REVIEW ARTICLE

Drugs 2007; 67 (3): 333-350
0012-6667/07/0003-0333/$49.95/0

Monoclonal Antibodies in the Treatment of Non-Hodgkin's Lymphoma

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Abstract

Antibody-based therapeutic approaches have had a significant impact in the treatment of non-Hodgkin's lymphoma (NHL). Rituximab's development as an anti-CD20 antibody heralded a new era in treatment approaches for NHL. While rituximab was first shown to be effective in the treatment of relapsed follicular lymphoma, it is now standard monotherapy for front-line treatment of follicular lymphoma, and is also used in conjunction with chemotherapy for other indolent, intermediate and aggressive B-cell lymphomas. The development of rituximab has led to intense interest in this type of therapeutic approach and to development and approval of the radioimmunoconjugates of rituximab, ^{90}Y-ibritumomab tiux-

etan and ^{131}I-tositumomab, which have added to the repertoire of treatments for relapsed follicular lymphoma and increased interest in developing other conjugated antibodies. Since rituximab is a chimeric antibody, there is a need to develop fully humanised antibodies, such as IMMU-106 (hA20), in order to minimise infusion reactions and eliminate the development of human antibodies against the drug.

Further clinical evaluation of antibodies has been based largely on our knowledge of antigen expression on the surface of lymphoma cells and has led to the development of antibodies against CD22 (unconjugated epratuzumab and calicheamicin conjugated CMC-544 [inotuzumab ozogamicin]), CD80 (galiximab), CD52 (alemtuzumab), CD2 (MEDI-507 [siplizumab]), CD30 (SGN-30 and MDX-060 [iratumumab]), and CD40 (SGN-40). Furthermore, the VEGF (vascular endothelial growth factor) inhibitor bevacizumab, which was first approved for the treatment of colon cancer is currently under investigation in NHL, and agonists rather than antibodies to TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) [rApo2L/TRAIL, HGS-ETR1{mapatumumab}, HGS-ETR2] are currently being investigated as treatments for both advanced solid tumours and NHL. Knowledge of the ability of cancer cells to become resistant to a targeted therapy by activating an alternative pathway to evade apoptosis has driven studies that combine antibodies such as epratuzumab plus rituximab (ER) or ER plus chemotherapy with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) [ER-CHOP], inotuzumab ozogamicin plus rituximab, alemtuzumab plus CHOP (CHOP-C), bevacizumab plus rituximab, and now the combination of rApo2L/TRAIL plus rituximab.

As a result of the expansion of research in this area, several treatment-specific adverse effects have been noted, including infusion-related reactions for rituximab, myelosuppression secondary to ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab, and immunosuppression leading to infectious complications for alemtuzumab. Also, soluble forms of the antigens (sCD30) are now being investigated as potential mechanisms of resistance to antibody treatments by binding the antibody before the drug can bind to the lymphoma cell. In addition, it has also become apparent that these antibodies often have a dose-dependent half-life (rituximab) or long half-lives of up to 2–3 weeks (epratuzumab and galiximab) with a consequent delay to a response, thus influencing how long we should wait for a response before declaring an antibody to be ineffective.

Antibody-based therapeutic approaches have already had a profound impact on the treatment of NHL, and it is almost certain that, as their clinical development progresses, we will continue to refine the optimum methods of incorporating these drugs in NHL treatment in order to offer our patients the best clinical benefits.

Until the end of the 20th century the principal modality of treatment for non-Hodgkin's lymphoma (NHL) was chemotherapy. Major advances in lymphoma biology and immunology, including tumour genetic fingerprinting, have introduced an exciting new era in NHL therapy. Monoclonal antibodies were first described in 1975.[1] They are generally produced by a single clone of B cells and are thus homogenous. Monoclonal murine antibodies were first developed by fusing B cells from mice immunised with human lymphoma cells. However, a concerning adverse effect was the potential for

humans to develop antimouse antibodies, which could cause allergic reactions as well as decrease the efficacy of the treatment.[2] Further technological developments in the field of recombinant DNA led to advances in the generation of chimeric antibodies (which are 65–90% human), partially humanised antibodies (which are 95% human) and, most recently, fully humanised antibodies.[3,4]

Antibody-based therapies are appealing and advantageous, since they target tumour cells while potentially sparing normal cells. This review focuses on monoclonal antibody therapy in NHL. Discussion includes both commercially available and US FDA-approved antibodies, as well as those currently being evaluated in clinical trials.

1. Targeting CD20

As a B-cell marker, CD20 is expressed throughout development and is present from the early pro-B cells through to the mature memory B cells. The function of CD20 is not yet completely understood, although it is known to be involved in the activation of B cells, the regulation of B-cell growth and control of calcium transportation. CD20 is a transmembrane protein, and studies have shown that both mouse and human CD20 complementary DNA (cDNA) encodes a membrane-embedded protein that has hydrophobic regions with the ability to pass through the cell membrane up to four times. In addition, the cytoplasmic domain is rich in serine and threonine, and has multiple sites for phosphorylation. Human CD20 has three isoforms because of differential phosphorylation of the serine and threonine residues.[5] Notably, the features of CD20 that drove the clinical development of targeted therapies are its high expression on B-cell lymphomas, as well as the fact that it does not rapidly modulate or become secreted or shed.[6]

1.1 Unconjugated Anti-CD20 Antibodies

1.1.1 Rituximab

Rituximab was approved in 1997 and was the first monoclonal antibody to be approved by the FDA. Its use has had a powerful impact on the treatment of NHL and also served as the driving force in our current focus on immunotherapy research in NHL and other malignancies. Its action appears to be mediated via three potential humoral and cell-mediated effector mechanisms, including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cellular cytotoxicity. Recent studies have shown that CDC appears to be the predominant mechanism by which rituximab exerts its therapeutic effect.[7,8] The most common adverse reactions of rituximab are considered to be infusion related and include chills, fever, hypotension, dyspnoea, hypoxia and arrhythmias. These symptoms occur twice as often during the first infusion compared with subsequent infusions (8% vs 4%). These infusion-related symptoms also occur more often in older patients and in those with higher levels of inflammatory cytokines. For this reason, rituximab infusion is begun at 50 mg/hour for the first dose and titrated upwards. In addition, premedication with paracetamol (acetaminophen) and diphenhydramine is common together with precautions against anaphylaxis.[9]

In a pivotal multicentre trial involving patients with relapsed follicular lymphoma, rituximab produced an overall response rate (ORR) of 50%, a complete remission (CR) rate of 6% and a median time to progression (TTP) of 13.1 months.[10] Retreatment of relapsed follicular lymphoma with rituximab was also shown to be effective, with an ORR of 40% and a median duration of response of 16.3 months.[11] Furthermore, several maintenance therapy regimens with rituximab in follicular lymphoma have been shown to improve treatment outcomes.[12-18] Rituximab has also been evaluated in combination with cytotoxic chemotherapeutic regimens, including fludarabine- and cyclophosphamide-based regimens, for the treatment of follicular lymphoma.

In a phase III trial for the front-line treatment of follicular lymphoma, rituximab plus cyclophosphamide, vincristine and prednisone (R-CVP) was demonstrated to have a response rate (RR) of 81% compared with 57% for patients treated with CVP

alone, with a median time to treatment failure of 27 months versus 7 months. The addition of rituximab to other chemotherapy regimens such as cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) and fludarabine-based regimens has also improved treatment outcomes.[19,20]

In elderly patients with previously untreated advanced diffuse large B-cell lymphoma, rituximab plus CHOP (R-CHOP) was superior to CHOP in achieving a CR (76% vs 63%) and in improving 5-year overall survival (OS) [58% vs 45%].[21] Similar outcomes were noted in the SWOG (Southwest Oncology Group)-0014 trial, which evaluated 3 cycles of R-CHOP followed by involved-field radiation treatment in diffuse large B-cell lymphoma patients with limited-stage disease, which was defined as stage I with one adverse risk factor of age >60 years, elevated serum lactate dehydrogenase (LDH), a performance status of 2 or higher, or stage II non-bulky disease. The 2-year progression-free survival (PFS) and OS were, respectively, 94% and 95%.[22] The R-CHOP data[21] compared with the SWOG 0014 data showed a 9% improvement in PFS and 2% improvement in OS at 2 years.[22]

CHOP-14 (CHOP cycles given every 14 days) was also compared with R-CHOP-14 in elderly patients with intermediate-to-aggressive B-cell NHL. In the RICOVER-60 (Rituximab with CHOP over 60) trial, elderly patients with stages I–IV diffuse large B-cell lymphoma received 6–8 cycles of CHOP-14 together with granulocyte-colony stimulating factor (G-CSF) support and with or without 8 treatments of rituximab. Radiotherapy was included for patients who had initial bulky disease and/or extranodal involvement. The primary endpoint of the trial was freedom from treatment failure (FFTF). Positive criteria for stopping the trial early on the basis of a favourable interim analysis were met. After a median observation of 26 months, a better FFTF rate for 8 cycles of CHOP-14 compared with 6 cycles of CHOP-14 was noted, but this was neutralised by the addition of rituximab with a 70% FFTF for both 6 and 8 cycles of R-CHOP-14. However, the advantage for OS at 26 months was not significant.[23] Whether this strategy is also beneficial to younger patients is currently unknown. Furthermore, it has not been determined whether R-CHOP-14 is superior to R-CHOP-21 (CHOP cycles given every 21 days), but this question is currently being addressed in a randomised trial conducted by GELA (Group d'Etude des Lymphome d'Adulte).

