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REVIEW

Novel antibodies in the treatment of non-Hodgkin's lymphoma

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ABSTRACT

Monoclonal antibodies (mAbs) have revolutionised the treatment of malignancies, especially non-Hodgkin's lymphoma (NHL). Antibody-based therapies target tumour cells expressing a specific antigen while sparing the majority of normal cells leading to a decrease in treatment-associated toxicity. Rituximab, a monoclonal antibody directed against CD20 on B cells, was the first monoclonal antibody to be approved by the US Food and Drug Association (FDA) in 1997 for the treatment of patients with relapsed/refractory, follicular or low-grade NHL. However, it was soon realised that not all patients respond to rituximab therapy and close to 60% of patients with follicular lymphoma who were previously sensitive to rituximab become 'resistant' to repeat rituximab therapy. This led to further attempts to improve the antitumour activity of anti-CD20 mAbs (i.e. 2nd/3rd generation anti-CD20s), and to identify additional potential targets on lymphoma cells other than CD20. A number of these antibodies directed against lymphoma cell targets other than CD20 are now undergoing development, many of which are currently in clinical trials. This manuscript focuses on an overview of these 'non-anti-CD20' novel mAbs for NHL.

KEYWORDS

CD20, lymphoma, monoclonal antibodies

MONOCLONAL ANTIBODIES

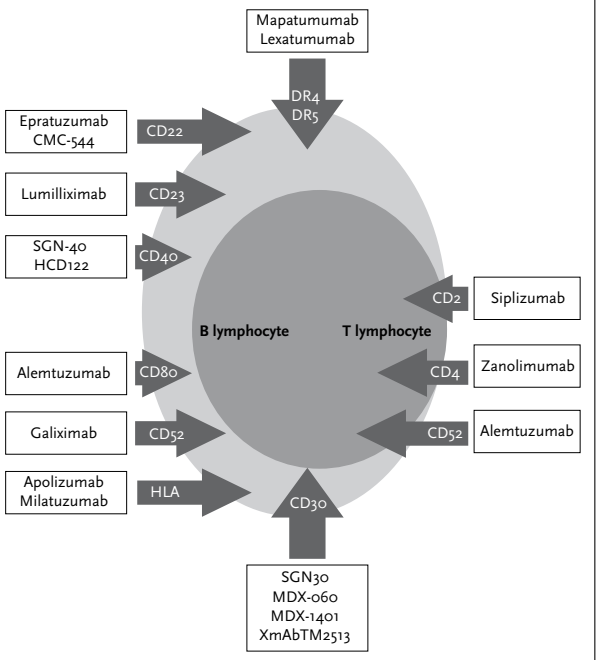
Monoclonal antibodies (mAbs) have revolutionised the treatment of malignancies, especially non-Hodgkin's lymphoma (NHL). Monoclonal antibodies were first described in 1975.[1] The first mAbs generated were non-human murine antibodies developed by fusing B cells from human lymphoma cell-immunised mice. However, humans often developed human antimouse antibodies (HAMA) to these agents, which can be associated with allergic reactions (sometimes severe), as well as a decrease in treatment efficacy by binding mAbs in the circulation prior to their reaching tumour target sites.[2] Developments in biotechnology have led to the development of chimeric antibodies (which are 65 to 90% human), partially humanised antibodies (which are 95% human) and, now, fully humanised antibodies.[3,4] Antibody-based therapies target tumour cells expressing a specific antigen while potentially sparing the majority of normal cells leading to a decrease in treatment-associated toxicity. The availability of mAbs has revolutionised the treatment of lymphomas since these cells express a number of potential target antigens (*figure 1*).

The properties of an ideal target antigen for antibody-based therapy are that: 1) it is selectively and highly expressed on neoplastic cells; 2) it is not secreted as free antigen in the blood; and 3) it does not undergo modulation following binding by the antibody. These characteristics allow recruitment of natural effectors and, subsequently, immunological attack against targeted tumour cells in the form of antibody-dependent cellular cytotoxicity (ADCC). Other antibody-associated mechanisms of antitumour activity include complement-dependent cytotoxicity (CDC), possible vaccine-like effect, and direct apoptosis.[5]

Rituximab, a chimeric monoclonal antibody composed of antigen-binding murine variable regions that are linked to a human backbone directed against CD20 on B cells, was the first monoclonal antibody to be approved by the US Food and Drug Administration (FDA) in 1997 on the basis of a study of 166 patients with relapsed or refractory, follicular or low-grade NHL.[6]

