complete response was observed in 39 patients (65%) after iodine ^{131}I-tositumomab, compared with 17 patients (28%) after their last qualifying chemotherapy ($P < 0.001$). ^{131}I-tositumomab therapy was shown to provide greatly superior relapse-free survival compared with the last qualifying chemotherapy. Gregory et al (2005) have demonstrated, in an analysis of large numbers of patients (> 1100), that superior outcomes were associated with earlier use of the drug and, conversely, that lower overall and complete response rates were observed in patients that were more extensively pre-treated.

Since 1990, well over 1000 patients with 'low-grade' and transformed lymphoma have been treated with ^{131}I-tositumomab. Long-term follow-up data were published by Fisher et al (2005) who performed an integrated efficacy analysis of five clinical trials of ^{131}I-tositumomab in 250 patients with

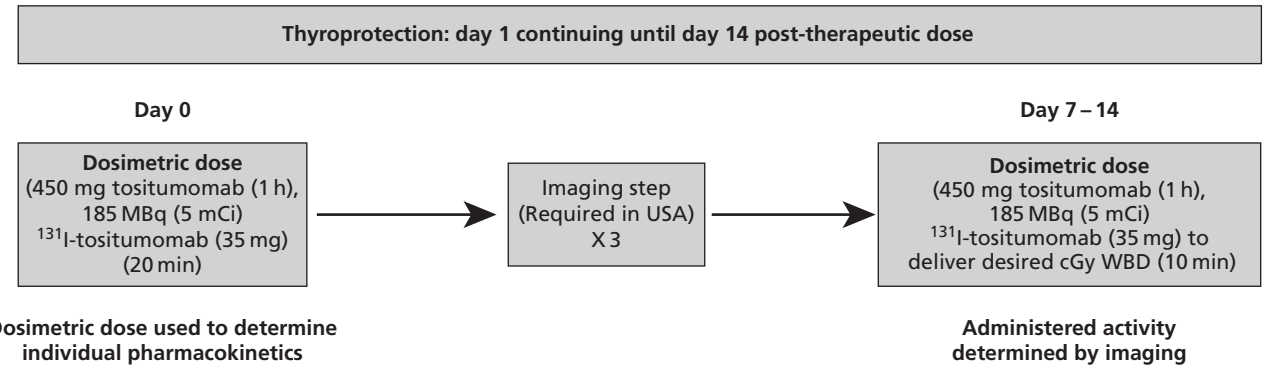

Figure 3 Treatment regimen for ^{131}I-tositumomab.

relapsed or refractory follicular or transformed follicular NHL, and the durability of these responses are discussed below.

Impressive response rates have also been seen in patients that were refractory to rituximab who were subsequently treated with ^{131}I-tositumomab. Horning et al (2005) used ^{131}I-tositumomab to treat 40 patients with low-grade NHL, 72% of whom had received four or more previous lines of therapy and 60% of whom had failed to respond to rituximab. An overall response rate of 68%, with a complete response rate of 30%, was noted and a median duration of response of 14.7 months was reported. Of the 12 complete responders, 9 remained in complete response at the time of presentation with a range of 12–26 months.

Kaminski and colleagues have shown highly promising results in the front-line treatment of previously untreated low-grade follicular lymphoma using ^{131}I-tositumomab (Kaminski et al 2005; Kaminski 2007). An encouraging overall response rate of 95% was seen, with 75% achieving complete response. PCR (polymerase chain reaction) was used to detect rearrangement of the *BCL2* gene, which revealed molecular responses in 80% of assessable patients who had a clinical complete response. The most recent update included 76 patients with a median follow-up of 5.1 years. The actuarial 5-year progression-free survival for all patients was 59%, with a median progression-free survival of 6.1 years. Haematological toxicity was moderate, with no patient requiring transfusion or G-CSF (granulocyte colony stimulating factor (Kaminski et al 2005), though 48 out of 76 (63%) patients developed detectable HAMA (human anti-mouse antibody) responses after a single-course of treatment with ^{131}I-tositumomab.