Rituximab combined with chemotherapy has also improved outcomes in aggressive mantle-cell lymphoma. Of particular benefit is the regimen of rituximab plus hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone) alternating with rituximab plus methotrexate and cytarabine. This regimen has dramatically improved outcomes compared with R-CHOP, with a CR rate of 87%, a 3-year PFS rate of 64% and an OS rate of 82% at 40 months. This intensive regimen is also an accepted approach for the treatment of the very aggressive Burkitt's and Burkitt's-like lymphomas.[24] Rituximab is also used in combination with salvage chemotherapy regimens such as R-ESHAP (rituximab plus etoposide, cisplatin, cytarabine and methylprednisolone) and R-ICE (rituximab plus ifosfamide, carboplatin and etoposide) for the treatment of relapsed intermediate-to-aggressive lymphomas. However, the benefit of adding rituximab to salvage regimens in patients whose disease relapses after receiving front-line rituximab-containing regimens remains unclear, although in theory it appears that there is a potential benefit.

1.1.2 Humanised Anti-CD20 Monoclonal Antibody: IMMU-106 (hA20)

Although rituximab has had a profound impact on the treatment of B-cell NHL, it is thought that the murine component of this chimeric antibody contributes to prolonged infusion times and infusion reactions. This spurred development of a humanised monoclonal anti-CD20 antibody. IMMU-106 or hA20 has a >90% humanised framework. A phase I/II dose-escalation study was performed in 20 patients with relapsed indolent or intermediate-to-aggressive B-cell NHL (16 grade 1–2 follicular lymphomas, one mantle-cell lymphoma, one small lymphocytic lymphoma, two marginal-zone lymphomas). Dosages included 120, 200, 375 or 750

mg/m^2 weekly for 4 weeks. The median number of prior treatments was 2.5 and 18 of the 20 patients had previously received a rituximab-based regimen. Infusion reactions were seen in 25% of patients but were mild to moderate, and infusion times were significantly shorter than with rituximab, with the average first infusion of the 375 mg/m^2 dose lasting only slightly more than 3 hours. Furthermore, tests for human anti-human antibodies (HAHA) have been negative to date. The ORR was 53% with a CR of 40%; CRs were seen at all dose levels, including three of seven patients at the 120 mg/m^2 dose level.[25] Thus, preliminary data are encouraging and provide evidence of the tolerability and efficacy of IMMU-106 and the shorter infusion times possible compared with rituximab. This trial is currently enrolling patients to expand the number at each dose level. If further data also show a positive response, it is likely that this will lead to investigations in frontline settings both alone and in combination with chemotherapy.

1.2 Radioimmunotherapy Using Anti-CD20 Antibodies

On the basis of the successful clinical use of rituximab, anti-CD20 antibodies were combined with the radioisotopes of yttrium (^{90}Y) and iodine (^{131}I), ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab, respectively, with the goal of potentially improving their therapeutic efficacy.[4] When infused into a patient, these radioimmunotherapies circulate until they bind to CD20+ cells, allowing the radiation via the radioactive isotopes to be targeted to the CD20+ cells. In addition, the radioisotope's path length causes a crossfire effect, which theoretically enables the emitted radiation to penetrate into bulky or poorly vascularised tumours. The approval of ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab have had a significant positive effect on the long-term management of follicular lymphoma in that they have expanded the repertoire of potentially successful therapies for relapsed follicular lymphoma and offer excellent response durations with a rather simple but elegant treatment strategy.[4]

1.2.1 ^{90}Y-Ibritumomab Tiuxetan

Yttrium-90 is a pure β-emitter. Multicentre trials have supported the efficacy of this therapy for relapsed low-grade or intermediate B-cell NHL, with an RR of 67%, a CR of 26%, and a median TTP of >12.9 months.[26] Notably, even for patients with rituximab-refractory follicular lymphoma, it has been shown that ^{90}Y-ibritumomab tiuxetan still induces an RR of 74% and a CR of 15%, with a TTP of 6.8 months.[27] Furthermore, a phase III trial supported its use in relapsed or refractory low-grade or transformed B-cell lymphomas with bone marrow involvement of <25% and a platelet count of >100 000/mm^3; data from the trial showed an 80% versus 56% RR and a 30% versus 16% CR, respectively, for ^{90}Y-ibritumomab tiuxetan versus rituximab. One interesting result of this trial was that the overall TTP was not significantly increased for all patients in the ^{90}Y-ibritumomab tiuxetan arm, although the TTP was longer in the follicular subgroups and those who achieved CR. Several explanations for this lack of overall benefit in TTP were described and included the fact that the trial was not powered to detect these differences, that the subjects treated with ^{90}Y-ibritumomab tiuxetan had a greater absolute shrinkage in lesions and thus could have disease progression of 50% with minor increases in tumour size, and that several patients treated in the rituximab arm were begun on other regimens but were not counted as having disease progression.[28]

1.2.2 ^{131}I-Tositumomab

Compared with ^{90}Y-ibritumomab tiuxetan, the radioisotope iodine-131 is a β- and γ-emitter, thus allowing imaging and therapy using the same antibody conjugate. Treatment with ^{131}I-tositumomab has shown a very good therapeutic benefit in patients with refractory low-grade or transformed B-cell lymphoma, with a 65% RR, 20% CR and a median duration of response of 6.5 months.[29]

As a consequence of the rituximab in the ^{90}Y-ibritumomab tiuxetan and the tositumomab in the ^{131}I-tositumomab treatment regimens, there have been notable descriptions of infusion-related symptoms similar to the previously described adverse effects for rituximab therapy (see section 1.1.1). In

addition, ^{90}Y-ibritumomab tiuxetan has also been associated with severe cutaneous and mucocutaneous skin reactions. Furthermore, haematological toxicities, while generally reversible in most patients, are significant with grade 3 or 4 neutropenia in 63 % of patients and grade 3 or 4 myelosuppression in 53 % of patients, with a time to nadir of 4–7 weeks and average duration of 30 days, although up to 5–7% of patients had grade 3–4 myelosuppression for >90 days.

Both ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab have been approved for use in previously treated patients. ^{131}I-tositumomab has also been shown to be an effective initial treatment for follicular lymphoma. Sixty-six patients with stage III or IV follicular lymphoma were treated with a single course of ^{131}I-tositumomab, with a high ORR of 95% and a CR in 75% of patients. At a median follow-up of 5 years, the PFS was 6.1 years. Haematological toxicities were moderate with no patients needing growth factors or transfusions, and no cases of myelodysplastic syndrome.[30] On the basis of these positive findings, a trial was conducted to evaluate the efficacy and safety of front-line treatment with CHOP followed by ^{131}I-tositumomab.

A phase II trial enrolled 90 patients with previously untreated advanced-stage follicular lymphoma. Patients received 6 cycles of CHOP followed 4–8 weeks later by ^{131}I-tositumomab. Treatment was well tolerated and reversible myelosuppression was more significant with CHOP than with ^{131}I-tositumomab. The ORR was 90% with a 67% CR and a 23% partial remission (PR) rate. Furthermore, 57% of the patients who did not achieve a CR with CHOP improved their remission status with ^{131}I-tositumomab. At a median follow-up of 2.3 years, the PFS was 81% and OS was 97%.[31,32] On the basis of these excellent data for the front-line use of radioimmunotherapy combined with chemotherapy for the treatment of follicular lymphoma, randomised trials are currently underway to further evaluate this treatment strategy.

2. Investigational Monoclonal Antibodies: Targeting Surface Antigens

The clear demonstration of the clinical efficacy of rituximab has driven further clinical research into how best to target the receptors expressed by lymphoma cells, including CD22, CD80, CD40 and CD52.

2.1 CD22 Antibodies

CD22 is a B-cell-restricted marker expressed only at the mature stages of differentiation. CD22 is expressed at high levels in NHL, with expression at >90% for both large B-cell and follicular lymphomas. The function of CD22 is thought to be as an antigen within the process of B-cell activation but remains not yet completely defined. *In vivo* work has shown that the B cells of CD22-deficient mice have a shorter life span and increased apoptosis. When CD22 binds to a natural ligand or antibody, it is rapidly internalised and appears to then provide pro-apoptotic signals within B NHL cells.[33]

2.1.1 Unconjugated Anti-CD22 Antibodies

Some of the first *in vitro* studies were performed using an LL2 antibody, which is an IgG2a mouse monoclonal antibody against CD22. LL2 was found to bind to almost all the B NHL cell lines evaluated, although the mechanism of its action could not be determined. The humanised IgG1 version of LL2 is epratuzumab, and initial studies with this humanised anti-CD22 antibody labelled with ^{131}I and ^{111}Indium/^{90}Y showed tumour localisation, accumulation and signs of anti-tumour activity. A phase I/II clinical trial with epratuzumab in patients with relapsed or refractory aggressive NHL enrolled a total of 56 patients who received 120–1000 mg/m^2 doses weekly for 4 weeks (table I). The patients receiving this protocol were heavily pretreated with a median of four prior regimens and most had bulky disease. Epratuzumab was well tolerated with no dose-limiting toxicities. The half-life for rituximab is dose dependent with a half-life of approximately 60 hours after the first infusion and 174 hours after the fourth infusion, while the half-life for epratuzumab is even longer at >3 weeks. ORR was noted in 10% of the

Table I. Unconjugated and conjugated anti-CD22[a] antibodies in clinical development