Although rituximab revolutionised the treatment of B-cell NHL, many patients do not respond to rituximab therapy: approximately 50% of patients with relapsed/

Figure 1. *Lymphocyte surface antigen targets*



refractory CD20+ follicular lymphoma (FL) previously treated with chemotherapy failed to respond to initial treatment with rituximab.[6] It has also been reported that close to 60% of previously rituximab-sensitive FL patients became 'resistant' to repeat rituximab therapy.[7] Ongoing attempts to improve the antitumour activity of anti-CD20 monoclonal antibodies include binding to a different epitope than that of rituximab; binding more tightly to CD20; increasing activation of ADCC and/or facilitating apoptosis. A number of second- and third-generation anti-CD20 candidate agents are currently in development. These 'newer' anti-CD20 antibodies may potentially prove to have augmented antitumour activity against CD20-+ B-cell neoplasms compared with rituximab; clinical trials with these novel agents are ongoing. Researchers have also identified several additional potential targets on lymphoma cells other than CD20, fostering the continued and concurrent development of a number of 'other' targeted antibodies, many of which are currently in clinical trials (*table 1*). This manuscript focuses on an overview of these non-anti-CD20 novel mAbs for NHL.

ANTIBODIES AGAINST TARGETS OTHER THAN CD20

CD22 is widely expressed on normal and malignant B cells, and its function appears to relate to B-cell activation and adhesion, modulation of antigen-receptor signalling, and cell-surface-receptor circulation.

EPRATUZUMAB

Epratuzumab (Immunomedics, Inc.) is a humanised IgG1 anti-CD22 antibody associated with both ADCC and direct cytotoxicity in preclinical studies. Phase I/II studies demonstrated objective responses across various dose levels in both relapsed/refractory follicular lymphoma (24%)[8] and diffuse large B-cell lymphoma (DLBCL, 15%).[9]
Toxicities were manageable and consisted primarily of infusion-related reactions; no dose-limiting toxicity was observed. Epratuzumab has also been combined with rituximab in phase II studies showing at least an additive

Table 1. *Novel antibodies for the treatment of non-Hodgkin lymphoma*

Antibody	Target	Type	Source	Trials
Epratuzumab	CD22	IgG1	Humanised	Phase I/II
CMC-544	CD22	IgG4	Humanised	Phase III
Lumiliximab	CD23	IgG1	Chimeric (macaque-human)	Phase III
SGN-30	CD30		Chimeric	Phase I/II
MDX-060	CD30	IgG1κ	Humanised	Phase I/II
MDX-1401	CD30		Humanised	Phase I
XmAbTM2513	CD30		Humanised	Phase I
SGN-40	CD40	IgGl	Humanised	Phase I
HCD122	CD40	IgGl	Humanised	Phase I
Alemtuzumab	CD52		Humanised	Phase II/III
Galiximab	CD80	IgG1λ	Chimeric (macaque-human)	Phase III
Siplizumab	CD2	IgG1κ	Humanised	Suspended
Apolizumab	HLADR	IgG1	Humanised	Suspended
Milatuzumab	CD74		Humanised	Phase I
Mapatumumab	DR		Humanised	Phase I/II
Lexatumumab	DR		Humanised	Phase I/II
Zanolimumab	CD4	IgG1κ	Humanised	Phase III

benefit while toxicities of the combination were comparable with those of single-agent rituximab.[10] In a recent international, multicentre trial[11] evaluating rituximab plus epratuzumab in patients with postchemotherapy relapsed/refractory, indolent NHL, an objective response (OR) was seen in 54% FL patients, (including 24% complete responses (CR) (CR/unconfirmed CR [CRu])), whereas, 57% small lymphocytic lymphoma (SLL) patients had ORs, (including 43% with CR/Cru). Rituximab-naive patients had an OR rate of 50%, whereas patients who previously responded to rituximab had an OR rate of 64%. An OR rate of 85% was observed in patients with FL who had Follicular Lymphoma International Prognostic Index (FLIPI) risk scores of 0 or 1, whereas 28 patients with intermediate or high-risk FLIPI scores (≥2) had an OR rate of 39%. The median duration of response was 13.4 months in patients with FL, and that duration increased to 29.1 months for ten patients who had a CR/CRu, including four patients who had durable responses with remissions that continued for >4 years. In patients with SLL, the median duration of response was 20 months, including one patient who had a response that continued for >3 years. Thus, the combination of epratuzumab and rituximab induced durable responses in patients with recurrent, indolent NHL. Epratuzumab is also being evaluated in combination with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and as a therapy in other B-cell neoplasms.[12]

