Pergamon

European Journal of Cancer, Vol. 35, No. 4, pp. 549–557, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(98)00420-1

Review

Monoclonal Antibody Therapy for B Cell Non-Hodgkin's Lymphomas: Emerging Concepts of a Tumour-targeted Strategy

C. Buske, M. Feuring-Buske, M. Unterhalt and W. Hiddemann

Department of Internal Medicine III, Ludwig-Maximilians, University of Munich, Munich, Germany

Although much progress has been made in the understanding of the pathobiology of malignant lymphomas in recent years, progress in the treatment of patients with this diagnosis has been limited. Monoclonal antibody therapy is an innovative and promising concept in the treatment of malignant lymphoma, and the current status of this treatment is reviewed here. Phase I/II clinical trials have proven the high antilymphoma activity of antibody-based therapeutic strategies. Radioimmunoconjugates with myeloablative activity have induced response rates of between 80 and 100% in heavily pretreated patients. The chimeric monoclonal antibody IDEC-C2B8 has shown high antilymphoma activity in patients with relapsed follicular lymphoma with an overall response rate of up to 50%. The combination of the IDEC-C2B8 antibody with standard chemotherapy has shown encouraging results with no increase in toxicity compared with chemotherapy alone. The introduction of antibody therapy promises to open new perspectives in the treatment of patients with malignant lymphoma. Prospective randomised clinical trials will define the patient who will gain maximal benefit from antibody-based therapy. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: malignant lymphoma, antibody therapy, chimeric antibody, radioimmunoconjugate, immunotoxin, anti-idiotype antibody
Eur J Cancer, Vol. 35, No. 4, pp. 549–557, 1999

INTRODUCTION—CURRENT THERAPEUTIC STRATEGIES

MALIGNANT LYMPHOMAS belong to the 10 most frequent types of cancers worldwide, with a steep increase in their prevalence of 7% per year. The age-adjusted incidence rates increased by 60% and the mortality rates by 32% from the late 1970s to the late 1980s [1–4]. Much progress has been made in unravelling the pathobiology of this heterogeneous group of malignancies, which is reflected in the recently proposed 'Revised European American Lymphoma' (REAL) classification, which—in contrast to the Working Formulation—defines each distinct lymphoma subentity by its specific morphology and immunophenotype and classifies the lymphomas according to cell lineage (T-/B-lineage) and differentiation stage (precursor/peripheral) [5, 6].

Correspondence to C. Buske at The Terry Fox Laboratory, 601 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3, e-mail: chris@terryfox.ubc.ca
Received 23 Jul. 1998; revised 26 Nov. 1998; accepted 1 Dec. 1998.

In contrast to the rapidly growing knowledge about the pathobiology of malignant lymphomas, progress in clinical management of these diseases has been limited. Patients with high-grade non-Hodgkin's lymphoma (NHL) are generally treated with curative intention, and although high initial response rates can be achieved by combination chemotherapy, 50–70% of patients relapse and subsequently die from their disease [7–10]. In patients suffering from low-grade B cell NHL, only a small proportion of patients with limited disease can be cured by extended-field or total nodal irradiation [11, 12]. For patients with advanced stage disease, i.e. for approximately 85% of cases, no established therapeutic strategy with curative potential exists and there is no consensus about their standard therapy [13–15].

The development of novel therapeutic strategies has concentrated on the intensification of therapy by myeloablative chemo- or radiochemotherapy supported by bone marrow (BMT) or peripheral blood stem cell transplantation (PBSCT). Although these therapeutic approaches may lead to more extensive disease eradication, the therapeutic principle

of dose intensification is limited by the lack of specificity of this approach, leading to increased therapy-related short- and long-term toxicity. In addition, the clinical benefit of these high-dose regimens is still unclear and the results of ongoing prospective randomised clinical trials have yet to prove their superiority in comparison with standard therapy [16–18].

Thus, one of the main goals of clinical research is the development of innovative therapeutic measures with increased lymphoma specificity. Immunobiological therapeutic approaches promise to fulfil these criteria and may synergise with conventional chemotherapy for lymphoma eradication. One of the most successful developments in this respect has been the introduction of the 'biological response modifier' interferon-alpha (IFN-α) in the treatment of follicular low-grade lymphomas. This cytokine prolongs disease-free survival in patients with advanced stage follicular lymphoma, when applied simultaneously with chemotherapy or as maintenance after initial cytoreductive treatment [19–23].

Recently, substantial progress has been made in antibody-based therapeutic strategies for patients with B cell NHL. This concept uses the expression of lymphoma-associated antigens and applies B cell-specific monoclonal antibodies (MAb) as therapeutic agents. Based on the promising data of phase I/II clinical trials in the last few years, this strategy has gained growing attraction and promises to open new perspectives for a lymphoma-targeted therapy with high specificity and low toxicity in patients with B cell NHL.

PRINCIPLES OF ANTIBODY THERAPY

Introduction

Like their normal counterparts, malignant B cell lymphomas are characterised by the surface expression of B cell antigens according to their differentiation stage of B cell ontogeny [24]. MAb specifically recognise their target structure. They can exert tumour-suppressive effects by at least three major mechanisms: an intrinsic cytotoxic activity; antibody-dependent cellular cytotoxicity (ADCC); and activation of complement-dependent cytolysis (CDC) [25–28].

The clinical efficacy of an antibody-based antilymphoma therapy depends first on the selected target antigen. The selected target antigen should be lymphoma specific or at least highly lymphoma associated. The selective expression of the antigen avoids binding of the MAb to normal non-haematopoietic tissue or to normal haematopoietic progenitor cells, thus reducing undesired non-specific toxicity. Furthermore, shedding of the antigen impairs antibody binding to surface structures by forming soluble circulating antibody–antigen complexes, thus reducing the pool of free MAb [29–31].

The second critical parameter, which dictates the clinical efficacy of antibody therapy is the individual characteristics of the antibody construct itself. Basically, MAbs can be applied unconjugated, as an immunoconjugate, linked to a plant or bacterial toxin, or as a radioconjugate, coupled to a radionucleide [32, 33].

Unconjugated antibodies

Murine antibodies, which were first used in antibody therapy, harbour the disadvantage of presenting foreign immunogenic protein, thus inducing human antimouse antibody formation (HAMA) with rapid clearance of the therapeutic MAb after repeated courses of therapy. In addition, murine antibodies are critically impaired by activating both ADCC and CDC in the human system. This is particularly of relevance for the clinical efficacy of murine antibodies, because most murine MAb have no, or only limited, endogenous cytotoxic activity. The limitations of murine MAb can be overcome by engineering 'chimeric' or 'humanised' antibodies. The chimeric construct combines the genes of the murine variable region with the constant region of the human immunoglobulins, maintaining the binding properties of the murine antigen and the effector properties of the human constant region (FC) [34]. The humanised antibody is composed of the original murine hypervariable sequences within a human V-region framework and constant region, thus further reducing the murine component of the antibody [35]. These constructs reduce significantly the immunogenicity of the MAb and are highly efficient in inducing CDC and ADDC (Figure 1) [36].