Antibody/regimen	Phase of trial	Patients	Disease type	Dose of antibody	Response	Reference
Epratuzumab	I/II	56	R/R NHL (35 DLBCL)	120–1000 mg/m^2 per wk × 4wk	ORR 10% 3 CRs TTP 35wk	34
Epratuzumab (E) + rituximab (R)	II	23	R/R B-NHL (15 FL/6 DLBCL)	E 360 mg/m^2 per wk × 4wk R 375 mg/m^2 per wk × 4wk	ORR 67% 9 CRs in FL 3 CRs in DLBCL TTP 17.8mo	35
ER-CHOP	Pilot	15	Newly diagnosed DLBCL	E 360 mg/m^2 + R 375 mg/m^2 + CHOP every 3wk for 6–8 cycles	ORR 86% 7 CRs F/U to date of 8.1mo with PD in 1/15	36
Conjugated ^{90}Y-epratuzumab	I/II	16	R/R NHL (8 indolent/8 aggressive)	185 MBq/m^2 of ^{90}Y-epratuzumab per w × 2–4w	ORR 62% 4 CRs EFS 14–41mo	37
Inotuzumab ozogamicin (CMC-544; calicheamicin conjugate)	I	34	R/R B-NHL (all B-NHL except Burkitt's or lymphoblastic)	0.4–2.4 mg/m^2 × 3–4wk	ORR 28% MTD 1.8 mg/m^2	38

a CD22 is a B-cell restricted marker expressed only at the mature stages of differentiation with >90% expression on follicular lymphomas and diffuse large B-cell lymphomas. These properties make it an appealing antigen for targeted therapeutic development. Data thus far have shown clinical activity for epratuzumab both alone and when combined with rituximab or with R-CHOP. Currently, there is much interest in further clinical development of the conjugated anti-CD22 antibodies, particularly inotuzumab ozogamicin (CMC-544).

B = B-cell;**CHOP** = cyclophosphamide, doxorubicin, vincristine and prednisone; **CR** = complete remission; **DLBCL** = diffuse large B-cell lymphomas; **EFS** = event-free survival; **ER** = epratuzumab plus rituximab; **FL** = follicular lymphomas; **F/U** = follow-up; **MTD** = maximum tolerated dose; **NHL** = non-Hodgkin's lymphoma; **ORR** = objective response rate; **PD** = progressive disease; **R/R** = relapsed/refractory; **TTP** = time to progression.

patients, with the highest level of response in the patients with diffuse large B-cell lymphoma; three CRs were observed. The median TTP was 26 weeks and two patients had ongoing responses at >4 weeks after completion of therapy, which could potentially be secondary to the long half-life of epratuzumab.[34]

Two recently completed trials support the rationale for combining epratuzumab with rituximab (ER) or ER plus CHOP (ER-CHOP). ER was evaluated in 23 patients with relapsed or refractory NHL with indolent or intermediate-to-aggressive B-cell NHL histologies. The RR for indolent lymphomas was 63%, with a 56% CR and a 66% RR for large B-cell lymphomas, which represented a favourable increase in the indolent lymphoma CR and large B-cell lymphoma RR compared with historical data for rituximab alone.[35] The preliminary data with ER provided the rationale to investigate ER-CHOP for 6–8 cycles in previously untreated patients with diffuse large B-cell lymphoma. The majority of the patients enrolled had advanced-stage disease. Patients were treated with epratuzumab 360 mg/m^2 in combination with the standard doses of R-CHOP. Preliminary data for the first 15 patients enrolled have been presented and have shown an ORR of 81% and a CR of 50%, and at a median follow-up of 8.1 months only 1 of 15 patients has shown disease progression.[36]

2.1.2 Conjugated Anti-CD22 Antibodies

There is growing interest in the ability to conjugate or link monoclonal antibodies to immunotoxins. These conjugated antibodies work as the drug delivery system for the attached conjugate. When the antibody binds to the antigen expressed on the lymphoma cell, the immunotoxin is released and subsequently acts with the antibody to destroy the cancer cell to which the antibody is bound; when conjugated to a radioisotope, as are ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab, a 'bystander effect' is also seen. The benefits of this approach are

that the immunotoxin conjugates add to the potency of the antibody, since they are often internalised into the targeted cells, thus increasing lymphoma cell death. However, potential drawbacks are that the conjugate can make the antibody less stable and the bystander effect seen for the radioisotope conjugates can lead to increased toxicity for surrounding normal cells.

A clinical trial investigated the outcomes of fractionated treatment with radioconjugated ^{90}Y-epratuzumab. In this study, three cohorts of six patients each received an infusion once per week for 2–4 weeks, with a total epratuzumab dose per infusion of 1.5 mg/kg. Two patients experienced haematological dose-limiting toxicities. This treatment showed a high RR in both indolent and aggressive lymphoma patients, with an ORR of 62% (75% ORR in indolent NHL, 50% ORR in intermediate-to-aggressive NHL). There was a durable CR in 25% of patients, with an event-free survival (EFS) of 14–41 months.[37]

Another conjugated anti-CD22 antibody of high clinical research interest is inotuzumab ozogamicin (CMC-544). This antibody specifically targets the CD22 antigen, but is also conjugated to calicheamicin, which is a potent antitumour antibiotic.[39] Calicheamicin is an established effective strategy for the treatment of acute myeloid leukaemia (AML) when targeted against CD33 as gemtuzumab ozogamicin, and acts by binding to DNA in the minor groove and causing double-strand DNA breaks. Calicheamicin, when conjugated to m5/44 (murine anti-CD22 monoclonal antibody), was shown to be effective in both *in vitro* and *in vivo* B-cell lymphoma models, and was noted to be even more active than conjugation of calicheamicin to rituximab.[40] Data from a phase I study of inotuzumab ozogamicin have further added to evidence of its efficacy. In this trial, 34 patients with B-type NHL, excluding Burkitt's and lymphoblastic lymphoma, were enrolled. The median number of prior therapies was four, and inotuzumab ozogamicin was administered every 3–4 weeks at doses of 0.4, 0.8, 1.34, 1.8 and 2.4 mg/m^2. The most common drug-related adverse effect was thrombocytopenia, with the nadir of the platelets occurring about 9 days from administration of inotuzumab ozogamicin. In addition, after the second and third doses, the half-life increased from 1 day to 4 days. Both of these effects appeared to be related to the calicheamicin component. CRs and PRs were observed in all except the 0.4 mg/m^2 cohort with a 28% ORR.[38]

Preclinical *in vivo* studies provide support for the further clinical development of inotuzumab ozogamicin, particularly in combination with rituximab. Lymphoma-bearing SCID (severe combined immunodeficiency) mice were treated intravenously with either saline, inotuzumab ozogamicin (40 μg/kg/dose), rituximab (10 mg/kg/dose) or rituximab alternating with inotuzumab ozogamicin. Antibodies were given every other day for 8 treatments. Results showed that mice treated with both rituximab and inotuzumab ozogamicin had the longest median survival of >90 days compared with 64 days for inotuzumab ozogamicin alone and 44 days for rituximab alone. While survival was longer in the mice treated with inotuzumab ozogamicin and rituximab, this did not correlate with an additive increase in ADCC when the two agents were combined, although both were noted to upregulate apoptosis through the generation of poly adenosine diphosphate-ribose polymerase (PARP) cleavage products.[41]

2.2 CD80 Antibodies

CD80 is a membrane-bound costimulatory molecule involved in regulating T-cell activation.[42,43] In healthy individuals, CD80 is transiently expressed on the surface of activated B cells, dendritic cells and T cells.[44] In contrast, CD80 is constitutively expressed on a variety lymphoid malignancies, including follicular lymphoma and Hodgkin's lymphoma (HL).[45-49] Compared with CD20 expression, CD80 is usually expressed at a lower density. Because of its rather restricted expression, CD80 was recently identified as a potential target for lymphoid malignancies. Preclinical studies demonstrated that anti-CD80 antibodies can inhibit lymphoma cell proliferation and induce ADCC.[50]

Galiximab is a primatised anti-CD80 (IgG1-λ) monoclonal antibody with human constant regions and primate (cynomolgus macaque) variable regions.[51] The single-agent activity of galiximab was recently examined in a multicentre study in patients with relapsed or refractory follicular B-cell lymphoma. In this phase I study, 37 patients received four weekly intravenous infusions of galiximab at doses of 125, 250, 375 or 500 mg/m^2. Therapy was safely administered with no major adverse effects observed, and no patients generated anti-galiximab antibodies. Unlike the chimeric antibody rituximab, which has a relatively short half-life, the galiximab half-life is long and similar to that of the anti-CD22 antibody epratuzumab, ranging from 2 to 4 weeks. Although the ORR was only 11% (with two CRs and two PRs), 49% of the patients showed a decrease in their tumour measurements. Interestingly, some responses were delayed, and one patient achieved a CR 1 year after starting therapy and two responders currently remain in the study, at 22 and 24.4 months out without progression.[52] Because the galiximab half-life is measured in a few weeks, the delayed response cannot be explained by a direct passive antibody effect, raising the possibility that galiximab may induce an active immune response. Interestingly, while all patients enrolled had CD80 expression of +1 to +2 by immunohistochemistry, the patients who responded to treatment did not have higher levels of expression of CD80 than the non-responders. Collectively, these data support investigation of treatment programmes that combine galiximab with other active agents such as rituximab.