CMC-544

CMC-544 (inotuzumab ozogamicin) is an immunoconjugate of calicheamicin and inotuzumab (humanised anti-CD22 antibody-G5/44). Calicheamicin is a potent antitumour antibiotic.[13] It acts by binding to the DNA and causing double-strand DNA breaks and is already being used in the treatment of acute myeloid leukaemia when conjugated to an anti-CD33 mAb. CMC-544 has shown preclinical efficacy against B-cell lymphoma both in *in vitro* and *in vivo* models. It inhibited the *in vitro* growth of a number of CD22+ cell lines (IC50s 6-300 pM) which was more potent than unconjugated calicheamicin alone, consistent with the active CD22-mediated cellular internalisation of the conjugate. It was also found to be more active than conjugation of calicheamicin to rituximab (i.e. which is not an 'internalising' mAb).[14] In *in vivo* models of human B-cell lymphomas, CMC-544 caused dose-dependent regression of B lymphoma xenografts.[15] It led to long-term survival of SCID mice with systemically disseminated human B-cell lymphoma.[16] Also, when suboptimal doses of CMC-544 were used in combination with suboptimal doses of rituximab, superior antitumour activity was derived by the combination than either drug administered alone.[17] These preclinical results were confirmed in a phase I study of inotuzumab ozogamicin adding further evidence to its efficacy. In this trial, 34 patients with relapsed/refractory B-cell NHL, excluding Burkitt's and lymphoblastic lymphoma, were enrolled who had an average of four prior therapies. Inotuzumab ozogamicin was administered every three to four weeks at five different doses. The most common adverse effect was thrombocytopenia with the nadir of the platelets occurring about nine days after administration of inotuzumab ozogamicin; it appeared to be related to calicheamicin. The overall response rate (ORR) was 28%; CRs and PRs were observed in all except one cohort.[18] CMC-544 is currently being evaluated in phase III clinical trials in patients with non-Hodgkin's B-cell lymphoma.

ANTI-CD23 MAB LUMILIXIMAB

CD23 is a low-affinity IgE receptor found to be highly expressed on chronic lymphocytic leukaemia (CLL) cells. Lumiliximab (Biogen Idec) is a genetically engineered primatised chimeric macaque-human anti-CD23 monoclonal antibody with a macaque variable region and human IgG1 constant region. Phase I clinical trials demonstrated that this mAb has an excellent safety profile but minimal single-agent activity in patients with CLL.[19] Since preclinical studies have shown that lumiliximab enhances the antitumour effect of fludarabine or rituximab, a phase I/II trial of lumiliximab in combination with fludarabine, cyclophosphamide and rituximab (FCR) for patients with recurring CLL was conducted. In this study, an ORR of 72% (52% CR, 10% PR, and 10% unconfirmed PR) was observed. When these outcomes were compared with published results using FCR, it seems that the addition of lumiliximab to FCR was better than FCR alone.[20] A phase III clinical trial comparing lumiliximab plus FCR *vs* FCR alone is ongoing.

ANTI-CD80 ANTIBODY

CD80 is a membrane-bound immune-costimulatory molecule involved in regulating T-cell activation. It is a member of the B7 family of costimulatory molecules.[21,22] CD80 is transiently expressed on the surface of activated B cells, dendritic cells and T cells in healthy individuals.[23] In contrast, a variety of lymphoid malignancies, including FL and Hodgkin's lymphoma (HL), constitutively express CD80, making it a suitable target for therapy.[24-28] In preclinical studies anti-CD80 antibodies demonstrated inhibition of lymphoma cell proliferation and induced ADCC.[29]

Galiximab (Biogen Idec) is a primatised anti-CD80 (IgG1λ) mAb with human constant regions and primate (cynomologous macaque) variable regions.[30] A multicentre

phase I dose-escalating study examined the single-agent activity of galiximab in patients with relapsed or refractory FL receiving four weekly intravenous infusions of galiximab at doses of 125, 250, 375 or 500 mg/m^2. Safety was established with no major adverse effects observed, and no patients were noted to have developed anti-galiximab antibodies. Tumour measurements decreased in 49% of the patients (with two CRs and two PRs) with an objective ORR of 11%.[31] Some responses were delayed, and one patient achieved a CR one year after starting therapy. Unlike rituximab, which has a relatively short half-life, the galiximab half-life is long and similar to that of epratuzumab, ranging from two to four weeks. The delayed antitumour responses cannot be easily explained as being secondary to a direct passive antibody effect, raising the possibility that galiximab may induce a 'unique' immune response. Another interesting observation was that the response did not correlate with the degree of CD80 expression; the patients who responded to treatment did not have higher levels of expression of CD80 than the non-responders. Preclinical data suggest synergy between rituximab and galiximab: a phase I/II study of galiximab together with rituximab was performed. The results showed that the combination therapy was superior to single agents.[32] In a recent study of the combination of galiximab and rituximab as initial therapy for FL, the Cancer and Leukaemia Group B (CALGB) reported a response in 69% of the study participants with 41% complete remissions.[33]

