Investigational Immunotherapeutics for B-Cell Malignancies

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A B S T R A C T

The use of rituximab-based chemoimmunotherapy regimens has remarkably improved the response rates, long-term outcomes, and quality of life of patients with B-cell malignancies. However, a substantial number of patients exhibit either primary or acquired resistance to rituximab, which suggests that novel immunotherapeutics with distinct mechanisms of action are necessary. A series of monoclonal antibodies with specificity against different surface antigens expressed on malignant B cells (eg, CD22, CD23, CD40, CD70) and novel immunotherapeutics (eg, bispecific monoclonal antibodies, small-modular immunopharmaceuticals, T-cell engagers) are currently in clinical or final preclinical stages of development. Although these agents offer reason for optimism, considerable challenges lie ahead in establishing their real clinical value, as well as in integrating them into current therapeutic algorithms for patients with B-cell malignancies. This review describes some of the most promising investigational immunotherapeutics for the treatment of B-cell malignancies.

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INTRODUCTION

Monoclonal antibodies (mAbs) have dramatically changed the management of patients with non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Since the approval of the human-murine immunoglobulin (Ig) G1 anti-CD20 mAb rituximab, multiple studies have evaluated the activity of this and other mAbs, either alone or in combination with chemotherapeutic backbones, for the treatment of B-cell malignancies. On binding to CD20, rituximab induces antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and apoptosis of CLL cells¹⁻³ and sensitizes malignant B cells to chemotherapy.⁴ The addition of rituximab to chemotherapeutic regimens (ie, cyclophosphamide, vincristine, and prednisone; cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]; fludarabine, mitoxantrone, and dexamethasone) produced a remarkable increase in overall response rates (ORR) and complete remission (CR) rates, as well as delay of time to progression (TTP).⁵ The addition of rituximab to CHOP chemotherapy (R-CHOP) improved CR rates and prolonged 5-year overall survival (OS) rates by more than 10% in patients with diffuse large B-cell lymphoma (DLBCL).⁶⁻⁹

Although single-agent rituximab renders modest results in CLL,¹⁰⁻¹⁴ likely owing to the low CD20 expression on CLL cells,¹⁵ combination chemoim-

muno-therapy with rituximab, fludarabine, and cyclophosphamide (FCR) results in higher ORR (95% v 88%), CR rates (52% v 27%), and improved progression-free survival (PFS; 76.6% v 62.3%) compared with fludarabine and cyclophosphamide therapy.¹⁶

Although targeting surface antigens with mAbs provides an efficacious option for the management of B-cell malignancies, it is clear that current immune approaches have limitations and that clinical outcomes can still be significantly improved.

ARE NOVEL AGENTS REALLY NEEDED IN B-CELL NHL AND CLL?

On re-treatment with a relapse therapy, most patients with B-cell malignancies may be considered for allogeneic transplantation. However, suitable donors are not always available, and transplantation-related complications remain a concern, which underscores the importance of developing novel chemoimmunotherapeutic regimens to improve CR rates, as well as TTP and OS in previously untreated patients. Approximately 50% of patients with relapsed/refractory CD20⁺ follicular lymphoma (FL) fail to respond to initial rituximab therapy,¹⁷ and nearly 60% of those with an initial response eventually become rituximab resistant.¹⁸ In addition, some patients exposed to rituximab-based chemoimmunotherapy fail to respond or experience relapse within 6 months, also suggesting

resistance to therapy. Furthermore, disorders such as CLL/small-cell lymphoma (SLL) are less responsive than other NHLs to standard-dose single-agent rituximab.

The success of rituximab in the treatment of B-cell malignancies, but also its recognized limitations, has spurred investigational efforts to engineer mAbs that target different surface antigens expressed on malignant B cells. One example of the latter is the development of alemtuzumab, an anti-CD52 mAb, for the treatment of CLL. In the international phase III randomized CAM307 trial, alemtuzumab rendered higher ORRs (83% *v* 55%), CR rates (22% *v* 2%), minimal residual disease (MRD) eradication (31% *v* 0%), and longer time to alternative treatment (23.3 *v* 14.7 months) and PFS compared with chlorambucil in patients with previously untreated CLL.¹⁹