2.3 CD52 Antibodies

The CD52 antigen is expressed on normal and neoplastic B and T lymphocytes, monocytes and natural killer (NK) cells. Alemtuzumab is a humanised therapeutic monoclonal antibody that is directed against CD52.[53] It first gained approval in the US and Europe as a treatment for chronic lymphocytic leukaemia (CLL) previously treated with alkylating agents and refractory to fludarabine. When used to treat patients with fludarabine-refractory CLL, an ORR was seen in an impressive 56% of patients.[54]

The major adverse effects of alemtuzumab relate to its infection risk and are associated with the decrease in CD4+ and CD8+ lymphocytes both during treatment and up to 9 months or more after completion of therapy. Infection risk is further increased in patients who have previously received purine analogues because of overlapping adverse effects of myelosuppression and lymphopenia. In addition to traditional bacterial infections, other more atypical infections are noted, including infections secondary to cytomegalovirus (CMV) or herpes simplex virus reactivation, *Pneumocystis (carinii) jeroveci* pneumonia and aspergillosis. Thus, prophylaxis is recommended with agents such as co-trimoxazole (trimethoprim/sulfamethoxazole) and valaciclovir.

Alemtuzumab has been evaluated for the treatment of advanced-stage mycosis fungoides/Sezary syndrome (MF/SS) and has been shown to be an effective treatment option, particularly for those with erythroderma or severe itching. Alemtuzumab 30mg intravenously was administered three times a week for up to 12 weeks. Data from a phase II trial in which 22 patients were treated showed an ORR of 55%; 32% of patients showed a CR and 23% a PR, with a median TTF of 12 months. These findings were supported by the clearance of Sezary cells from the blood in 86% of the patients. There was also a symptomatic improvement in itching. Adverse effects of treatment included CMV reactivation in 18% of patients as well as herpes simplex infections and *Mycobacterium* pneumonia. However, these infectious adverse events generally occurred in patients who had already received three or more prior regimens.[55] Another study showed more modest results in terms of the effectiveness of alemtuzumab with a similar dosage regimen for the treatment of advanced MF/SS, but was limited to a cohort of eight patients who had refractory disease after many previous therapies. The ORR was 38% and the median TTP was short at 4 months.[56]

A pilot study has also been completed to asses the activity of alemtuzumab in the treatment of relapsed

or refractory peripheral T-cell lymphomas (PTCL). A total of 14 patients with relapsed or refractory stage III or IV PTCL were treated with alemtuzumab 30mg intravenously three times a week for a maximum of 12 weeks. All patients received prophylactic co-trimoxazole and valaciclovir prophylaxis. The ORR was 36% and three patients achieved a CR, with the durations of the CR ranging from 2–12 months. While treatment was effective, significant haematological and infectious complications were noted, including CMV reactivation, pulmonary aspergillosis and pancytopenia.[57]

The potential of maintaining effectiveness while decreasing toxicity with a reduced dosage of alemtuzumab was examined in a phase II study. Alemtuzumab 10mg intravenously three times a week for 4 weeks was administered to patients with relapsed/refractory T-cell lymphoma. Preliminary observations for ten patients receiving this protocol have demonstrated an OR of 60% (two CRs, four PRs) with CMV reactivation in one patient.[58]

In addition, data on subcutaneous as an alternative to intravenous administration of alemtuzumab have been reported. The subcutaneous delivery trial used a dose-escalation scheme of 3mg, 10mg, 30mg for the first week and then 30mg subcutaneously three times a week for up to 12 weeks. Twenty patients were given this regimen (13 patients with CLL, one with CLL/AML, three with cutaneous T-cell lymphoma [CTCL], and three with PTCL). While CMV reactivation, bacterial pneumonia and herpes zoster still occurred, grades 3 and 4 infusion reactions were notably less than with intravenous administration and the ORR to therapy was 60%.[59]

The data from these two trials show that both reduced-dose intravenous and subcutaneous alemtuzumab are generally safe, well tolerated and effec-

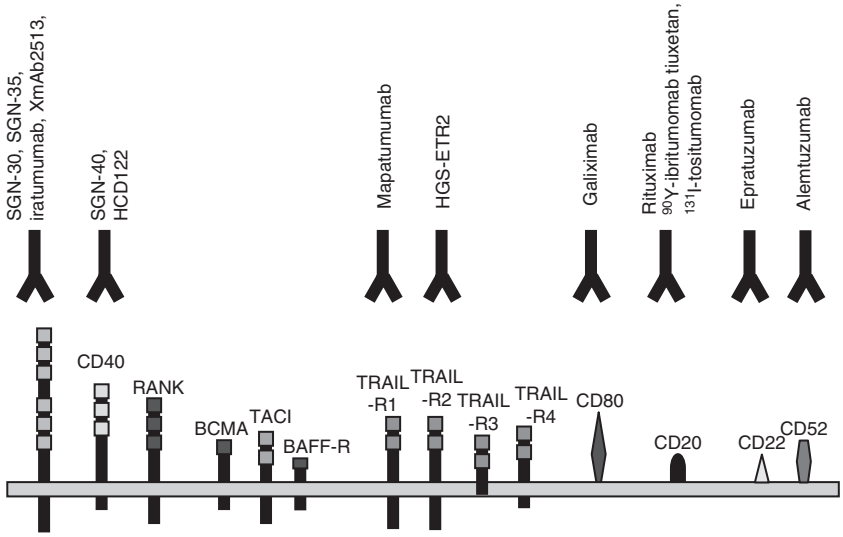

Fig. 1. Selected monoclonal antibodies currently in use for the treatment of a variety of non-Hodgkin's lymphoma (NHL) types. Antibodies targeting CD20 and CD52 are currently approved by the US FDA for the treatment of B-cell NHL and chronic lymphocytic leukaemia (CLL), respectively. (CD30 = expressed in Hodgkin's lymphoma (HL), anaplastic large cell lymphoma, and tonsillar tissue; **CD40** = expressed on normal B lymphocytes, monocytes, dendritic cells, several types of epithelial and endothelial cells, B- and T-cell lymphomas, HL, and several types of carcinomas; **RANK** (receptor activator nuclear factor-κ B) = expressed on osteoclast precursors; **BCMA** (B-cell maturation antigen)/**TACI** (transmembrane activator and calcium modulator and cyclophylin ligand interactor)/**BAFF-R** (B-cell activating factor) = expressed on B cells; **TRAIL** (tumour necrosis factor-related apoptosis-inducing ligand)-R1-4 = TRAIL-R3 and TRAIL-R4 expressed on all cells, while TRAIL-R1 and TRAIL-R2 are expressed on cancer cells; **CD80** = transiently expressed on the surface of activated B cells, dendritic cells and T cells, while being constitutively expressed on a variety of lymphoid malignancies including follicular lymphoma and HL; **CD20** = B-cell marker expressed throughout development from the early pro-B cells through the mature memory B cells; **CD22** = B-cell marker only expressed at the mature stages of differentiation; **CD52** = expressed on normal and neoplastic B and T lymphocytes, monocytes and natural killer cells.

Table II. Selected investigational antibodies, targeting tumour necrosis factor (TNF) receptor family members, currently in clinical trials

Target	Monoclonal antibody (type/action)	Phase of trial	Disease type	Reference
CD30	SGN-30 (chimeric/antagonistic)	I, I/II	HL, ALCL	65,66
CD30	Iratumumab [MDX-060] (fully human/ antagonistic)	I, II	HL, ALCL	67
CD40	SGN-40 (fully human/antagonistic)	I	B-NHL, MM	68
CD40	HCD122 [Chir12.12] (fully human/ antagonistic)	I	CLL, MM	69
TRAIL receptor R1 and TRAIL-R2	Mapatumumab [HGS-ETR1] and HGS-ETR2 (fully human/agonistic)	I, II	NHL	70,71

ALCL = anaplastic large cell lymphoma; **B** = B-cell; **CLL** = chronic lymphocytic leukaemia; **HL** = Hodgkin's lymphoma; **MM** = multiple myeloma; **NHL** = non-Hodgkin's lymphoma; **TRAIL** = TNF-related apoptosis-inducing ligand.

tive alternatives to full-dose intravenous administration.

One trial has also investigated whether front-line treatment with CHOP plus alemtuzumab (CHOP-C) would be an effective option for patients with PTCL. A total of 20 patients were treated with CHOP, preceded on day −1 with alemtuzumab 30mg subcutaneously. After a median follow-up of >8 months, five patients had died of lymphoma with no toxicity deaths recorded and, for the 15 of 20 patients who were still alive, eight had a CR and one had a PR. Therefore, CHOP-C appears to be a feasible treatment option for PTCL, although infectious complications exist.[60]

Thus, alemtuzumab has documented clinical efficacy for the treatment of relapsed/refractory MF/SS and PTCL, although infusion-related toxicities and infectious adverse effects are common. Trials now underway include investigations for the treatment of relapsed/refractory HL, and further larger cohort investigations are planned for the treatment of newly diagnosed HL.

2.4 CD2 Antibodies

CD2 is a transmembrane glycoprotein with an important role in both T-cell and NK-cell functions. CD2 has dual roles as an adhesion molecule and a costimulatory molecule via its actions with its ligand CD58. CD2 antigen is thus a rationally good target for the treatment of T-cell lymphoma.[61] Siplizumab (MEDI-507) is a humanised IgG1κ monoclonal antibody that binds to human CD2 antigen. Preclinical studies demonstrated that siplizumab kills target cells by ADCC. Treatment of mice bearing adult human T-cell leukaemia/lymphoma cells with siplizumab antibody significantly prolonged their survival and led to depletion of T and NK cells.[62] This antibody is currently being evaluated in a phase I study in patients with adult T-cell leukaemia and peripheral T-cell lymphoma. Hopefully, this antibody will prove to be both safe and effective, enabling combination trials with chemotherapy in the future.