ANTI-CD52 ANTIBODY

The CD52 antigen is expressed on normal and malignant B and T lymphocytes, monocytes and natural killer (NK) cells. Alemtuzumab (Bayer HealthCare Pharmaceuticals), also known as CAMPATH, is a humanised monoclonal antibody against CD52. It was approved as a treatment for CLL previously treated with alkylating agents and refractory to fludarabine showing an ORR of 56%.[34]

Alemtuzumab has also been evaluated for the management of advanced-stage mycosis fungoides/Sezary syndrome (MF/SS). In a phase II trial, intravenous alemtuzumab, 30 mg three times a week for up to 12 weeks, showed an ORR of 55%, with a median time to treatment failure (TTF) of 12 months; 32% of patients showed a CR and 23% a PR. Sezary cells were cleared from the blood in 86% of the patients.[35]

To assess the effectiveness of alemtuzumab in the treatment of relapsed or refractory peripheral T-cell lymphomas (PTCL), a total of 14 patients were treated with alemtuzumab intravenously. The ORR was 36% and three patients achieved a CR, with the durations of the CR ranging from two to 12 months.[36] Frontline treatment with CHOP plus alemtuzumab (CHOP-C) as an effective option for patients with PTCL has been investigated. A total of 20 patients were treated with CHOP, preceded on day -1 with alemtuzumab 30 mg 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 suggesting that CHOP-C appears to be a feasible option for PTCL.[37]

The major adverse effect of alemtuzumab is an increased risk of infections, largely a consequence of a dramatic decrease in CD4+ and CD8+ lymphocytes during treatment and lasting up to nine months or more after completion of therapy. This risk is further increased in patients who have previously received purine analogues with their associated myelosuppression and lymphopenia. The spectrum of infections extends from bacterial infections, atypical infections including cytomegalovirus (CMV) or herpes simplex virus reactivation, *Pneumocystis (carinii) jeroveci* pneumonia, and aspergillosis. Thus, prophylaxis is recommended with agents such as co-trimoxazole (trimethoprim/sulphamethoxazole), valaciclovir, and fluconazole.

In an attempt to maintain effectiveness while decreasing toxicity, a reduced dosage of alemtuzumab was examined in a phase II study. Alemtuzumab 10 mg intravenously three times a week for four weeks was administered to patients with relapsed/refractory T-cell lymphoma. Results from ten patients receiving this protocol have demonstrated an OR of 60% with CMV reactivation in one patient.[38] Altering the route of administration has been examined as a strategy to reduce the risk of acute infusion reactions while maintaining effectiveness. Ther are data on subcutaneous administration of alemtuzamab, using a dose-escalation scheme of 3 mg, 10 mg, 30 mg for the first week, and then 30 mg subcutaneously three times a week for up to 12 weeks. Twenty patients were given this regimen (13 patients with CLL, one with CLL/acute myeloid leukaemia (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%.[39]

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.

CD2 ANTIBODIES

CD2 is a transmembrane glycoprotein with a dual role as an adhesion molecule and a costimulatory molecule via its actions with its ligand CD58. It is important in both T-cell and NK-cell functions. CD2 antigen is thus a potential

target for the treatment of T-cell lymphoma.[40] Siplizumab (MEDI-507) is a humanised IgG1κ monoclonal antibody against CD2 antigen. Preclinical studies demonstrated that siplizumab induces ADCC.[41] This antibody was being evaluated in a phase I study in patients with adult T-cell leukaemia and peripheral T-cell lymphoma. However, recently an increased incidence of Epstein-Baar virus (EBV)-induced B-cell lymphoproliferative disease (LPD) in patients treated with siplizumab has been reported. Although initial responses were encouraging, four (13.7%) patients developed EBV-LPD and the trial was stopped. In those patients developing EBV-LPD, a significantly greater reduction in NK cell number and CD2 expression on T cells was seen.[42]