Another important determinant for the clinical efficacy of the antibody is its pharmacokinetic characteristics, which decide the bioavailability of the antibody at the target cell. Early studies with radiolabelled antibodies demonstrated that less than 0.02% of the applied MAb binds to the tumour target. One critical parameter for the capability of the MAb to penetrate into the tumour is its size. Using genetic technology, the expression of small 25 kDa constructs as single chain fragments of the hypervariable binding region (scFv fragments) has become possible. These antibody fragments are characterised by an improved capacity to penetrate the tumour with high local bioavailability [37]. However, the bioavailability of the MAb at the tumour cell is also strongly influenced by external factors: key obstacles for targeting are intrinsic tumour characteristics such as non-uniform tumour vascularisation, capillary permeability or differences in the interstitial pressure [38]. In addition, the binding site barrier, caused by the binding of MAb to peripheral tumour antigens, is responsible for delayed penetration of the MAb into the tumour [39].

A strategy to optimise the antilymphoma activity of native MAb is the construction of bi-specific antibodies. These antibodies are frequently directed against the TcR/CD3 complex and a B cell antigen as the lymphoma-associated target structure. This strategy recruits CD4- and CD8-positive cytotoxic T cells and induces T cell-mediated specific tumour lysis by simultaneous binding to the CD3 complex and the tumour-associated antigen [40–43]. A different bi-specific construct uses the FcγR as the cytotoxic trigger molecule on the effector cell and a tumour-associated antigen to optimise antibody-mediated tumour lysis [44]. Apart from the FcγRI (CD64), other antigens expressed on cytotoxic subpopulations, such as the FcγFIIIa CD16 or the FcαRI (CD89), are interesting trigger molecules for bi-specific antibodies [45].

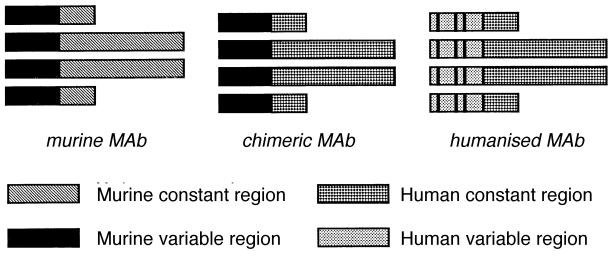

Figure 1. Structure of monoclonal antibodies.

A different approach to improve the therapeutic efficacy of native MAb is the simultaneous application of a different lymphoma-suppressive agent such as IFN-α or by adding immunostimulatory agents, thus activating immunological cell-mediated antilymphoma activity in addition to the inherent antibody-mediated cytotoxicity [21–23]. A recent elegant study demonstrated that the efficacy of an antibody-mediated antilymphoma therapy can be considerably augmented by the addition of an immunostimulatory oligodeoxynucleotide, activating natural killer (NK) cells and macrophages by the CpG motif in a mouse model [46].

Conjugated antibodies

The cytolytic activity of the native MAb can be enhanced by coupling the antibody with a plant or bacterial toxin (immunotoxin) or with a radionucleide (radioimmunoconjugate). The basic idea of this strategy is to use the specificity of the MAb to transfer the toxicity of the cytotoxic reagent to the target cell [32, 33].

In first and second generation immunotoxins, the plant toxin, ricin, was the most widely used toxic component of the construct. Ricin consists of two chains, the toxic A chain with its blocking effect on protein synthesis and the B chain, which is responsible for binding the toxin to the ubiquitous galactose residues on mammalian cells [47]. In immunotoxins linked to the A chain, the B chain is replaced by the antibody or the Fab fragment to minimise non-specific binding of the construct. In immunotoxins which are coupled to the 'blocked ricin (bR)' the non-specific binding sites are masked by oligosaccharides. This construct exploits the internalisation function of the B chain and *in vitro* data have shown significantly reduced non-specific toxicity of the bR compared with native ricin [48]. The 'third generation' immunotoxins are chimeric proteins, consisting of the coding regions of ADP-ribosylating toxins such as *pseudomonas* exotoxin or *diphtheria* toxin fused to single-stranded variable regions of immunoglobulin light chains by a synthetic linker. The advantage of these constructs is the possibility of large scale production by recombinant genetic technology and their improved capacity to penetrate the tumour by their reduced size [49].

NHL are highly radiosensitive and, therefore, ideal candidates for radiation therapy. Radiolabelled MAbs deliver constant low-dose radiation compared with external beam radiation. Theoretically, constant radiation reduces the chance of tumour escape and permits synchronisation of the lymphoma population into the radiosensitive G_2 phase of the cell cycle. In addition, lymphoma subclones with low antigen or no antigen expression are hit by radiation from antigen-positive lymphoma cells coated by the radiolabelled antibodies [50]. The most frequent radionucleides used are ^{131}iodine (^{131}I) and yttrium 90 (^{90}Y). ^{90}Y has theoretically several advantages over ^{131}I. It has no long-range γ-irradiation as has ^{131}I, but is an intermediate-energy β emitter with a maximum energy of 2.28 MeV and a range of between 1 and 1000 cell diameters. The maximal B energy is substantially higher than the maximal B energy of ^{131}I with 0.61 MeV, leading to a more effective killing of adjacent antigen-negative tumour cells. Because ^{90}Y is a pure β emitter ^{90}Y-labelled MAb permit out-patient treatment. Additionally, the radionucleide has a shorter half-life of 64.2 h compared with 8.1 days of ^{131}I, reducing non-specific toxicity [51, 52]. However, application of ^{90}Y does not allow imaging, in contrast to ^{131}I, which is the radionucleide most often used in clinical trials. In addition, studies in human colon cancer xenograft models have proven a higher effectiveness of ^{131}I compared with ^{90}Y when a three-dimensional dose calculation for interpretation of tumour dosimetry was performed [53]. The attraction of radioimmunotherapy with α-particle emitting radioimmunoconjugates was recognised for many years, but technical obstacles prevented the clinical use of α emitters until a few years ago. α emitters such as ^{211}At and ^{212}Bi are attractive for targeted tumour therapy as they show a substantially enhanced specificity compared with β emitters: they deliver radiation with high linear activity, killing the target cell by one or two hits compared with several thousand hits, which are necessary for cell killing by β emission. Moreover, the range of α particles is 50–7 μm compared with the 1–10 mm range of β particles, reducing toxicity to normal bystander cells. Both ^{211}A and ^{212}Bi have a favourable short half-life, 7.2 h and 60.6 min, respectively. Although *in vitro* studies and animal models have demonstrated a high tumour toxicity and first clinical applications in patients with relapsed or refractory acute myeloid leukaemia (AML) were promising, the clinical applicability of α-particle emitting radionuclides has yet to be proven. In addition, there is still a shortage in the supply of α emitters such as ^{212}Bi, which so far impedes the evaluation of this innovative radioimmunological concept in large clinical trials [54].