3. Targeting the TNF Receptor Family

The tumour necrosis factor (TNF) family is large and is currently described as including 26 receptors and 18 ligands. They exist as divergent forms, as either membrane bound or soluble.[63,64] When activated, these ligands form protein trimers with the receptors, which have cysteine-rich extracellular domains. The diversity of the TNF family has made it a pivotal grouping of receptors for the development of monoclonal antibodies for the treatment of NHL (figure 1) [table II].

3.1 CD30 Antibodies

In healthy individuals, CD30 expression is restricted to a small number of activated B and T lymphocytes. CD30 is expressed in the malignant Hodgkin's and Reed-Sternberg (HRS) cells of classical HL and anaplastic large cell lymphoma (ALCL).

Phase I results of two clinical trials of anti-CD30 antibodies in patients with CD30+ haematological malignancies were recently reported. The chimeric antibody SGN-30 was reported to induce cell-cycle

arrest and apoptosis in Hodgkin's-derived cell lines *in vitro*.[65] This antibody was recently evaluated in a phase I study in patients with relapsed HL or ALCL.[66] A total of 24 patients were treated with escalating doses ranging from 1 to 15 mg/kg by weekly intravenous infusion for 6 consecutive weeks. Early results showed one CR and one minor remission. Iratumumab (MDX-060), a fully human, monoclonal anti-CD30 antibody, was also evaluated in a phase I study in a similar patient population. A different dosage escalation scheme (range, 0.1–15 mg/kg) was used to treat 72 patients with weekly doses for 4 consecutive weeks. Four patients achieved a CR (two HL and two ALCL) and three patients achieved a PR (two HL and one ALCL). Additionally, since this antibody was fully humanised there were no infusion reactions noted and no generation of HAHAs.[67]

The XmAb2513 is a second-generation anti-CD30 antibody with enhanced effector function through mutagenesis at the Fc-receptor binding interface. This antibody has a 4-fold higher affinity than its chimeric parent anti-CD30 antibody.[72] XmAb2513 takes the humanised variable domain of the antibody and then pairs it with a modified Fc region. This pairing has a notable 20-fold increase in the affinity for the FcγRIIIA receptor. These *in vitro* data support the rationale for further *in vivo* analysis and the potential further clinical development of this antibody, as well as the technology used to develop it.

CD30 can be shed in a soluble form (sCD30) detectable in the serum of most patients with CD30-expressing lymphomas. Clinically, sCD30 seems to predict a poorer prognosis, with 41% of advanced-stage HL patients having serum sCD30 levels ≥100 IU/mL, and this level correlated with a 5-year EFS of 51%. However, the half-life of sCD30 is not known. sCD30 can bind to CD30L with high affinity and is able to block its biological function.[73,74] Thus, it is possible that soluble CD30 may play a role in reducing the clinical efficacy of anti-CD30 antibodies by neutralising them in the circulation and preventing them from reaching HRS cells. This potential issue may be resolved by careful engineering of the anti-CD30 antibody so it can bind only to the transmembrane form. In fact, recent data suggest that sCD30 may lack certain epitopes associated with membrane-bound CD30, thus allowing engineering of monoclonal antibodies that preferentially target CD30+ cells without being neutralised by sCD30.[75]

3.2 CD40 Antibodies

CD40 is expressed by normal B lymphocytes, monocytes and dendritic cells, in addition to several types of epithelial and endothelial cells. B- and T-cell lymphomas, HRS cells and several types of carcinomas also express CD40. CD40L (CD154) is predominantly expressed by activated T lymphocytes. CD40L has diverse physiological functions, including priming dendritic cells to activate CD8+ cytotoxic T cells, B-cell selection and survival, and immunoglobulin isotype switching. CD40L is less frequently expressed, however, by activated B lymphocytes, NK cells, monocytes, basophils, eosinophils, dendritic cells, platelets, and endothelial and smooth muscle cells. Soluble CD40L (sCD40L) can be detected in the serum of patients with lymphoma, CLL, autoimmune diseases and essential thrombocythaemia.[76] The role of sCD40L has yet to be completely determined. Preliminary data have shown that high levels of sCD40 appear to be an independent risk factor for a poor prognosis in multiple myeloma and acute myelogenous leukaemia, but not in mantle-cell lymphoma.[77]

Because CD40 and CD40L can be co-expressed by several types of B-cell malignancies, an autocrine-paracrine CD40L/CD40 survival loop has been proposed to play a role in the pathogenesis and survival of some B-cell neoplasms.[78] Two antibodies targeting CD40 (SGN-40 and HCD122 [Chir12.12]) are currently being evaluated in clinical trials. SGN-40 is a humanised IgG1 antihuman CD40 antibody. Preclinically, SGN-40 has demonstrated potent inhibition of proliferation, and induced apoptosis and ADCC in a panel of high-grade B-cell lymphoma lines. In addition, SGN-40 demonstrated activity similar to that of rituximab in xenograft CD40 tumour models.[79] SGN-40 has

been evaluated in the phase I setting in NHL. Preliminary data show that disease was stabilised in one of six patients treated with doses of 2 mg/kg/week for 4 weeks, while another patient showed symptomatic improvement. A parallel study of SGN-40 using the next dose level of 4 mg/kg resulted in severe headaches, which have been postulated to be secondary to cytokine release of TNFα and interleukin-6. However, no patients treated in the 2 mg/kg cohort developed headaches.[68] The protocol was amended to address the concern regarding first-dose cytokine release and continued dose escalation. A humanised IgG1 anti-CD40 antagonistic monoclonal antibody, HCD122, has also demonstrated *in vitro* activity in both CLL and NHL cells. Interestingly, when rituximab and HCD122 were compared for their ADCC activity using malignant human B-cell lymphoma lines expressing CD20 and CD40, HCD122 was superior.[69] HCD122 is currently being investigated in phase I trials for the treatment of B-cell CLL.

3.3 TRAIL and its Receptors

TRAIL (TNF-related apoptosis-inducing ligand)/Apo2 is a death protein that belongs to the TNF family. It has four exclusive receptors: TRAIL-R1 (DR4), -R2 (DR5, KILLER, TRICK2), -R3 (DcR1, TRID, LIT), and -R4 (DcR2 TRUNDD).[64,80,81] TRAIL also binds to osteoprotegerin at a lower affinity.[82] TRAIL-R1 and -R2 are death receptors, whereas TRAIL-R3 and -R4 are frequently called decoy receptors.[83] TRAIL preferentially kills cancer cells while sparing normal tissue, making it potentially useful in cancer therapy. This preferential killing is due in part to the differential expression of its receptors. Normal tissues usually do not express the death receptors TRAIL-R1 and TRAIL-R2 and, therefore, are protected from TRAIL-induced apoptosis. In contrast, most tumours express TRAIL-R1 and TRAIL-R2, making them more sensitive to TRAIL-induced apoptosis. In fact, triggering TRAIL death receptors by TRAIL protein or by agonistic antibodies has been shown to induce cell death in a variety of tumours, including lymphoma.[84-86]

Both anti-TRAIL-R1 and -R2 demonstrated activity against a wide variety of cultured and primary lymphoma cells *in vitro*.[86] Preclinical evaluations and clinical trials using TRAIL/Apo2L or anti-TRAIL-R1 (mapatumumab [HGS-ETR1]) and -R2 (HGS-ETR2) fully human monoclonal antibodies are ongoing. Preclinical work has shown that mapatumumab and HGS-ETR2 are able to induce apoptosis, upregulate caspase 8 and cleave PARP, as well as enhance doxorubicin and bortezomib activity. Furthermore, *in vivo* work with TRAIL/Apo2L has shown activity in xenograft models of lymphoma, lung cancer and breast cancer.[80,87] A phase II trial of mapatumumab in patients with relapsed or refractory NHL was completed with 40 patients enrolled. Patients were treated with two dose levels of 3 or 10 mg/kg every 21 days for a maximum of 6 cycles. There was one CR and two PRs in patients with follicular lymphoma, and no patients discontinued therapy because of toxicity associated with the drug.[70] A phase Ia trial enrolled patients with advanced solid tumours or NHL and treated them for 5 consecutive days with recombinant Apo2L/TRAIL (AMG 655) every 3 weeks for 8 cycles at dose levels of up to 15 mg/kg. A staggered dosage format was used to enrol patients with and without liver metastases to ascertain potential hepatotoxicity. Fifty-one patients have been enrolled with no dose-limiting or clearly attributable toxicities. Fifty-three percent of patients had stable disease and one patient with chondrosarcoma had an objective PR at a dose of 8 mg/kg.[88] Further efforts have investigated the potential of synergistic activity by targeting the TRAIL-R1 and CD20 antigen by combining mapatumumab and rituximab. Data showed that the combination of the two resulted in 30–50% apoptosis and 90% inhibition of proliferation, which was markedly higher than use of either agent alone.[71] Thus, these data provide the rationale for future clinical trials investigating combined targeted approaches with TRAIL-based therapies.