CD4 ANTIBODIES

Zanolimumab (Genmab) is an anti-CD4 human monoclonal IgG1κ antibody (also known as HuMax-CD4). The CD4 receptor is expressed on most T lymphocytes and to a lesser degree on macrophages. It is also highly expressed on malignant T-cell lymphoma cells. This antibody interferes with interaction between the CD4 receptor and the major histocompatibility complex (MHC) class II molecule preventing T-cell activation. Currently, it is under investigation for the treatment of $CD4^+$ malignancies, mainly CTCL in early and advanced stages and other noncutaneous PTCL. *In vitro* studies demonstrated that zanolimumab depleted CD4+ T cells via ADCC.[43] In a phase II trial in refractory CTCL a safe toxicity profile and a favourable response of 40% were observed.[44] Another phase II trial of HuMax-CD4 in noncutaneous PTCL presented by D'Amore *et al.*[45] demonstrated an ORR of 62.5% in the first eight patients enrolled in the trial with only one case of febrile neutropenia. In two open-label phase II clinical trials evaluating the efficacy of zanolimumab in early and late-stage CTCL, 38 patients with mycosis fungoides (MF) and nine patients with Sezary syndrome (SS) were treated with zanolimumab. Objective responses were seen in 15% patients with MF receiving low-dose zanolimumab (280 mg). An increased response rate of 56% was observed with high-dose treatment (560 mg or 980 mg). In this high-dose group, responses occurred early with 90% of responses already present within eight weeks of treatment initiation. These responses were durable with a median response duration of 81 weeks. Zanolimumab demonstrated a favourable safety profile with the most frequent AEs being inflammatory skin reactions and low-grade infections.[46] Based on these results a blinded, randomised phase III trial comparing two different dosings of zanolimumab (8 mg/m^2 *vs* 14 mg/m^2) in previously treated MF was initiated.[47]

TNF RECEPTOR FAMILY

The tumour necrosis factor (TNF) family of proteins is implicated in the regulation of essential cell processes such as survival, proliferation, differentiation and cell death. Altered expression of TNF family members is often associated with pathological conditions such as autoimmune disease and cancer.[48]

CD30 ANTIBODIES

CD30 is a member of the TNF family of proteins. Its expression is restricted in normal healthy individuals to a small number of activated B and T lymphocytes. CD30 is expressed on Reed-Stemberg cells of classical Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL).[49] Patients with high-risk, relapsed or refractory HL, systemic anaplastic large-cell lymphoma, and primary cutaneous CD30-positive disorders have CD30 as a common marker, which can serve as a therapeutic target.

SGN-30 (Seattle Genetics, Inc.) is a chimeric antibody against CD30. There are *in vivo* and *in vitro* data that SGN-30 may be synergistic with chemotherapy.[50] A phase I study confirmed the safety and tolerability of SGN-30.[51] Preliminary results of phase II studies of SGN-30 and MDX-060 confirm some efficacy of these anti-CD30 monoclonal antibodies in HL and ALCL. Encouraging results were seen in patients with relapsed or refractory systemic anaplastic large cell lymphoma with objective responses in both systemic and cutaneous variants of the disease.[52-54]

MDX-060 (Medarex, Inc.) is a human anti-CD30 IgG1κ monoclonal antibody that inhibits growth of CD30-expressing tumour cells in preclinical models. Phase I and II studies were performed to determine the safety and efficacy of MDX-060 in patients with relapsed or refractory CD30+ lymphomas.[55] MDX-060 was well tolerated at doses up to 15 mg/kg, and a maximum tolerated dose was not identified. Only 7% of patients experienced grade 3 or 4 treatment-related adverse events. Among the 72 patients treated, clinical responses were observed in six. Twenty-five patients had stable disease, including five who remained free-from-progression one year after treatment, although MDX-060 demonstrated limited activity as a single agent. The minimal toxicity observed and the significant proportion of patients with prolonged stable disease suggest that further study of MDX-060 in combination with other therapies is warranted.