ANTILYMPHOMA THERAPY: CLINICAL RESULTS

Immunotoxins

Most of the clinical experience with immunotoxins in antilymphoma therapy stems from trials using ricin, *diphtheria* toxin, and *pseudomonas* exotoxin A as the toxic component of the conjugate. So far, mainly phase I/II trials of patients with relapsed or refractory lymphoma have been conducted (Table 1). In an early study, 15 patients with refractory high-, intermediate- and low-grade B cell NHL were treated with an FAB′ anti-CD22 (RFB4)-ricin A-chain conjugate. The majority of patients (14/15) were in an advanced stage of their disease and presented with bulky manifestations. This approach resulted in 6 (40%) partial remissions (PR), but the median time to progression was short at only 1.8 months. Non-specific toxicity was observed consisting of pulmonary oedema, capillary leak syndrome, rhabdomyolysis and expressive aphasia [55]. In a different setting, Grossbard and colleagues used altered ricin with blocked galactose binding sites as the immunotoxin, conjugated to the anti-B4 (CD19) antibody in a phase I dose escalating study. Continuous 7 day intravenous infusion induced 2 complete remissions (CR), 3 PRs and 11 transient responses in 34 patients with relapsed or refractory B cell malignancies (26 NHL, 4 chronic lymphocytic leukaemia (CLL), 4 acute lymphoblastic leukaemia (ALL)). The major toxicity was hepatotoxicity, thrombocytopenia and capillary leak syndrome. The MAb construct induced HAMA in 19 patients [56]. In a phase I clinical trial a deglycosylated ricin A chain immunotoxin, coupled to a murine anti-CD19 MAb demonstrated only limited clinical activity in patients with heavily pretreated advanced low/intermediate-grade B cell NHL with 1 CR and 2 PRs in 31 evaluable patients with B cell lymphoma. The induction of HAMA and/or human antiricin A chain antibody (HARA) was observed in up to 30% of the patients [57]. In the recombinant fusion protein DAB_{486}IL-2 the binding site of

Table 1. *Immunotoxins*

Author [Ref.]	Immunotoxin	Phase	Disease	No. of patients	CR rate (%)	PR rate (%)
Vitetta *et al.* [55]	FAB′-anti-CD22-ricin A	I	Refractory B cell NHL	15	0 (0)	6 (40)
Grossbard *et al.* [56]	Anti-CD19-blocked ricin (anti-B4-bR)	I	Relapsed/refractory B cell malignancies	34	2 (6)	3 (9)
Stone *et al.* [57]	Anti-CD19-ricin A (IgG-HD37-SMPT-dgA)	I	Refractory low/intermediate-grade B cell NHL	31	1 (3)	2 (7)
Le Maistre *et al.* [58]	DAB_{486}IL-2	I	IL-2-receptor-positive NHL, HD, CLL, CTCL	18	1 (6)	2 (11)
Nichols *et al.* [59]	DAB_{486}IL-2	I/II	CTCL, NHL	53	6 (11)	9 (17)

CR, complete remission; PR, partial remission; NHL, non-Hodgkin's lymphoma; IL-2, interleukin-2; HD, Hodgkin's disease; CLL, chronic lymphocytic leukaemia; CTCL, cutaneous T cell lymphoma.

the *diphtheria* toxin was substituted by interleukin-2 (IL-2), targeting only cells with the high-affinity IL-2 receptor. In a phase I study, 18 patients with heavily pretreated IL-2 receptor-positive haematological malignancies (9 NHL, 3 Hodgkin's disease (HD), 5 CLL, 1 cutaneous T cell lymphoma (CTCL)) were treated with the immunotoxin. It induced 1 CR for over 18 months and 2 PRs for more than 12 months and 5 months. The attraction of the conjugate was hampered by the very short half-life of 5 min and the high incidence of antibodies to the *diphtheria* toxin and the DAB_{486}IL-2 construct that were observed in 50% of patients at the end of the study [58, 60]. This construct showed remarkable activity in a recent study comprising 35 patients with CTCL and in 18 patients with NHL. In the CTCL group, 5 CRs which lasted for more than 20, 11, 7, 5 and 4 months and 7 PRs (duration 3–20 months), were obtained. 1 patient with Lennert's lymphoma achieved a CR for over 20 months and 2 patients with B cell NHL achieved PRs of 2 and 9 months' duration [59]. These encouraging results were recently tested in two multicentre phase III studies randomising between placebo and two dose levels of the immunotoxin in patients with CTCL [59, 61, 62].

As most immunotoxins have limited capacity to penetrate large tumour masses, one of the most attractive clinical settings for the use of immunoconjugates is the final eradication of residual disease. This strategy has been analysed in a phase I study, in which 21 patients with low/intermediate-grade B cell NHL in sensitive relapse underwent anti-B cell purged autologous bone marrow transplantation (ABMT). 12 patients were in CR after ABMT and eligible for subsequent therapy with the immunotoxin anti-CD19-bR. Antibody therapy was conducted between day + 61 and day + 208. 11 patients stayed in CR for between 13 and 26 months (median 17 months) after ABMT plus immunotoxin infusion [63]. This promising approach was subsequently tested in a phase III study: 157 patients were randomised to receive either two courses of anti B4-bR after ABMT or no further treatment. No significant difference in disease-free survival between the two treatment arms was observed [64]. In conclusion, the clinical trials demonstrated a clear inherent antilymphoma activity of immunotoxins. However, the induction of a considerable non-specific toxicity, the immunogenicity of the constructs and the often rapid clearance *in vivo* are key obstacles for the broad clinical acceptance of this antilymphoma strategy.

Radioimmunoconjugates

Lymphomas are highly radiosensitive and, therefore, an appropriate target for the use of MAbs conjugated to radionuclides (Table 2). In an early study Press and colleagues demonstrated considerable clinical activity for the anti-CD37 MAb MB-1 coupled to ^{131}I: 4 patients with advanced refractory NHL and favourable biodistribution of the radioimmunoconjugate were treated with a dose delivery of 232–606 mCi. All patients achieved a CR. The first 2 patients relapsed after 4 and 6 months, the other two stayed in continuous CR (CCR) for 11 + and 8 + months. The main toxicity was severe myelosuppression requiring platelet transfusions in all patients and autologous bone marrow reinfusion in 1 case [65]. Using an identical radioimmunoconjugate, Kaminski and associates investigated its antilymphoma activity in a non-myeloablative dose delivery ranging from 25 to 161 mCi: therapeutic doses were applied to 10 patients with relapsed or refractory B cell NHL: the conjugate induced 1 CR and 1 PR lasting 2–6 months [66]. Another MAb applied in several studies is the B1 (anti-CD20)-MAb conjugated to ^{131}I. Kaminski and associates exposed 9 patients with relapsed or refractory B cell NHL to

Table 2. *Radioconjugates*

Author [Ref.]	Radioconjugate	Phase	Disease	Total activity (mCi)	No. of patients	CR rate (%)	PR rate (%)
Press *et al.* [65]	^{131}I-anti-CD37	I	Relapsed/refractory NHL	232–608	4	4 (100)	0 (0)
Kaminski *et al.* [66]	^{131}I-anti-CD37	I	B cell NHL	25–161	10	1 (10)	1 (10)
Kaminski *et al.* [67]	^{131}I-anti-CD20	I	Relapsed/refractory B cell NHL	34–66	9	4 (44)	2 (22)
Kaminski *et al.* [68]	^{131}I-anti-CD20	I	B cell NHL	34–161	28	14 (50)	8 (29)
Kaminski *et al.* [69]	^{131}I-anti-CD20	II	Untreated follicular lymphoma	ND	21	15 (71)	6 (29)
Press *et al.* [70]	^{131}I-anti-CD20	I	Relapsed B cell NHL	234-777	19	16 (84)	2 (11)
Press *et al.* [71]	^{131}I-anti-CD20	II	Relapsed B cell NHL	345–785	21	16 (76)	2 (10)
Czuczman *et al.* [72]	^{131}I-anti-CD21	I	Recurrent/refractory NHL	90–200	18	0 (0)	1 (6)

CR, complete remission; PR, partial remission; NHL, non-Hodgkin's lymphoma; ND, not determined.