3.4 BAFF, APRIL and their Receptors

BAFF (B-cell activating factor; also known as BLyS, TALL-1, ZANK, zTNF4 and TNFS 13B) is

expressed by macrophages, monocytes and dendritic cells, but not by benign B or T lymphocytes.[89] BAFF binds to three receptors that are preferentially expressed on B cells: BAFF-R (also known as BR-3); TACI (transmembrane activator and calcium modulator and cyclophylin ligand interactor); and BCMA (B-cell maturation antigen). TACI and BCMA are also shared with a related TNF family member called APRIL (a proliferation-inducing ligand; also called TRDL-1 or TALL-2).[90,91] These three receptors are almost exclusively expressed by B lymphocytes, although TACI transcripts have been observed in T lymphocytes. BAFF is an important survival factor for both benign and malignant B lymphocytes.[89,92,93] APRIL, a secreted soluble protein that shares the highest sequence homology with BAFF, is expressed in monocytes, macrophages, dendritic cells and T lymphocytes.[90,94] APRIL binds to TACI and BCMA receptors.

Dysregulated BAFF and APRIL expression has been observed in patients with a variety of NHL malignancies, and it has been noted to be Epstein-Barr virus-inducible.[95-99] It has been demonstrated that serum levels of BAFF or BLyS have prognostic significance, especially in patients with HL.[100] Furthermore, HRS cells express BAFF and APRIL in addition to TACI and BCMA receptors, suggesting that this pathway may play an important role in supporting HRS cell survival.[101] Collectively, these data show the prognostic significance of BAFF and APRIL, and demonstrate that blocking the BAFF and APRIL survival pathway may be of therapeutic value in patients with B-cell lymphoid malignancies.

4. Targeting Angiogenesis

4.1 VEGF Antibodies

VEGF (vascular endothelial growth factor; also called VEGF-A), a secreted dimeric protein that promotes the growth and survival of embryonic and newly formed endothelial cells in adults, belongs to a gene family that includes VEGF-B, -C and -D. VEGF primarily regulates angiogenesis, whereas VEGF-C and -D are involved in regulating lymphangiogenesis.[102,103] VEGF binds to two receptors, VEGFR-1 (also known as Flt-1) and VEGFR-2 (also known as KDR or Flk-1), whereas VEGF-B binds only to VEGFR-1.[104] Both VEGFR-1 and VEGFR-2 have tyrosine kinase activity in their cytoplasmic domains that can be inhibited by small molecules. Increased angiogenesis has been observed in all types of lymphoma, but more prominently in those patients with aggressive histologies.[105-107] Furthermore, high levels of soluble angiogenic factors in the sera of patients with lymphoma correlated with poor treatment outcomes.[108] The anti-VEGF antibody bevacizumab has been investigated in a phase II trial for the treatment of patients with advanced-stage aggressive NHL in first or second relapse. Patients received bevacizumab 10 mg/kg intravenously every 2 weeks. Of the 46 patients treated, two patients had a PR and eight had stable disease. The median TTP was 5 months in these ten patients.[109] Molecular studies were also performed on the tumours of patients treated according to this protocol and showed that VEGF and VEGFR-1 expression was restricted to malignant cells, while VEGFR-2 was predominantly expressed in the endothelial cells in the lymphoma specimens. In addition, a positive correlation was observed between VEGF tissue expression and plasma levels of VEGF, and plasma levels of VEGF expression decreased significantly during bevacizumab administration.[110] A clinical trial is currently evaluating the combination of bevacizumab and rituximab for the treatment of relapsed patients with diffuse large B-cell lymphoma and mantle-cell lymphoma, as is the SWOG S0515 trial, which combines R-CHOP plus bevacizumab in the treatment of advanced-stage diffuse large B-cell lymphoma.

5. Conclusion

Monoclonal antibody-based therapies have already had a profound impact on the treatment of NHL with their initial use as monotherapy for relapsed follicular lymphoma. To date, they appear to be most effective when combined with standard chemotherapy, particularly in patients with intermediate-to-aggressive NHL. In addition to expanding

the repertoire of monoclonal antibodies available for treatment, it is important to increase our understanding of the best methods of optimising their use. Key issues with regard to maximising the benefits of monoclonal antibody therapy include continuing to refine its optimal timing with chemotherapy, duration of use, maintenance therapy and whether it should be continued at disease progression. Finally, an important avenue of investigation is elucidating the mechanisms of action and the potential additive or synergistic benefits of combining monoclonal antibodies with other antibodies or targeted therapies in addition to traditional chemotherapy.

Acknowledgements

No sources of funding were used to assist in the preparation of this review. The authors have no conflicts of interest that are directly relevant to the content of this review.

References

1. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256 (5517): 495-7
2. Reilly RM, Sandhu J, Alvarez-Diez TM, et al. Problems of delivery of monoclonal antibodies: pharmaceutical and pharmacokinetic solutions. Clin Pharmacokinet 1995; 28 (2): 126-42
3. Winter G, Harris WJ. Humanized antibodies. Immunol Today 1993; 14 (6): 243-6
4. Levene AP, Singh G, Palmieri C. Therapeutic monoclonal antibodies in oncology. J R Soc Med 2005; 98 (4): 146-52
5. Uchida J, Lee Y, Hasegawa M, et al. Mouse CD20 expression and function. Int Immunol 2004; 16 (1): 119-29
6. Cragg MS, Walshe CA, Ivanov AO, et al. The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun 2005; 8: 140-74
7. Harjunpaa A, Junnikkala S, Meri S. Rituximab (anti-CD20) therapy of B-cell lymphomas: direct complement killing is superior to cellular effector mechanisms. Scand J Immunol 2000; 51 (6): 634-41
8. Golay J, Manganini M, Facchinetti V, et al. Rituximab-mediated antibody-dependent cellular cytotoxicity against neoplastic B cells is stimulated strongly by interleukin-2. Haematologica 2003; 88 (9): 1002-12
9. Byrd JC, Murphy T, Howard RS, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001; 19 (8): 2153-64
10. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16 (8): 2825-33
11. Davis TA, Grillo-Lopez AJ, White CA, et al. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol 2000; 18 (17): 3135-43
12. Ghielmini M, Schmitz S-FH, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood 2004; 103 (12): 4416-23
13. Hainsworth JD, Litchy S, Shaffer DW, et al. Maximizing therapeutic benefit of rituximab: maintenance therapy versus retreatment at progression in patients with indolent non-Hodgkin's lymphoma: a randomized phase II trial of the Minnie Pearl Cancer Research Network. J Clin Oncol 2005; 23 (6): 1088-95
14. Hochster HS, Weller E, Gascoyne RD, et al. Maintenance rituximab after CVP results in superior clinical outcome in advanced follicular lymphoma (FL): results of the E1496 phase III trial from the Eastern Cooperative Oncology Group and the Cancer and Leukemia Group B [abstract no. 349]. Blood 2005; 106 (11): 106a
15. Hiddemann W, Forstpointner R, Dreyling M, et al. Rituximab maintenance following a rituximab containing chemotherapy significantly prolongs the duration of response in patients with relapsed follicular and mantle cell lymphomas: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG) [abstract no. 6527]. J Clin Oncol 2005; 23 (16S Pt 1): 566s
16. Colocci N, Weller E, Hochster HS, et al. Prognostic significance of the follicular lymphoma international prognostic index (FLIPI) in the E1496 trial of chemotherapy with or without maintenance rituximab [abstract no. 6526]. J Clin Oncol 2005; 23 (16S Pt 1): 566s
17. Hiddemann W, Forstpointner R, Dreyling M, et al. Rituximab maintenance prolongs response duration after salvage therapy with R-FCM in patients with relapsed follicular lymphomas and mantle cell lymphomas: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG) [abstract 920]. Blood 2005; 106 (11): 170a
18. Van Oers MHJ, Van Glabbeke M, et al. Chimeric anti-CD20 monoclonal antibody (rituximab; mabthera) in remission induction and maintenance treatment of relapsed/resistant follicular non-Hodgkin's lymphoma: final analysis of a phase III randomized intergroup clinical trial [abstract no. 353]. Blood 2005; 106 (11): 107a
19. Czuczman MS, Weaver R, Alkuzweny B, et al. Prolonged clinical and molecular remission in patients with low-grade or follicular non-Hodgkin's lymphoma treated with rituximab plus CHOP chemotherapy: 9-year follow-up. J Clin Oncol 2004; 22 (23): 4711-6
20. McLaughlin MA, Rodriguez FB, Hagemeister J, et al. Stage IV indolent lymphoma: a randomized study of concurrent vs. sequential use of FND chemotherapy (fludarabine, mitoxantrone, dexamethasone) and rituximab (R) monoclonal antibody therapy, with interferon maintenance [abstract no. 2269]. 39th Annual Meeting of the American Society of Clinical Oncology; 2003 May 31-Jun 3; Chicago (IL). Proc Am Soc Clin Oncol 2003; 22: 564
21. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002; 346 (4): 235-42
22. Miller TP, Unger JM, Spier C, et al. Effect of adding rituximab to three cycles of CHOP plus involved-field radiotherapy for limited-stage aggressive diffuse B-cell lymphoma (SWOG-0014) [abstract no. 158]. Blood 2004; 104 (11): 48a