MDX-1401 is a nonfucosylated fully human monoclonal antibody that binds to human CD30; it was compared with MDX-060 *in vitro* and *in vivo*.[56] MDX-1401 greatly improved ADCC activity as evidenced by a decrease in half-maximal effective concentration (EC50) and an increase in

maximum cell lysis when compared with MDX-060. Increased ADCC activity was observed among a panel of cell lines, including one with very low CD30 antigen expression in which parental antibody failed to induce any detectable ADCC. Thus, the low doses of antibody required for ADCC activity irrespective of donor genotype, the ability to mediate ADCC in target cells expressing low levels of CD30, and increased *in vivo* efficacy support the development of MDX-1401 for treatment of malignant lymphoma. Preliminary data from an ongoing phase I clinical trial of MDX-1401 in patients with relapsed or refractory HL was presented at the recent Annual Meeting of the American Association for Cancer.[57]

XmAb™2513 is a novel humanised monoclonal antibody that binds to CD30 and demonstrates anti-proliferative activity against CD30-positive (CD30+) cell lines. XmAb2513 also has an engineered Fc region to enhance cell killing activity via recruitment of effector cells through increased binding affinity to Fcγ receptors. Consequently, XmAb2513 exhibits superior antibody-dependent cell mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP), when compared with a native IgG1 (unengineered) version of the antibody.[58] Xencor, Inc., a company developing protein and antibody therapeutics, has initiated a phase I clinical trial with its lead product candidate XmAb™2513 in patients with HL and anaplastic large cell lymphoma (ALCL).

ANTI-CD40 ANTIBODIES

CD40 is also a member of the tumour necrosis factor receptor family. CD40 is expressed by normal B lymphocytes, monocytes and dendritic cells, as well as some epithelial and endothelial cells. B- and T-cell lymphomas, Hodgkin and Reed-Sternberg cells and several types of carcinomas also express CD40. CD40L (CD154) is predominantly expressed by activated T lymphocytes. The various biological functions of CD40L include priming dendritic cells to activate CD8-cytotoxic T cells, B-cell selection and survival, and switching of immunoglobulin isotype. However, CD40L is less frequently expressed by activated B lymphocytes, NK cells, monocytes, eosinophils, basophils, dendritic cells, platelets, and endothelial and smooth muscle cells. Soluble CD40L (sCD40L) can be detected in the serum of patients with lymphoma, CLL, essential thrombocythaemia and autoimmune diseases.[59] The role of sCD40L has not been 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.[60]

Two antibodies targeting CD40 (SGN-40 and HCD122 [Chirl2.12]) are currently being evaluated in clinical trials. SGN-40-Dacetuzumab (Seattle Genetics) is a humanised IgGl antihuman CD40 antibody. Preclinical data have shown SGN-40 to cause potent inhibition of proliferation, and induction of apoptosis and ADCC in high-grade B-cell lymphoma lines. Activity similar to that of rituximab was seen in xenograft CD40 tumour models treated with SGN-40.[61] Preliminary data from phase I trials in NHL show that disease was stabilised in one of six patients treated with doses of 2 mg/kg/week for four weeks, while another patient showed symptomatic improvement.[62] Another humanised IgGl anti-CD40 monoclonal antibody, HCD122 (Novartis), is also being tested and has *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.[63] HCD122 is currently being investigated in phase I trials for the treatment of B-cell CLL.

TRAIL RECEPTOR

Tumour necrosis factor-related apoptosis-inducing ligand or Apo2 ligand (TRAIL/Apo2L) is also a member of the tumour necrosis factor (TNF) superfamily of proteins that induces apoptosis upon binding to its death domain-containing transmembrane receptors: death receptors 4 and 5 (DR4, DR5). Importantly, TRAIL preferentially induces apoptosis in cancer cells while sparing normal cells.[64] This preferential killing is partly due to the differential expression of its receptors. Normal tissues do not usually express the death receptors TRAIL-Rl and TRAIL-R2 and, therefore, are protected from TRAIL-induced apoptosis. In contrast, most tumours express TRAIL-Rl and TRAIL-R2, making them more sensitive to TRAIL-induced apoptosis. Agonistic monoclonal antibodies targeting TRAIL-death receptors (TRAIL-Rs) have been developed and are currently being used in clinical trials. Binding of these antibodies to TRAIL-R1 and TRAIL-R2 results in death-inducing signalling complex (DISC) formation and induction of apoptosis. These novel fully humanised compounds have been combined with conventional agents in the treatment of advanced solid malignancies, including different types of lymphoma.[65] Preclinical studies and clinical trials using TRAIL/Apo2L ligand or anti-TRAIL-Rl (mapatumumab [HGS-ETRl]) and -R2 (HGS-ETR2) fully human monoclonal antibodies are ongoing. Phase Ia studies indicate that mapatumumab is well tolerated, and the maximum tolerated dose (MTD) has yet to be reached.[66,67] A phase II study using mapatumumab as a single-agent in NHL has yielded 8% objective responses.[68] This monotherapy was well tolerated in the phase II setting, with a single drug-related serious adverse event (i.e. vomiting) reported. Similar results have been seen with single-agent lexatumumab,

with several patients experiencing stable disease in a phase Ia study, although no objective tumour responses have been observed to date.[69,70]