non-myeloablative doses ranging from 34 to 66 mCi. 6 of the 9 patients responded with 4 CRs and 2 PRs. 3 of 4 patients were in CCR at 11, 9 and 8 months. Toxic side-effects were minimal. In spite of the low number of treated patients, these results pointed to a high antilymphoma activity of this conjugate [67]. In a phase I study, a total of 28 patients received therapeutic doses ranging from 34 to 161 mCi. 14 patients achieved a CR, 8 patients a PR. 13 of 13 low-grade lymphomas responded with 10 CRs. Transformed lymphomas were also sensitive to treatment in 6 of 8 cases and chemotherapy-refractory lymphomas responded in 13 of 19 cases. The median duration of CR exceeded 16.5 months [68]. The activity of this construct was reconfirmed in a recent phase II trial: 21 patients with previously untreated follicular lymphoma were evaluable for clinical response to the ^{131}I-labelled B1 antibody. A therapeutic dose of 75 cGy to the whole body was delivered. 15 of the 21 patients achieved a CR (71%), including 62% with bulky disease [69]. 4 of 12 patients converted to consistent polymerase chain reaction (PCR) negativity for the t(18;14) translocation. This radionucleide also demonstrated significant activity in a myeloablative approach: in a phase I dose escalation study, the radioimmunoconjugate was applied in a myeloablative dose ranging from 234 to 777 mCi to 19 patients with refractory low/intermediate-grade B cell lymphoma followed by autologous bone marrow support. A CR was induced in 16 patients, a PR in 2 patients. 9 cases remained in CCR for 3–53 months [70]. In an update, a total of 21 patients with relapsed B cell lymphoma were treated with therapeutic doses of the ^{131}I-labelled antibody followed by autologous bone marrow or stem cell reinfusion: 18 patients showed an objective response with 16 CRs and 2 PRs. 15 patients remained disease free without further therapy after 3–23 months and 17 patients (81%) were progression free with an overall survival of 95% after a median follow-up of more than 1 year [71]. In contrast, the ^{131}I-labelled anti-CD21 antibody OKB7 had limited efficacy with 1 PR and 12 mixed responses in 18 patients with recurrent or refractory NHL in a phase I dose escalation study. In addition, a high frequency of HAMA (12 of 16 patients) was observed [72].

The use of chimeric or humanised antibodies labelled with radionucleides is a promising approach to combine antibody-mediated cytotoxicity with the radiotoxicity of the isotope and to reduce immunogenicity. Clinical experience with these constructs is still limited and further studies are required to evaluate the therapeutic potential of this approach [73]. Nevertheless, the clinical trials conducted so far have demonstrated that radioimmunotherapy is an innovative promising therapeutic modality, which can induce tumour responses in heavily pretreated or chemorefractory patients with B cell lymphoma. Ongoing trials are now analysing the clinical efficacy of high-dose radioimmunotherapy plus high-dose chemotherapy followed by autologous transplantation for final disease eradication [74].

Unconjugated MAbs

Early clinical trials using unlabelled native MAbs in B cell lymphoma therapy have shown only limited success (Table 3). In one early trial the anti-CD20 MAb 1F5, although rapidly clearing circulating lymphoma cells in the peripheral blood, induced only 1 PR lasting 6 weeks in 1 of 4 patients with refractory B cell NHL [75]. A different, very elegant approach used the idiotype of an individual B cell lymphoma as a unique tumour-specific marker for targeted antibody therapy. Although the mechanism of lymphoma suppression is not understood in detail, it is now clear that anti-idiotype antibodies can suppress lymphoma growth by a direct tumour-suppressive activity and the induction of ADCC [85, 86]. This promising therapeutic strategy requires that the specific anti-idiotypic MAb is generated for each individual B cell lymphoma. Technical difficulties in producing these antibodies are a major obstacle for a broad acceptance of this therapy outside specialised centres. In addition, the time needed for the development of the antibodies does not favour their use in rapidly growing high-grade lymphomas. However, clinical results with anti-idiotype antibodies are encouraging. 14 patients (13 patients with follicular lymphoma (FCL)) were treated with anti-idiotype antibodies and 8 cases responded with 1 CR lasting for 6 years and 7 PRs with a time to disease progression between 1 and 25+ months [76]. The emergence of idiotype-mutated or negative subclones was identified as one of the key tumour-escape mechanisms in this setting. The effect of a concomitant application of IFN-α or chlorambucil was tested for suppression of idiotype-negative clones in two clinical trials. 11 patients with FCL received anti-idiotype antibodies plus

Table 3. Unconjugated monoclonal antibodies

Author [Ref.]	Antibody	Phase	Disease	No. of patients	CR rate (%)	PR rate (%)
Press and colleagues [75]	Anti-CD20 (1F5)	I	Relapsed B cell NHL	4	0 (0)	1 (25)
Brown and colleagues [76]	Anti-id	I	B cell NHL	14	1 (7)	7 (50)
	Anti-id + IFN-α			11	2 (18)	7 (64)
Maloney and colleagues [77]	Anti-id + Cb	I	Relapsed low-grade B cell NHL	13	1 (8)	8 (62)
Maloney and colleagues [78]	Anti-CD20 (IDEC-C2B8)	I	Relapsed B cell NHL	15	0 (0)	2 (13)
Maloney and colleagues [79]	Anti-CD20 (IDEC-C2B8)	I	Relapsed B cell NHL	18	0 (0)	6 (33)
Maloney and colleagues [80]	Anti-CD20 (IDEC-C2B8)	II	Relapsed low-grade B cell NHL	37	3 (8)	14 (38)
Davis and colleagues [81]	Anti-CD20 (IDEC-C2B8)	II	Refractory/relapsed B cell NHL	166	10 (6)	70 (42)
Coiffier and colleagues [82]	Anti-CD20 (IDEC-C2B8)	II	Intermediate/high-grade B cell NHL (previously treated)	47	4 (9)	11 (23)
Link and colleagues [83]	Anti-CD20 (IDEC-C2B8) + CHOP	II	Intermediate/high-grade B cell NHL (previously untreated)	30	19 (63)	10 (33)
Davis and colleagues [84]	Anti-CD20 (IDEC-C2B8) IFN-α	II	Low-grade B cell NHL	26	2 (8)	13 (50)

id, idiotype; Cb, chlorambucil; NHL, non-Hodgkin's lymphoma; CR, complete remission; PR, partial remission; IFN-α, interferon-alpha; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone.

IFN-α. 2 CRs and 7 PRs were induced with a median response duration of 7 months (range 2–24 months). However, clinical response was not different to antibody therapy alone and the emergence of idiotype-negative lymphoma cells was not suppressed by IFN-α [76]. The concomitant application of chlorambucil also did not prevent the development of idiotype-mutated or negative subclones: 1 CR and 8 PRs were achieved in 13 pretreated patients with low-grade B cell NHL with a median time to progression of 7 months. No selective elimination of idiotype-negative lymphoma cells by chlorambucil was observed [77]. Recently, Caspar and colleagues demonstrated in an elegant *in vivo* mouse model, that it is possible to induce a polyclonal immune response by active immunisation with the tumour-specific idiotype. The immune response is also directed against the mutated idiotype and thus may prevent tumour escape [87]. This is supported by the observation of a recently published clinical trial: 41 patients after standard chemotherapy received serial vaccination with the individual idiotype protein. 20 patients generated a specific anti-idiotype immune response. The overall survival and the median duration of freedom from disease progression of all 20 patients with an anti-idiotype immune response were statistically significantly prolonged compared with the patient group without immune response [88]. These promising data have now to be validated by a prospective clinical investigation. The attractiveness of the concept of anti-idiotype MAb was enhanced by the identification of shared antigenic protein sequences in the variable regions of the immunoglobulins, so-called 'shared idiotypes'. In theory, this allows the generation of a set of MAb which recognise the idiotypes of B cell lymphomas in 30% of patients at diagnosis, and promises a more rapid treatment of this subset of patients by the use of the pre-existing panel of MAb [32, 89]. However, the concept of 'shared idiotypes' still faces logistical problems, which limit its broad clinical application to date.