23. Pfreundschuh M, Kloess M, Schmits R, et al. Six, not eight cycles of bi-weekly CHOP with rituximab (R-CHOP-14) is the preferred treatment for elderly patients with diffuse large B-cell lymphoma (DLBCL): results of the RICOVER-60 trial of the German high-grade non-Hodgkin lymphoma Study Group (DSHNHL) [abstract no. 13]. Blood 2005; 106 (11): 9a
24. Romaguera JE, Fayad L, Rodriguez MA, et al. High rate of durable remissions after treatment of newly diagnosed aggressive mantle-cell lymphoma with rituximab plus hyper-CVAD alternating with rituximab plus high-dose methotrexate and cytarabine. J Clin Oncol 2005; 23 (28): 7013-23
25. Morschhauser F, Leonard J, Coiffier B, et al. Initial safety and efficacy results of a second-generation humanized anti-CD20 antibody, IMMU-106 (hA20), in non-Hodgkin's lymphoma [abstract no. 2428]. Blood 2005; 106 (11): 683a
26. Witzig TE, White CA, Wiseman GA, et al. Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma. J Clin Oncol 1999; 17 (12): 3793-803
27. Witzig TE, Flinn IW, Gordon LI, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. J Clin Oncol 2002; 20 (15): 3262-9
28. Witzig TE, Gordon LI, Cabanillas F, et al. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol 2002; 20 (10): 2453-63
29. Kaminski MS, Zelenetz AD, Press OW, et al. Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. J Clin Oncol 2001; 19 (19): 3918-28
30. Kaminski MS, Tuck M, Estes J, et al. 131I-tositumomab therapy as initial treatment for follicular lymphoma. N Engl J Med 2005; 352 (5): 441-9
31. Kaminski MS, Estes J, Regan D. Front-line treatment of advanced B-cell low-grade lymphoma with radiolabelled anti-B1 antibody: initial experience [abstract no. 51]. 33rd Annual Meeting of the American Society of Clinical Oncology; 1997 May 17-20; Denver (CO). Proc Am Soc Clin Oncol 1997; 16: 15a
32. Press OW, Unger JM, Braziel RM, et al. A phase 2 trial of CHOP chemotherapy followed by tositumomab/iodine I 131 tositumomab for previously untreated follicular non-Hodgkin lymphoma: Southwest Oncology Group Protocol S9911. Blood 2003; 102 (5): 1606-12
33. Sato S, Tuscano JM, Inaoki M, et al. CD22 negatively and positively regulates signal transduction through the B lymphocyte antigen receptor. Semin Immunol 1998; 10 (4): 287-97
34. Leonard JP, Coleman M, Ketas JC, et al. Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results. Clin Cancer Res 2004; 10 (16): 5327-34
35. Leonard JP, Coleman M, Ketas J, et al. Combination antibody therapy with epratuzumab and rituximab in relapsed or refractory non-Hodgkin's lymphoma. J Clin Oncol 2005; 23 (22): 5044-51
36. Micallef IN, Kahl BS, Gayko U, et al. Initial results of a pilot study of epratuzuamab and rituximab in combination with cehmotherapy (ER-CHOP) in previously untreated patients with diffuse large B-cell lymphoma [abstract no. 6580]. 40th Annual Meeting of the American Society of Clinical Oncology; 2004 Jun 5-8; New Orleans (LA). Proc Am Soc Clin Oncol 2004; 23: 575
37. Linden O, Hindorf C, Cavallin-Stahl E, et al. Dose-fractionated radioimmunotherapy in non-Hodgkin's lymphoma using DOTA-conjugated, 90Y-radiolabeled, humanized anti-CD22 monoclonal antibody, epratuzumab. Clin Cancer Res 2005; 11 (14): 5215-22
38. Advani A, Gine E, Gisselbrecht C, et al. Preliminary report of a phase I study of CMC-544, an antibody-targeted chemotherapy agent, in patients with B-cell non-Hodgkin's lymphoma (NHL) [abstract no. 230]. Blood 2005; 106 (11): 71a
39. McCarron PA, Olwill SA, Marouf WMY, et al. Antibody conjugates and therapeutic strategies. Mol Interv 2005; 5 (6): 368-80
40. DiJoseph JF, Armellino DC, Doughter MM, et al. Antibody-targeted chemotherapy with immunoconjugates of calicheamicin: differential anti-tumor activity of conjugated calicheamicin targeted to B-cell lymphoma via B-cell lineage specific molecules CD19, CD20, and CD22 [abstract no. 2490]. Blood 2004; 104 (11): 683a
41. Hernandez-Ilizaiturri FJ, Devineni S, Arora S, et al. Targeting CD20 and CD22 with rituximab in combination with CMC-544 results in improved anti-tumor activity against non-Hodgkin's lymphoma (NHL) pre-clinical models [abstract no. 1473]. Blood 2005; 106 (11): 425a
42. Schultze J, Nadler LM, Gribben JG. B7-mediated costimulation and the immune response. Blood Rev 1996; 10 (2): 111-27
43. June CH, Bluestone JA, Nadler LM, et al. The B7 and CD28 receptor families. Immunol Today 1994; 15 (7): 321-31
44. Vyth-Dreese FA, Dellemijn TA, Majoor D, et al. Localization in situ of the co-stimulatory molecules B7.1, B7.2, CD40 and their ligands in normal human lymphoid tissue. Eur J Immunol 1995; 25 (11): 3023-9
45. Dorfman DM, Schultze JL, Shahsafaei A, et al. In vivo expression of B7-1 and B7-2 by follicular lymphoma cells can prevent induction of T cell anergy but is insufficient to induce significant T cell proliferation. Blood 1997; 90: 4297-306
46. Trentin L, Zambello R, Sancetta R, et al. B lymphocytes from patients with chronic lymphoproliferative disorders are equipped with different costimulatory molecules. Cancer Res 1997; 57 (21): 4940-7
47. Vooijs WC, Otten HG, van Vliet M, et al. B7-1 (CD80) as target for immunotoxin therapy for Hodgkin's disease. Br J Cancer 1997; 76 (9): 1163-9
48. Nozawa Y, Wakasa H, Abe M. Costimulatory molecules (CD80 and CD86) on Reed-Sternberg cells are associated with the proliferation of background T cells in Hodgkin's disease. Pathol Int 1998; 48 (1): 10-4
49. Munro JM, Freedman AS, Aster JC, et al. In vivo expression of the B7 costimulatory molecule by subsets of antigen-presenting cells and the malignant cells of Hodgkin's disease. Blood 1994; 83 (3): 793-8
50. Plumas J, Chaperot L, Jacob M, et al. Malignant B lymphocytes from non-Hodgkin's lymphoma induce allogeneic proliferative and cytotoxic T cell responses in primary mixed lymphocyte cultures: an important role of co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) in stimulation by tumor cells. Eur J Immunol 1995; 25 (12): 3332-41
51. Younes A, Hariharan K, Allen RS, et al. Initial trials of anti-CD80 monoclonal antibody (galiximab) therapy for patients with relapsed or refractory follicular lymphoma. Clin Lymphoma 2003; 3 (4): 257-9
52. Czuczman MS, Thall A, Witzig TE, et al. Phase I/II study of galiximab, an anti-CD80 antibody, for relapsed or refractory follicular lymphoma. J Clin Oncol 2005; 23 (19): 4390-8