Interim results from a phase Ib study of rhApo2L/TRAIL ligand plus rituximab in patients with low-grade NHL who had previously failed rituximab-containing therapy have shown the combination to be well tolerated and active, with two (25%) complete responses, one (13%) PR, and five (63%) SDs achieved.[71]

HLA CLASS II ANTIGENS

HLA class II antigens are expressed on B cells throughout differentiation and play a key role in cell cycling and proliferation. Anti-class II antibodies inhibit B-cell proliferation and induce apoptosis, in part through induction of the Fas–Fas ligand pathway or activation of Akt. Apolizumab (Hu1D10) is a humanised anti-HLADR antibody capable of inducing complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and programmed cell death. Its limited clinical activity and unexpected increased risk for clot development suspended its further development.[72] Other anti-HLA antibodies are currently in development. CD74 is an integral membrane protein that functions as a major histocompatibility complex class II chaperone. It has recently also been shown to have a role as an accessory-signalling molecule and has been implicated in malignant B-cell proliferation and survival. These biological functions combined with expression of CD74 on malignant B cells and limited expression on normal tissues implicate CD74 as a potential therapeutic target. The anti-CD74 monoclonal antibody LL1 has been humanised (hLL1: milatuzumab or IMMU-115) and can provide the basis for novel therapeutic approaches to B-cell malignancies, particularly because this antibody shows rapid internalisation into CD74+ malignant cells. Studies show that unconjugated hLL1 and conjugates of hLL1 constructs with radioisotopes, doxorubicin, and frog RNase have high antitumour activity. CD74 is a new candidate target for the immunotherapy of neoplasms expressing this antigen, which can be exploited using either a naked antibody or conjugating it to isotopes, drugs, or toxins.[73] Phase I trials of milatuzumab are now underway in human subjects with lymphoma and multiple myeloma.

TARGETING ANGIOGENESIS

Tumour-induced angiogenesis is necessary for their growth and metastasis. Of the different inducers of tumour-induced angiogenesis, vascular endothelial growth factor A (VEGF) plays the most important role.[74] Increased VEGF expression has been found in many tumour types, including NHL.[75-77] Elevated levels of VEGF correlate with a worse overall survival and increased aggressiveness in patients with NHL.[77-79] Bevacizumab is a humanised monoclonal antibody that recognises all known isoforms of VEGF.[80] Southwest Oncology Group (SWOG) initiated the phase II study, S0108, to test biweekly intravenous bevacizumab as single agent therapy for patients with relapsed, aggressive NHL.[81] Bevacizumab as a single agent was not effective therapy in this population. Results from animal models and clinical trials suggest this agent is best utilised when combined with other effective antitumour therapies.[82,83] A clinical trial testing standard CHOP-rituximab (R-CHOP) therapy with bevacizumab in 13 patients with DLBCL has been published documenting the tolerability and feasibility of this treatment.[84] SWOG has recently completed S0515, a phase II trial of R-CHOP plus bevacizumab in 70 patients with untreated, advanced DLBCL. A phase III international trial is also currently enrolling patients to R-CHOP with or without bevacizumab in *de novo* patients with DLBCL. These trials will determine the ultimate role of this agent in aggressive NHL.

RADIOIMMUNOTHERAPY

Radioimmunotherapy with radiolabelled mAbs is an emerging and promising treatment option for non-Hodgkin's lymphoma and has lately gained momentum due to results from recent trials. In his original work, DeNardo *et al.* provided an early example of this concept, targeting HLA antigens in aggressive non-Hodgkin's lymphoma with radiolabelled monoclonal antibodies against Lym-1 showing some complete responses.[85] Convincing data supporting the benefits of radiolabelled consolidation immunotherapy are available in FL. Other lymphomas, such as relapsed diffuse large B-cell lymphoma,[86-90] mantle-cell lymphoma,[91-94] HL[95] and marginal zone lymphoma[96] have also been evaluated with positive results.