The most encouraging development in antibody therapy of NHL in recent years has been based on the generation of chimeric MAbs (Table 3). The IDEC-2B8 antibody is a chimeric anti-CD20 antibody, which consists of the heavy and light chain variable regions from the murine IgG1 anti-CD20 MAb and of the human IgG1κ constant region. In preclinical studies this antibody demonstrated a 1000-fold enhanced capacity to activate human CDC and ADCC as compared with the unmodified murine MAb [90]. In addition, the antibody directly induced apoptosis in B cell NHL cell lines [91]. In the first phase I dose escalating study, 15 patients with relapsed low-grade B cell NHL were treated with a single infusion of the antibody. In all patients peripheral circulating B cells were depleted for 1–3 months. Although only one single dose was administered, a PR was documented in 2, minor responses in 4 of the 15 heavily pretreated patients. The antibody was well tolerated up to a dose of 500 mg/m^2 with grade II fever after infusion as the main side-effect. No human antichimeric antibodies (HACA) were detected [78]. The MAb demonstrated high clinical activity when applied at a dose of 375 mg/m^2 weekly for 4 weeks in 20 patients with recurrent low-grade or intermediate/high-grade lymphomas: 6 of 18 evaluable patients responded with a PR, 5 patients showed a minor response. The MAb was also well tolerated in this multiple dose trial [79]. In a recent study this treatment schedule was selected to analyse the activity of the MAb in 37 patients with recurrent low-grade or follicular B cell NHL in a phase II multicentre study. The antibody induced a CR in 3 and a PR in 14 patients with a median time to disease progression of 10.2 months [80]. The high antilymphoma activity of the MAb in heavily pretreated patients combined with a low toxicity profile and immunogenicity were confirmed in a recent integrated analysis of 166 patients with refractory or relapsed low-grade B cell NHL with an overall response rate of 48% (6% CRs, 42% PRs) [81]. The excellent clinical activity of this antibody is emphasised by the observation that patients with FCL, who were initially bcl-2 PCR positive, converted to PCR negativity in 68% of cases in the peripheral blood and in 50% in the bone marrow [92]. Molecular remission was also induced in 5 of 5 evaluable patients with previously untreated or first relapse of FCL by a combination of six cycles of standard CHOP plus six doses of 375 mg/m^2 IDEC-C2B8 intravenously [93]. However, individual patients with PCR-negative bone marrow had no clinical CR with persistent lymphadenopathy, which suggests that the antibody is less efficient in eradicating malignant cells in lymphoma bulks than in the bone marrow compartment.

Although the clinical efficacy seems to be most pronounced in patients with FCL, the MAb at a dose of 375 mg/m^2 weekly for 8 weeks or 1×375 mg/m^2 followed by 7×500 mg/m^2 weekly also proved to be active in pretreated intermediate- and high-grade B cell lymphoma: in 47 evaluable patients treatment with IDEC-C2B8 induced 9% CRs and 23% PRs with an overall response rate of 32%. 3 of 11 patients (1 CR, 2 PRs) with mantle cell lymphoma responded to therapy [82]. In a recent phase II pilot study the clinical activity of IDEC-C2B8 in combination with standard CHOP chemotherapy was tested in patients with previously untreated intermediate- or high-grade NHL: 30 patients were evaluable for response. The overall response rate was 96% with 63% CRs and 33% PRs. Combination therapy did not cause an enhanced frequency of serious adverse events as compared with conventional CHOP alone [83]. Another promising strategy combined the antilymphoma activity of the antibody and the biological response modifier IFN-α: in a recently published interim analysis, 31 patients with relapsed or refractory FCL were treated with 5 million units/day square metre 3×week IFN-α for 3 months and with IDEC-C2B8 375 mg/m^2 weekly at weeks 5–8. 26 patients were evaluable for response with an overall response rate of 58% (8% CRs, 50% PRs) [84]. With regard to the reported data, the IDEC-C2B8 antibody is one of the most promising developments in the field of antibody-based therapeutic strategies as it combines a high antilymphoma activity with a moderate toxicity. Although prospective randomised data are still needed, the antibody is a highly attractive candidate for a combination therapy with standard chemotherapy or as a maintenance therapy for final lymphoma eradication in chemosensitive patients after successful initial cytoreduction.

FUTURE PERSPECTIVES

Further progress has been made in the development of novel antibody production techniques or antibody-based strategies. Recently, the technology of antibody production by 'phage libraries' has been introduced, which permits genetic technological production of human antibodies against non-immunogenic antigens [94]. The innovative antibody-based 'antibody directed enzyme prodrug therapy' (ADEPT) promises further progress in the clinical management of

NHL. This concept uses an antibody directed against a tumour antigen as a vector to deliver an enzyme to the malignant cell. After clearance of this antibody from the periphery and non-malignant tissue a non-toxic prodrug is applied, which is converted to a cytotoxic agent by the enzyme at the tumour cell [95, 96]. In addition, substantial progress has been made in optimising pretargeting strategies in radioimmunological approaches. One of the major problems of radioimmunotherapy is the high radioactive background with a subsequent low tumour to background ratio when the tumour is pretargeted prior to the application of the therapeutic radiolabelled conjugate. Promising concepts to solve this problem are the two-step or three-step pretargeting strategies. In the two-step protocol, a biotinylated antitumour antibody is applied as the first conjugate and allowed to bind to the tumour antigen. In a second step, radiolabelled streptavidin is infused when the first conjugate is cleared from the plasma and normal tissue. Compared with the direct 'one-step' protocol a 2–3-fold increase in the tumour to blood ratio has been reported [97]. In the three-step protocol, a biotinylated MAb binds to the tumour antigen in the first step. In the second step, free tumour-unbound biotinylated MAb is rapidly cleared from the circulation by injected avidin as a 'chase' molecule. In the third step, a radiolabelled biotinylated conjugate binds to avidin, which is attached to the first biotinylated MAb at the tumour surface. In animal models, up to a 10-fold increase in the tumour:blood ratio has been achieved and this pretargeting strategy has already been successfully applied to cancer patients [98, 99]. These strategies will help to improve the specificity of pretargeting strategies and, thus, the clinical applicability of radioimmunotherapy.

CONCLUSION

Despite the considerable progress in understanding the pathobiology of B cell NHL, fewer advances have been made in improving the clinical outcome of patients with these diseases in the last few decades. Thus, there is an urgent need for the development of innovative therapeutic strategies, which can synergise with conventional chemotherapy based on a different mode of action. The concept of antibody therapy as an innovative immunobiological lymphoma-targeted strategy may play an important role for the clinical management of B cell NHL. Although MAb therapy still faces limitations, including non-specific toxicity, high immunogenicity, rapid clearance *in vivo* or technical difficulties in production, significant progress has been made to surmount these difficulties, for instance by the generation of highly effective chimeric MAbs, the production of 'third generation' immunotoxins by recombinant DNA technology or the use of anti-idiotype antibodies directed against 'shared idiotypes'. Based on these current achievements, antibody therapy for patients suffering from NHL promises to open new perspectives in the treatment of these diseases in the near future. In particular, the therapeutic concept of antibody therapy is highly attractive for combination with standard chemotherapy, based on the different underlying mechanisms of action of the two concepts, and as maintenance therapy for final lymphoma eradication in patients with residual disease after successful cytoreduction. Further prospective clinical trials will help to define the patient groups who will gain the maximal clinical benefit from antibody-based strategies.