Contribution of targeted radiation to clinical response

The contribution of targeted radiation to the overall response seen in RIT has been addressed with two randomized studies comparing the radioimmunoconjugates ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab with the unlabelled mAbs (Witzig et al 2002b; Davis et al 2004). Both studies have shown greatly superior clinical responses of RIT over the unlabelled mAb. The study of ^{90}Y-ibritumomab tiuxetan versus rituximab is described above and the second study, comparing treatment outcomes for unlabelled tositumomab (pre-dose) and ^{131}I-tositumomab with those for an equivalent total dose of unlabelled tositumomab, involved 78 patients with refractory or relapsed low-grade NHL (Davis et al 2004). The investigators reported an overall response rate of 55% versus 19% ($P=0.002$) with a complete response rate of 33% versus 8% ($P=0.012$) in ^{131}I-tositumomab versus unlabelled tositumomab groups, respectively. The median duration of the overall response was not reached for ^{131}I-tositumomab and was 28.1 months for unlabelled tositumomab. The median duration of complete response was not reached in either arm and the median time to progression was 6.3 versus 5.5 months ($P=0.031$), respectively. Although haematological toxicity was more severe and non-haematological side-effects were more frequent after ^{131}I-tositumomab than after tositumomab alone, there were no serious infectious or bleeding complications. The frequency of developing HAMA was similar in the two arms at 27% (^{131}I-tositumomab group) versus 19% (tositumomab alone group). This study demonstrated that although unlabelled tositumomab showed single-agent activity, the conjugation of iodine-131 to tositumomab significantly enhanced the therapeutic efficacy and improved responses were observed in the cross-over part of the study whereby patients who had failed to respond to unlabelled tositumomab subsequently responded to ^{131} I-tositumomab (Davis et al 2004).

Radioimmunotherapy induces durable remissions

Perhaps the most impressive finding to emerge from these maturing data using RIT in follicular lymphoma is the remarkable duration of response enjoyed by some patients. Fisher et al (2005) performed an analysis of the long-term follow-up of patients treated with ^{131}I-tositumomab. The overall response rate was 47–68%, with complete response rates of 20–38%. At a median follow-up of 5.3 years, 5-year progression-free survival was 17% and 81 of 250 patients (32%) had a time-to-progression of ≥ 1 year. These patients were termed the durable response population. For the durable response population, 44% had not progressed at ≥ 2.5 to ≥ 9.5 years, with a median duration of response of 45.8 months and, impressively, the median duration of those patients who had achieved a complete response had not been reached. Interestingly many of the patients who enjoyed these long durable responses had many poor prognostic characteristics, including bone-marrow involvement (41%), bulky disease ≥ 5 cm (49%) and transformed histology (23%). Forty-three percent of the patients had been treated with more than four previous therapies and 36% had not responded to their most recent therapy. The durability of the responses seen with RIT has also been observed with ^{90}Y-ibritumomab tiuxetan (Gordon et al 2004). In all of the RIT studies, around 70% of those patients who achieved a complete response remained in remission for years (Witzig 2003; Gordon et al 2004). Further, some patients treated in the early studies have now been in remission for more than 5 years afterwards and have a median follow-up of almost 4 years (Table 2). An analysis of long-term responders underscores the potential of ^{90}Y-ibritumomab tiuxetan to achieve durable remissions with an observed median duration of response approaching 2 years and responses greater than 6 years being observed in some patients (Gordon et al 2004).

An analysis of prognostic factors has confirmed that this remarkable durability of response is unlikely to be accounted for by patient selection as most of these durable remissions have been achieved in heavily chemotherapy pre-treated and chemo-refractory patients with validated poor prognostic factors, such as extensive previous therapy (1–9 regimens), chemo-refractory disease, high lactate dehydrogenase level and extra-nodal disease. The only clinical factor that was found to correlate to clinical response to RIT was the maximum dimension of the largest tumour. Patients with tumours that had a maximum dimension of <5 cm had an overall response rate of 90% ($P<0.001$), whereas patients that had tumours more bulky than this were less likely to respond (Witzig et al 2003).