At present, two radioimmunoconjugates that target CD20 are approved for use in patients with relapsed or refractory follicular or low-grade lymphoma: yttrium-90 (90Y) labelled ibritumomab tiuxetan (Zevalin, Cell Therapeutics) and iodine-131 (131)-labelled tositumomab (Bexxar, GlaxoSmithKline). Other radiolabelled immunotherapies currently being evaluated in B-cell NHL, include: LL2 anti-CD22, conjugated to either 131I or 90Y; Lym-1 HLA-DR, conjugated to 90Y or 67Cu; rituximab anti-CD20, conjugated to 211At, 186Re, or 227^{Th}; or B4 anti-CD19, conjugated to 90Y.[97-106] Witzig *et al.*[107] demonstrated that radioimmunotherapy with 90Y-ibritumomab tiuxetan produces statistically significantly higher overall response rates (80 *vs* 56%, p=0.002) and complete response rates (30

vs 16%, p=0.04) compared with standard immunotherapy using rituximab. Myelosuppression was the primary toxicity noted which was reversible. In a recent meta-analysis[108] of patients with recurrent/refractory NHL treated with 90Y-ibritumomab tiuxetan, long-term response, defined as time to treatment progression of 12 months or longer, was seen in 37% of patients. At a median follow-up of 53.5 months the median duration of response was 28.1 months and the median TTP was 29.3 months. One-third of these patients had been treated with three or more earlier therapies, and 37% had not responded to their last therapy. An interesting finding was that a single dose of 90Y-ibritumomab tiuxetan yielded durable responses and prolonged overall survival in a substantial number of patients not responding to earlier therapies.

The use of 90Y-labelled ibritumomab tiuxetan is being evaluated in aggressive lymphomas. It induced high response rates in relapsed DLBCL and in patients refractory to CHOP chemotherapy; interestingly lower responses were observed after failure of R-CHOP than after failure of CHOP alone.[109] In a recent phase II study of CHOP chemotherapy followed by 90Y-ibritumomab tiuxetan as frontline therapy in patients with DLBCL[110] ORR to 90Y-ibritumomab tiuxetan was 100%, including 95% CR and 5% partial remission, and four of the five patients who achieved less than a CR with CHOP improved their remission status after 90Y-ibritumomab tiuxetan therapy. In another study, consolidation of frontline chemotherapy with 90Y-labelled ibritumomab tiuxetan improved the rate of complete remission and prolonged progression-free survival.[111] 90Y-labelled ibritumomab is also being evaluated prior to transplantation in NHL patients.[112-114]

131I-labelled tositumomab is a conjugate of the murine anti-CD20 antibody tositumomab and iodine-131. In one study, two thirds of patients with chemotherapy-refractory low-grade non-Hodgkin's lymphoma had a response with 20% complete remissions.[115] Among patients with rituximab-refractory lymphoma the rate of response to 131I-labelled tositumomab was 63% with 29% having complete remissions.[116] In another study, 95% of patients with newly diagnosed non-Hodgkin's lymphoma had responses to 131I-labelled tositumomab used as frontline treatment, including 75% complete remissions.[117] This treatment has also been administered as consolidation after chemotherapy, resulting in durable responses with conversion of partial remission to complete remissions.[118]

Radioimmunotherapy options for T-cell lymphomas (T-NHL) are limited. Anti-CD45-RIT is being evaluated in human and murine T-NHL. CD45 was shown to be highly expressed on T-NHL patient samples. This high CD45 expression of T-NHL may allow reliable tumour targeting and disease control supporting anti-CD45 RIT for T-NHL patients.[119]

CONCLUSIONS

Therapeutic monoclonal antibodies have provided significant benefit for patients with NHL. Virtually all patients with B-cell lymphoma receive rituximab at variable times over their treatment course. Radiolabelled antibodies may be effective in rituximab-resistant and chemotherapy-resistant disease, but their clinical use is much more limited today compared with the use of unlabelled mAbs. While significant efforts continue in this area, the logistics, haematological toxicity and other factors have limited the use of concurrent chemotherapy plus radioimmunotherapy; however, recent data suggest that sequential radioimmunoherapy following chemotherapy may have significant clinical value.[111] Novel anti-CD20 agents offer the potential for enhanced activity relative to that of rituximab, while agents directed against unique non-CD20 targets offer the possibility of combining mAbs against CD20 and other antigens concurrently. However, many challenges exist in the clarification of the optimal use of such novel agents. Whether new anti-CD20s are better than rituximab requires randomised comparative trials or definitive demonstration of improved effectiveness in rituximab-refractory patients. The promise of antibody-based therapeutics in lymphoma has already been demonstrated and suggests that the further development of such agents offers the potential for increasing clinical benefit for NHL patients and improving outcomes with less toxicity than that associated with historical therapy.

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