1. Devesa SS, Blot WJ, Stone BJ, Miller BA, Tarone RE, Fraumeni JF Jr. Recent cancer trends in the United States. *J Natl Cancer Inst* 1995, **87**, 175–182.
2. DeVesa SS, Fears T. Non-Hodgkin's lymphoma time trends. United States and international data. *Cancer Res* 1992, **52**(Suppl. 19), 5432–5440.
3. Coleman MP, Esteve J, Damiecki P, Arslan A, Renard H. Non-Hodgkin's lymphoma (ICD9 200, 202) in trends in cancer incidence and mortality, 1993. *IARC Publication* no. 121, Lyon, 1993, 641–653.
4. Parker S. Cancer statistics. *CA Cancer Journal for Clinicians* 1996, **65**, 5–27.
5. Harris NL, Jaffe ES, Stein H, *et al*. A revised European–American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994, **84**, 1361–1392.
6. Hiddemann W, Longo DL, Coiffier B, *et al*. Lymphoma classification—the gap between biology and clinical management is closing. *Blood* 1996, **88**, 4085–4089.
7. Longo DL, Glatstein E, Duffey PI, *et al*. Treatment of localized aggressive lymphomas with combination chemotherapy followed by involved-field radiation therapy. *J Clin Oncol* 1989, **7**, 1295–1302.
8. Kaiser U, Trümper L, Pfreundschuh M, Havemann K. Behandlung aggressiver Non-Hodgkin-Lymphome. *Internist* 1997, **38**, 135–142.
9. Armitage JO. Treatment of non-Hodgkin's lymphoma. *N Engl J Med* 1992, **328**, 1023–1030.
10. Fisher RI, Gaynor ER, Dahlberg S, *et al*. Comparison of CHOP vs m-BACOD vs ProMACE-CytaBOM vs MACOP-B in patients with intermediate- or high-grade non Hodgkin's lymphoma. *N Engl J Med* 1993, **328**, 1002–1006.
11. Epelbaum R, Kuten A, Coachman NM, *et al*. Stage I–II low-grade non-Hodgkin's lymphoma: prognostic factors and treatment results. *Strahlenth Onkol* 1992, **168**, 66–72.
12. Stuschke M, Hoederath A, Sack H, *et al*. Extended field and total central lymphatic radiotherapy in the treatment of early stage lymph node centroblastic-centrocytic lymphomas: results of a prospective multicenter study. Study Group NHL-fruhe Stadien. *Cancer* 1997, **80**, 2273–2284.
13. Longo D. What's the deal with follicular lymphoma? *J Clin Oncol* 1993, **11**, 202–208.
14. Hiddemann W. Non-Hodgkin's lymphomas—current status of therapy and future perspectives. *Eur J Cancer* 1995, **31**, 2141–2145.
15. Young RC, Longo DL, Glatstein E, Ihde DC, Jaffe ES, DeVita VT Jr. The treatment of indolent lymphomas: watchful waiting v. aggressive combined modality treatment. *Semin Hematol* 1988, **25**(Suppl. 2), 11–16.
16. Haioun C, Lepage E, Gisselbrecht C, *et al*. Comparison of autologous bone marrow transplantation with sequential chemotherapy for intermediate-grade and high-grade non-Hodgkin's lymphoma in first complete remission. A study of 464 patients. *J Clin Oncol* 1994, **12**, 2543–2551.
17. Gisselbrecht C, Lepage E, Morel P, *et al*. Short and intensified treatment with autologous stem cell transplantation versus ACVB regimen in poor prognosis aggressive lymphoma. Prognostic factors of induction failure. *Ann Oncol* 1996, **7**(Suppl. 3), 18.
18. Bierman PJ, Vose JM, Anderson JR, Bishop MR, Kessinger A, Armitage JO. High-dose therapy with autologous hematopoietic rescue for follicular low-grade non-Hodgkin's lymphoma. *J Clin Oncol* 1997, **15**, 445–450.
19. Solal-Celigny P, Lepage E, Brousse N, *et al*. Recombinant interferon alfa-2b combined with a regimen containing doxorubicin in patients with advanced follicular lymphoma. *N Engl J Med* 1993, **329**, 1608–1614.
20. Decaudin D, Lepage E, Solal-Celigny P, *et al*. Low-grade follicular lymphoma: multivariate analysis of prognostic factors in 520 patients. *Blood* 1997, **90**(Suppl. 1), 392a.
21. Unterhalt M, Herrmann R, Tiemann M, *et al*. Prednimustine, mitoxantrone (PmM) versus cyclophosphamide, vincristine, prednisone (COP) for the treatment of advanced low grade non Hodgkin lymphoma. *Leukemia* 1996, **10**, 836–843.
22. Unterhalt M, Herrmann R, Koch P, *et al*. Prognostic determinants for long term outcome of low grade follicular lymphomas after cytoreductive, chemotherapy and interferon alpha