Integration into treatment algorithms for NHL

The high response rates and durable remissions achieved with either ^{90}Y-ibritumomab or ^{131}I-tositumomab make single-agent RIT an attractive treatment option for many patients with relapsed follicular lymphoma. Furthermore, the impressive duration of response seen after achieving a complete response is achieved with a treatment that is completed within a week; the treatment is very well tolerated, has minimal non-myelotoxic toxicity and easily manageable myelotoxicity.

The introduction of RIT has some parallels with the introduction of rituximab into clinical practice in the late 1990s. There is no doubt that RIT drugs are highly active but considerable uncertainty remains as to when and how best to integrate RIT into clinical practice, even within the licensed indication of relapsed low-grade (in the US) or relapsed rituximab failure or refractory follicular lymphoma (within the EU). The treatment is well tolerated by older patients and RIT makes this approach a strong recommendation for relapsed follicular NHL.

In the education session on follicular NHL at the American Society of Hematologists (2004), an algorithm was suggested for follicular lymphoma, where RIT was recommended upon the first relapse after a rituximab–chemotherapy combination (Figure 4) (Winter et al 2004). Currently this seems a reasonable treatment approach to follicular lymphoma as it does not exclude transplantation options at a later date especially if, as recommended, progenitor stem cell collection is performed at the time of the initial remission. Although data is emerging to suggest RIT can be given after transplantation (see above) this is inevitably at lower doses. Further clinical trials are needed to further define the role of RIT in the treatment of other NHL, although the recent randomized data from the FIT trial suggest that RIT may play a useful part in consolidating response from partial to complete and thereby potentially increasing the progression-free survival and perhaps even overall survival, although this remains unproven.

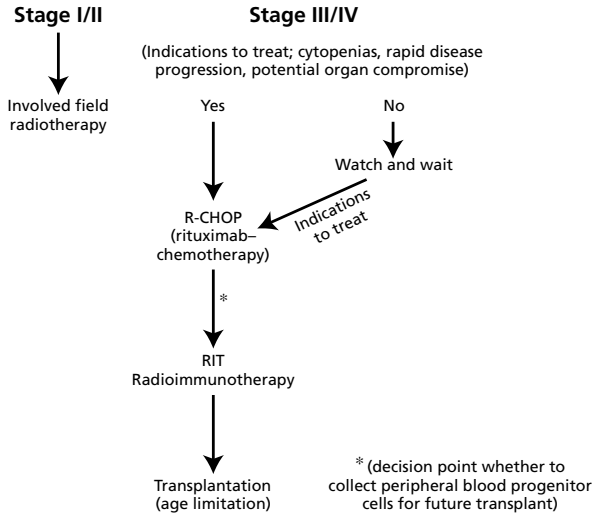

Figure 4 A proposed treatment algorithm for follicular lymphoma patients after first relapse following a rituximab–chemotherapy combination (Winter et al 2004).

Conclusion

In summary, the exquisite sensitivity of haematological malignancies to targeted radiation, alongside the impressive results achieved by the pioneers in this field, suggests that radioimmunotherapy is likely to be a productive area for future clinical research. Despite these clinical advances there remains much to discover about the mechanisms of action in successful radioimmunotherapy. Recent experimental work has demonstrated that the combination of targeted radiation and antibody effector mechanisms are critical to long-term clearance of tumour. Further work is required to enhance our understanding and determine whether a prolonged host immune response is induced by RIT and whether this underlies the long durable remissions over many years that some patients enjoy. The huge progress made over the last decade with the development of RIT in the treatment of haematological malignancies leads to distinct optimism that further development of RIT over the next five years will lead to significant improvement in clinical outcome for patients.

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