53. Wierda WG, Kipps TJ, Keating MJ. Novel immune-based treatment strategies for chronic lymphocytic leukaemia. J Clin Oncol 2005; 23 (26): 6325-32
54. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002; 99 (10): 3554-61
55. Lundin J, Hagberg H, Repp R, et al. Phase 2 study of alemtuzumab (anti-CD52 monoclonal antibody) in patients with advanced mycosis fungoides/Sezary syndrome. Blood 2003; 101 (11): 4267-72
56. Kennedy GA, Seymour JF, Wolf M, et al. Treatment of patients with advanced mycosis fungoides and Sezary syndrome with alemtuzumab. Eur J Haematol 2003; 71 (4): 250-6
57. Enblad G, Hagberg H, Erlanson M, et al. A pilot study of alemtuzumab (anti-CD52 monoclonal antibody) therapy for patients with relapsed or chemotherapy-refractory peripheral T-cell lymphomas. Blood 2004; 103 (8): 2920-4
58. Zinzani PL, Alinari L, Tani M, et al. Preliminary observations of a phase II study of reduced-dose alemtuzumab treatment in patients with pretreated T-cell lymphoma. Haematologica 2005; 90 (5): 702-3
59. Bubis JA, Schaal AD, Kimtis EA, et al. Subcutaneous administration of alemtuzumab: a single institution experience [abstract no. 5029]. Blood 2005; 106 (11): 5029
60. Gallamini A, Zaja F, Gargantini L, et al. CHOP chemotherapy plus campath-1H (CHOP-C) as first line treatment in patients with peripheral T-cell lymphoma (PTCL) [abstract no. 3345]. Blood 2005; 106 (11): 935a
61. King PD, Sadra A, Han A, et al. CD2 signaling in T cells involves tyrosine phosphorylation and activation of the Tec family kinase, EMT/ITK/TSK. Int Immunol 1996; 8 (11): 1707-14
62. Zhang Z, Zhang M, Ravetch JV, et al. Effective therapy for a murine model of adult T-cell leukemia with the humanized anti-CD2 monoclonal antibody, MEDI-507. Blood 2003; 102 (1): 284-8
63. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 2001; 104 (4): 487-501
64. Younes A, Aggarwall BB. Clinical implications of the tumor necrosis factor family in benign and malignant hematologic disorders. Cancer 2003; 98 (3): 458-67
65. Cerveny CG, Law C-L, McCormick RS, et al. The anti-CD30 monoclonal antibody SGN-30 inhibits Hodgkin's disease growth and sensitizes cells to established chemotherapeutics [abstract no. 2639]. American Society of Hematology Annual Meeting abstracts. Blood 2004; 104: 2639
66. Bartlett NL, Bernstein SH, Leonard JP, et al. Antitumor activity and pharmacokinetics of six weekly doses of SGN-30 (anti-CD30 monoclonal antibody) in patients with refractory or recurrent CD30+ hematologic malignancies [abstract no. 2390]. Blood 2003; 102 (11): 647a
67. Ansell SM, Byrd JC, Horwitz SM, et al. Phase I/II, open-label, dose-escalating study of MDX-060 administered weekly for 4 weeks in subjects with refractory/relapsed CD30 positive lymphoma [abstract no. 2636]. Blood 2004; 104 (11): 721a
68. Advani RH, Furman RR, Rosenblatt JD, et al. Phase I study of humanized anti-CD40 immunotherapy with SGN-40 in non-Hodgkin's lymphoma [abstract no. 1504). Blood 2005; 106 (11): 433a
69. Lugman M, Tong X, Niu X, et al. CHIR-12.12, an antagonist anti-CD40 antibody, exhibits greater ADCC than rituximab against a variety of malignant B cells: evaluation of FcγRIIIa polymorphism and ADCC response [abstract no. 1472]. Blood 2005; 106 (11): 424a
70. Younes A, Vose JM, Zelenetz AD, et al. Results of a phase 2 trial of HGS-ETR1 (agonistic human monoclonal antibody to TRAIL receptor 1) in subjects with relapsed/refractory non-Hodgkin's lymphoma [abstract no. 489]. Blood 2005; 106 (11): 146a
71. Czuczman MS, Maddipatia S, Knight J, et al. In vitro synergistic anti-tumor activity by targeting TRAIL-R1 and CD20 antigen by combining HGS-ETR1 (agonistic human monoclonal antibody to TRAIL receptor 1) and rituximab monoclonal antibody against non-Hodgkin's lymphoma cells (NHL) [abstract no. 1475]. Blood 2005; 106 (11): 425a
72. Hammond PW, Vafa O, Jacinto J, et al. A humanized anti-CD30 monoclonal antibody, XmAb2513, with enhanced in vitro potency against CD30-postive lymphomas mediated by high affinity Fc-receptor binding [abstract no. 1470]. Blood 2005; 106 (11): 424a
73. Hargreaves P, Al-Shamkhani A. Soluble CD30 binds to CD153 with high affinity and blocks transmembrane signaling by CD30. Eur J Immunol 2002; 32 (1): 163-73
74. Younes A, Consoli U, Snell V, et al. CD30 ligand in lymphoma patients with CD30+ tumors. J Clin Oncol 1997; 15 (11): 3355-62
75. Nagata S, Ise T, Onda M, et al. Cell membrane-specific epitopes on CD30: potentially superior targets for immunotherapy. Proc Natl Acad Sci U S A 2005; 102 (22): 7946-51
76. van Kooten C, Banchereau J. CD40-CD40 ligand. J Leukoc Biol 2000; 67 (1): 2-17
77. Hock BD, McKenzie JL, Patton NW, et al. Circulating levels and clinical significance of soluble CD40 in patients with hematologic malignancies. Cancer 2006; 106 (10): 2148-57
78. Younes A. The dynamics of life and death of malignant lymphocytes. Curr Opin Oncol 1999; 11 (5): 364-9
79. Law CL, Gordon KA, Collier J, et al. Preclinical antilymphoma activity of a humanized anti-CD40 monoclonal antibody, SGN-40. Cancer Res 2005; 65 (18): 8331-8
80. Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2002; 2 (6): 420-30
81. Bhardwaj A, Aggarwal BB. Receptor-mediated choreography of life and death. J Clin Immunol 2003; 23 (5): 317-32
82. Emery JG, McDonnell P, Burke MB, et al. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J Biol Chem 1998; 273 (23): 14363-7
83. Degli-Esposti M. To die or not to die: the quest of the TRAIL receptors. J Leukoc Biol 1999; 65 (5): 535-42
84. Pukac L, Kanakaraj P, Humphreys R, et al. HGS-ETR1, a fully human TRAIL-receptor 1 monoclonal antibody, induces cell death in multiple tumour types in vitro and in vivo. Br J Cancer 2005; 92 (8): 1430-41
85. Snell V, Clodi K, Zhao S, et al. Activity of TNF-related apoptosis-inducing ligand (TRAIL) in haematological malignancies. Br J Haematol 1997; 99 (3): 618-24
86. Georgakis G, Li Y, Humphreys R, et al. Activity of selective fully human agonistic antibodies to the TRAIL death receptors TRAIL-R1 and TRAIL-R2 in primary and cultured lymphoma cells: induction of apoptosis and enhancement of doxorubicin- and bortezomib-induced cell death. Br J Haematol 2005; 130 (4): 501-10
87. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998; 28 (5381): 1305-8

88. Herbst RS, Mendolson DS, Ebbinghaus S, et al. A phase I safety and pharmacokinetic (PK) study of recombinant Apo2L/TRAIL, an apoptosis-inducing protein in patients with advanced cancer [abstract no. 3013]. J Clin Oncol 2006; 24 (18S Pt 1): 124s

89. Schneider P, Tschopp J. BAFF and the regulation of B cell survival. Immunol Lett 2003; 88 (1): 57-62

90. Medema JP, Planelles-Carazo L, Hardenberg G, et al. The uncertain glory of APRIL. Cell Death Differ 2003; 10 (10): 1121-5

91. Mackay F, Schneider P, Rennert P, et al. BAFF AND APRIL: a tutorial on B cell survival. Annu Rev Immunol 2003; 21: 231-64

92. Kern C, Cornuel JF, Billard C, et al. Involvement of BAFF and APRIL in the resistance to apoptosis of B-CLL through an autocrine pathway. Blood 2004; 103 (2): 679-88

93. He B, Chadburn A, Jou E, et al. Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL. J Immunol 2004; 172 (5): 3268-79

94. Mackay F, Ambrose C. The TNF family members BAFF and APRIL: the growing complexity. Cytokine Growth Factor Rev 2003; 14 (3-4): 311-24

95. Klein B, Tarte K, Jourdan M, et al. Survival and proliferation factors of normal and malignant plasma cells. Int J Hematol 2003; 78 (2): 106-13

96. Kolb JP, Kern C, Quiney C, et al. Re-establishment of a normal apoptotic process as a therapeutic approach in B-CLL. Curr Drug Targets Cardiovasc Haematol Disord 2003; 3 (4): 261-86

97. Novak AJ, Bram RJ, Kay NE, et al. Aberrant expression of B-lymphocyte stimulator by B chronic lymphocytic leukemia cells: a mechanism for survival. Blood 2002; 100 (8): 2973-9

98. Moreaux J, Legouffe E, Jourdan E, et al. BAFF and APRIL protect myeloma cells from apoptosis induced by IL-6 deprivation and dexamethasone. Blood 2004; 103 (8): 3148-57

99. Novak AJ, Darce JR, Arendt BK, et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. Blood 2004; 103 (2): 689-94

100. Oki Y, Georgakis GV, Migone T, et al. Serum BLyS Level as a prognostic marker in patients with lymphoma [abstract no. 1926]. Blood 2005; 106 (11): 546a

101. Chiu A, Qiao X, He B, et al. The TNF family members BAFF and APRIL play an important role in Hodgkin lymphoma [abstract no. 22]. Blood 2005; 106 (11): 11a

102. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003; 9 (6): 669-76

103. Plate K. From angiogenesis to lymphangiogenesis. Nat Med 2001; 7 (2): 151-2

104. Olofsson B, Pajusola K, Kaipainen A, et al. Vascular endothelial growth factor B, a novel growth factor for endothelial cells. Proc Natl Acad Sci U S A 1996; 93 (6): 2576-81

105. Ribatti D, Vacca A, Bertossi M, et al. Angiogenesis induced by B-cell non-Hodgkin's lymphomas: lack of correlation with tumor malignancy and immunologic phenotype. Anticancer Res 1990; 10 (2A): 401-6

106. Ribatti D, Vacca A, Marzullo A, et al. Angiogenesis and mast cell density with tryptase activity increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Int J Cancer 2000; 85 (2): 171-5

107. Vacca A, Ribatti D, Roncali L, et al. Angiogenesis in B cell lymphoproliferative diseases: biological and clinical studies. Leuk Lymphoma 1995; 20 (1-2): 27-38

108. Salven P, Orpana A, Teerenhovi L, et al. Simultaneous elevation in the serum concentrations of the angiogenic growth factors VEGF and bFGF is an independent predictor of poor prognosis in non-Hodgkin lymphoma: a single-institution study of 200 patients. Blood 2000; 96 (12): 3712-8

109. Stopeck AT, Bellamy W, Unger J, et al. Phase II trial of a single agent bevacizumab (Avastin) in patients with relapsed, aggressive non-Hodgkin's lymphoma (NHL): Southwest Oncology Group Study S0108 [abstract no. 6592]. J Clin Oncol 2005; 23 (16 Suppl.): 583s

110. Stopeck A, Iannone M, Rimsza L, et al. Expression of VEGF, VEGF receptors, and other angiogenic markers in relapsed aggressive non-Hodgkin's lymphoma: correlative studies from the SWOG S0108 Trial [abstract no. 2288]. Blood 2004; 104 (11): 629a

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