maintenance. Results of the German Low Grade Lymphoma Study Group (GLSG). *Blood* 1997, **90**(Suppl. 1), 1743a.
23. Hiddemann W, Unterhalt M, Herrmann R, *et al.* Mantle cell lymphomas have more widespread disease and a slower response to chemotherapy as compared to follicle center lymphomas. Result of a prospective comparative analysis of the German Low Grade Lymphoma Study Group. *J Clin Oncol* 1998, **16**, 1922–1930.
24. Anderson KC, Bates MP, Slaughenhoupt BL, Pinkus GS, Schlossmann SF, Nadler LM. Expression of human B-cell associated antigens on leukemias and lymphomas: a model of human B-cell differentiation. *Blood* 1984, **63**, 1424–1433.
25. Roitt I, Brostoff J, Make D. *Immunology*, 3rd edn. London, Gower Medical Publishing, 1991.
26. Taylor C, Linden H, Rubin M, *et al.* Phase I trial of mouse IgA anti-transferrin receptor monoclonal antibody 42/6 in patients with cancer. *Proc Am Assoc Cancer Res* 1994, **52**, 377–384.
27. Vuist WMJ, Levy R, Maloney DG. Lymphoma regression induced by monoclonal anti-idiotypic antibodies correlates with their ability to induce Ig signal transduction and is not prevented by tumor expression of high levels of bcl-2 protein. *Blood* 1994, **83**, 899–906.
28. Maloney DG, Smith B, Appelbaum FR. The anti-tumor effect of monoclonal anti-CD20 antibody therapy includes direct anti-proliferative activity and induction of apoptosis in CD20 positive non-Hodgkin's lymphoma cell lines. *Blood* 1996, **83**, 637a.
29. Jurcic JG, Caron PC, Scheinberg DA. Monoclonal antibody therapy of leukemia and lymphoma. *Adv Pharm* 1995, **33**, 287–314.
30. LoBuglio AF, Mansoor NS. Advances in monoclonal antibody therapy of cancer. *Am J Med Sci* 1992, **304**, 214–224.
31. Grossbard ML, Nadler LM. Monoclonal antibody therapy for indolent lymphomas. *Sem Oncol* 1993, **5**(Suppl. 5), 118–135.
32. Vitetta ES, Fulton RJ, May RD, Till M, Uhr JWI. Redesigning nature's poisons to create antitumor reagents. *Science* 1987, **238**, 1098–1104.
33. Order SE, Sleeper AM, Stillwagon GB, Klein JL, Leichner PK. Radiolabeled antibodies: results and potential in cancer therapy. *Cancer Res* 1990, **50**, 1011s–1013s.
34. Morrison SL. Transfectomas provide novel chimeric antibodies. *Science* 1986, **229**, 379–393.
35. Jones PT, Dear PH, Foots J, Neuberber MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* 1986, **321**, 522–525.
36. LoBuglio AF, Wheeler RH, Trang J, *et al.* Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. *Proc Natl Acad Sci* 1989, **86**, 4220–4224.
37. Epenetos AA, Snook D, Durbin H, Johnson PM, Taylor-Papadimitriou J. Limitations of radiolabeled monoclonal antibodies for localization of human neoplasms. *Cancer Res* 1986, **46**, 3183–3191.
38. Strand SE, Zanzonico P, Johnson TK. Pharmacokinetic modelling. *Med Phys* 1993, **20**, 515–527.
39. Juweid M, Neumann R, Paik C, *et al.* Micropharmacology of monoclonal antibodies in solid tumors: direct experimental evidence for a binding site barrier. *Cancer Res* 1992, **52**, 5144– 5153.
40. Skerra A. Bacterial expression of immunoglobulin fragments. *Curr Opin Immunol* 1993, **5**, 256–262.
41. Weiner GJ, Hillstrom JR. Bispecific anti-idiotype/anti-CD3 antibody therapy of murine B cell lymphoma. *J Immunol* 1991, **147**, 4035–4044.
42. Demanet C, Brissinck J, Leo O, Moser M, Thielemans K. Role of T-cell subsets in the bispecific antibody (anti-idiotype×anti-CD3) treatment of the BCL-1 lymphoma. *Cancer Res* 1994, **54**, 2973–2978.
43. Haagen I-A, Geerars AJ, de Lau WB, *et al.* Killing of autologous B-lineage malignancy using CD3×CD19 bispecific monoclonal antibody in end stage leukemia and lymphoma. *Blood* 1994, **84**, 556–563.
44. Deo YM, Graziano RF, Repp R, Van de Winkel JGJ. Clinical significance of the IgG receptors and FcγR-directed immunotherapies. *Immunol Today* 1997, **18**, 127–135.
45. Valerius T, Stockmeyer B, van Spriel AB, *et al.* FcαRI (CD89) as a novel trigger molecule for bispecific antibodies. *Blood* 1998, **90**, 4485–4492.
46. Wooldridge JE, Ballas Z, Krieg AM, Weiner GJ. Immunostimulatory oligodeoxynucleotides containing CpG motifs enhance the efficacy of monoclonal antibody therapy of lymphoma. *Blood* 1997, **89**, 2994–2998.
47. Eiklid K, Olsnes S, Pihl A. Entry of lethal doses of abrin, ricin and modeccin in the cytosol of HeLa cells. *Exp Cell Res* 1980, **126**, 321–326.
48. Roy DC, Griffin JD, Belvin M, Blattler WA, Lambert JM, Ritz J. Anti-My 9-blocked ricin: an immunotoxin for selective targeting of acute myeloid leukemia cells. *Blood* 1991, **77**, 2404–2412.
49. Brinkmann U, Pastan I. Recombinant immunotoxins: from basic research to cancer therapy. *Methods* 1995, **8**, 143–156.
50. Corcoran MC, Eary J, Bernstein I, Press OW. Radioimmunotherapy strategies for non-Hodgkin's lymphomas. *Ann Oncol* 1997, **8**(Suppl. 1), S133–S138.
51. Anderson-Berg WT, Squire RA, Strand M. Specific radioimmunotherapy using 90-Y labeled antibody in erythroleukemic mice. *Cancer Res* 1987, **47**, 1905–1912.
52. Order SE, Klein JL, Leichner PK, Frincke J, Lollo C, Carlo DJ. ^{90}Yttrium antiferritin—a new therapeutic radiolabeled antibody. *Int J Radiat Oncol Biol Phys* 1986, **12**, 277–281.
53. Buchsbaum DJ, Lawrence TS, Roberson PL, Heidorn DB, Ten Haken RK, Steplewski Z. Comparison of ^{131}I- and ^{90}Y-labeled monoclonal antibody 17-1A for treatment of human colon cancer xenografts. *Int J Radiat Oncol Biol Phys* 1993, **25**, 629–638.
54. Kotz D. Alpha particle therapy poised to become new line of cancer treatment. *J Nucl Med* 1998, **39**, 17N–19N.
55. Vitetta ES, Stone M, Amlot P, *et al.* Phase I immunotoxin trial in patients with B-cell lymphoma. *Cancer Res* 1991, **51**, 4052–4058.
56. Grossbard ML, Lambert JM, Goldmacher VS, *et al.* Anti-B4-blocked ricin: a phase I trial of 7-day continuous infusion in patients with B-cell neoplasms. *J Clin Oncol* 1993, **11**, 726–737.
57. Stone MJ, Sausville EA, Fay JW, *et al.* A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. *Blood* 1996, **88**, 1188–1197.
58. LeMaistre CF, Meneghetti C, Rosenblum M, *et al.* Phase I trial of an interleukin-2 (IL-2) fusion toxin (DAB_{486}IL-2) in hematological malignancies expressing the IL-2 receptor. *Blood* 1992, **79**, 2547–2554.
59. Nichols J, Foss F, Kuzel TM, *et al.* Interleukin-2 fusion protein: an investigational therapy for interleukin-2 receptor expressing malignancies. *Eur J Cancer* 1997, **33**(Suppl. 1), S34–S36.
60. Schwartz G, Tepler I, Charette J, *et al.* Complete response of a Hodgkin's lymphoma in a phase I trial of DAB_{486}IL-2, an IL-2-diphtheria fusion toxin. *Blood* 1991, **78**(Suppl.), 175a.
61. Kuzel T, Olsen E, Martin A, *et al.* Pivotal phase III trial of two dose levels of DAB_{486}IL-2 (OntakTM) for the treatment of mycoses fungoides (MF). *Blood* 1997, **90**(Suppl. 1), 586a.
62. Frankel AE, FitzGerald D, Siegall C, Press OW. Advances in immunotoxin biology and therapy: a summary of the fourth international symposium on immunotoxins. *Cancer Res* 1996, **56**, 926–932.
63. Grossbard ML, Gribben JG, Freedman AS, *et al.* Adjuvant immunotoxin therapy with anti B4-blocked ricin after autologous bone marrow transplantation for patients with B-cell non-Hodgkin's lymphoma. *Blood* 1993, **81**, 2263–2271.
64. Grossbard ML, Niedzwiecki D, Nadler LM, *et al.* Anti-B4-blocked ricin (Anti-B4-bR) adjuvant therapy post-autologous bone marrow transplant (ABMT)(CALGB 9254): a phase III intergroup study. *Proc Am Soc Clin Oncol* 1998, **17**, 3a.
65. Press OW, Eary JF, Badger CC, *et al.* Treatment of refractory non-Hodgkin's lymphoma with radiolabeled MB-1 (anti-CD37) antibody. *J Clin Oncol* 1989, **7**, 1027–1038.
66. Kaminski MS, Fig LM, Zasadny KR, *et al.* Imaging, dosimetry, and radioimmunotherapy with iodine-131-labeled anti-CD37 antibody in B-cell lymphoma. *J Clin Oncol* 1992, **10**, 1696–1711.
67. Kaminski MS, Zasadny KR, Francis IR, *et al.* Radioimmunotherapy of B-cell lymphoma with [^{131}I] anti-B1 (anti-CD20) antibody. *N Engl J Med* 1993, **329**, 459–465.
68. Kaminski MS, Zasadny KR, Francis IR, *et al.* Iodine-131-anti B1 radioimmunotherapy for B-cell lymphoma. *J Clin Oncol* 1996, **14**, 1974–1981.
69. Kaminski MS, Gribbin T, Estes J, *et al.* I-131 anti-B1 antibody for previously untreated follicular lymphoma (FL): clinical and molecular remissions. *Proc Am Soc Clin Oncol* 1998, **17**, 2a.
70. Press OW, Eary JF, Appelbaum FR, *et al.* Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N Engl J Med* 1993, **329**, 1219–1924.

71. Press OW, Eary JF, Appelbaum FR, *et al.* Phase II trial of ^{131}I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet* 1995, **346**(8971), 336–340.
72. Czuczman MS, Straus DJ, Divgi CR, *et al.* Phase I dose-escalation trial of iodine 131-labeled monoclonal antibody OKB7 in patients with non-Hodgkin's lymphoma. *J Clin Oncol* 1993, **11**, 2021–2029.
73. Juweid M, Sharkey RM, Markowitz A, *et al.* Treatment of non-Hodgkin's lymphoma with radiolabeled murine, chimeric, or humanized LL2, an anti-CD22 monoclonal antibody. *Cancer Res* 1995, **55**(23 Suppl.), 5899s–5907s.
74. Corcoran MC, Eary J, Bernstein I, Press OW. Radioimmunotherapy strategies for non-Hodgkin's lymphoma. *Ann Oncol* 1997, **8**(Suppl. 1), 133–138.
75. Press OW, Appelbaum F, Ledbetter JA, *et al.* Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. *Blood* 1987, **69**, 584–591.
76. Brown SL, Miller RA, Horning SJ, *et al.* Treatment of B-cell lymphomas with anti-idiotype antibodies alone and in combination with alpha interferon. *Blood* 1989, **73**, 651–661.
77. Maloney DG, Brown S, Czerwinski DK, *et al.* Monoclonal anti-idiotype antibody therapy of B-cell lymphoma: the addition of a short course of chemotherapy does not interfere with the anti-tumor effect nor prevent the emergence of idiotype-negative variant cells. *Blood* 1992, **80**, 1502–1510.
78. Maloney DG, Liles TM, Czerwinski DK, *et al.* Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. *Blood* 1994, **84**, 2457–2466.
79. Maloney DG, Grillo-Lopez AJ, Bodkin D, *et al.* IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. *J Clin Oncol* 1997, **15**, 3266–3274.
80. Maloney DG, Grillo-Lopez AJ, White CA, *et al.* IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 1997, **90**, 2188–2195.
81. Davis T, Levy R, White CA, *et al.* Retreatments with RITUXAN™ (Rituximab, IDEC-C2B8) have significant efficacy, do not cause hama and are a viable minimally toxic alternative in relapsed or refractory non-Hodgkin's lymphoma (NHL). *Blood* 1997, **90**(Suppl. 1), 509a.
82. Coiffier B, Ketterer N, Haioun C, *et al.* A multicenter, randomized phase II study of rituximab (chimeric anti-CD20 mAb) at two dosages in patients with relapsed or refractory intermediate or high-grade NHL (IHG-NHL) or in elderly patients in first-line therapy. *Blood* 1997, **90**(Suppl. 1), 510a.
83. Link BK, Grossbard ML, Fisher RI, *et al.* Phase II pilot study of the safety and efficacy of rituximab in combination with CHOP chemotherapy in patients with previously untreated intermediate- or high-grade NHL. *Proc Am Soc Clin Oncol* 1998, **17**, 3a.
84. Davis T, Maloney D, White CA, *et al.* Combination immunotherapy of low grade or follicular (LG/F) non-Hodgkin's lymphoma (NHL) with Rituximab and alpha interferon: interim analysis. *Proc Am Soc Clin Oncol* 1998, **17**, 11a.
85. Kaminski MS, Kitamura K, Maloney DG, Campbell MJ, Levy R. Importance of antibody isotype in monoclonal anti-idiotype therapy of a murine B cell lymphoma. A study of hybridoma class switch variants. *J Immunol* 1986, **136**, 1123–1130.
86. van Endert PM, Heilig B, Hammerling G, Moldenhauer G. Monoclonal antibodies to idiotype inhibit *in vitro* growth of human B-cell lymphomas. *Blood* 1992, **79**, 129–137.
87. Caspar CB, Levy S, Levy R. Idiotype vaccines for non-Hodgkin's lymphoma induce polyclonal immune response that cover mutated tumor idiotypes: comparison of different vaccine formulations. *Blood* 1997, **90**, 3699–3706.
88. Hsu FJ, Caspar CB, Czerwinsky D, *et al.* Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma—long-term results of a clinical trial. *Blood* 1997, **89**, 3129–3135.
89. Swisher EM, Shawler DL, Collins HA, *et al.* Expression of shared idiotypes in chronic lymphocytic leukemia and small lymphocytic lymphoma. *Blood* 1991, **77**, 1977–1982.
90. Reff ME, Carner K, Chambers KS, *et al.* Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994, **83**, 435–445.
91. Maloney DG, Smith B, Appelbaum FR. The anti-tumor effect of monoclonal anti-CD20 antibody therapy includes direct anti-proliferative activity and induction of apoptosis in CD20 positive non-Hodgkin's lymphoma cell lines. *Blood* 1996, **88**(Suppl.), 637a.
92. Cabanillas F, Grillo-Lopez AJ, McLaughlin P, *et al.* Anti-CD20 antibody (MAB), IDEC-C2B8: clearance of bcl-2 t(14;18) positive cells from peripheral blood (PB) and bone marrow (BM) in patients (pts) with relapsed low-grade or follicular lymphoma (LG/F NHL). *Blood* 1996, **88**(Suppl. 1), 91a.
93. Rogers J, Jackson J, Rosenberg J, *et al.* Clearance of bcl-2 (t14; 18) from peripheral blood (PB) and bone marrow (BM) in patients (pts) with relapsed low-grade or follicular (IWF: A-D) lymphoma (NHL) following single-agent therapy with the chimeric anti-CD20 antibody (MAB) IDEC-C2B8. *Ann Oncol* 1996, **7**, 108a.
94. Marks C, Marks JD. Phage libraries—a new route to clinically useful antibodies. *N Engl J Med* 1996, **335**, 730–733.
95. Senter PD, Saulnier MG, Schreiber GJ, *et al.* Anti-tumor effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate. *Proc Natl Acad Sci* 1988, **85**, 4842–4846.
96. Bakina E, Wu Z, Rosenblum M, Farquhar D. Intensely cytotoxic anthracycline prodrugs: glucuronides. *J Med Chem* 1997, **40**, 4013–4018.
97. Saga T, Weinstein JN, Jeong JM, *et al.* Two-step targeting of experimental lung metastases with biotinylated antibody and streptavidin-biotin binding. *Cancer Res* 1994, **54**, 2160–2165.
98. Kobayashi H, Sakahara H, Hosono M, *et al.* Improved clearance of radiolabeled biotinylated monoclonal antibody following the infusion of avidin as a 'chase' without decreased accumulation in the target tumor. *J Nucl Med* 1994, **35**, 1677–1684.
99. Paganelli G, Magnani P, Zito F, *et al.* Three-step monoclonal antibody tumor targeting in carcinoembryonic antigen-positive patients. *Cancer Res* 1991, **51**, 5960–5966.