Several mechanisms of resistance may prevent some patients from responding to therapy. For instance, the presence of membrane-complement regulatory proteins such as CD55 and/or CD59 on CLL cells may potentially impair complement activation and reduce the formation of the membrane attack complex, thus protecting the tumor cell from antibody-mediated CDC.^{1,2,15} However, a clear correlation between the expression of CD55 and CD59 with resistance to rituximab-induced cell killing and clinical response has not been consistently established.¹ In addition, pharmacokinetic factors, down-regulation or modification of target surface antigens, or limited proapoptotic activity may also play a role in the resistance to currently available mAbs.²⁰⁻²² Therefore, novel immunotherapeutics with different mechanism of action are required to improve the current therapies for B-cell malignancies.

INVESTIGATIONAL mAbs IN CLINICAL DEVELOPMENT

The success of rituximab therapy has validated CD20 as a therapeutic target in CLL and B-cell NHL and mAbs as effective therapies. A series of novel mAbs with specificity against a variety of antigens on the surface of B cells are being evaluated (Table 1). Among

the latter, ofatumumab (anti-CD20) and lumilixumab (anti-CD23) have reached advanced stages of clinical development.

Ofatumumab

Ofatumumab (HuMax CD20) is a fully humanized monoclonal IgG1 that targets a unique epitope located in the smaller extracellular loop of CD20 closer to the cell membrane and different from that bound by rituximab.²³ Ofatumumab binds CD20 with increased stability and affinity, thus enhancing its capacity to induce CDC. The fact that the binding of ofatumumab to CD20 occurs in close proximity to the cell membrane ensures that complement fixation occurs closer to the site of action and further away from C1-inhibitor.²³ As a consequence, ofatumumab is effective at inducing CDC of rituximab-resistant cells and of malignant B cells with low CD20 expression.²⁴ Ofatumumab induces faster killing than rituximab of ARH77 cells and B cells obtained from patients with CLL.²⁵ In monkeys, B-cell repopulation was only detectable when the plasma levels of ofatumumab decreased to less than 10 $\mu\text{g/mL}$.²⁶ On CD20 saturation, ofatumumab levels of 5 to 10 $\mu\text{g/mL}$ are sufficient to exert a sustained *in vivo* biologic activity.²⁶

Phase I/II clinical data in 40 patients with relapsed/refractory FL using intravenous (IV) ofatumumab 300 to 1,000 mg weekly for 4 weeks resulted in an ORR of 63% without dose-limiting toxicity.²⁷ A pivotal trial that was recently completed evaluated the activity of ofatumumab in patients with FL refractory to rituximab. Patients received an initial dose of 300 mg followed by 7 weekly infusions of either 500 or 1,000 mg. Ofatumumab (NCT00394836) is currently being evaluated as a single agent in patients with DLBCL (NCT00622388) and Waldenström macroglobulinemia (NCT00811733), as well as in combination with chemotherapy in DLBCL (NCT00823719) and CHOP chemotherapy in patients with FL (NCT00494780). In a recently completed multicenter phase I/II study, 33 patients with relapsed/refractory CLL received four weekly infusions of ofatumumab at different dose schedules: three patients

Table 1. Investigational Immunotherapeutics Currently Being Evaluated for the Treatment of B-Cell Malignancies

Agent	Type of Therapeutic	Description	Antigen	Development Status
Lumiliximab	mAb	Chimeric	CD23	Clinical trials
Ofatumumab	mAb	Humanized	CD20	Clinical trials
AME-133	mAb	Humanized	CD20	Clinical trials
GA101	mAb	Humanized	CD20	Preclinical
Veltuzumab	mAb	Humanized	CD20	Clinical trials
Epratuzumab	mAb	Humanized	CD22	Clinical trials
Apolizumab	mAb	Humanized	HLA-DR β	Clinical trials
HCD122	mAb	Humanized	CD40	Clinical trials
SGN-40	mAb	Humanized	CD40	Clinical trials
MDX-1411	mAb	Humanized	CD70	Clinical trials
Milatumumab	mAb	Humanized	CD74	Clinical trials
Galiximab	mAb	Chimeric	CD80	Clinical trials
Anti-CD20/22 bsAbs	Bispecific mAb	Humanized	CD20 and CD22	Preclinical
Bi20	Bispecific mAb	Humanized	CD20 and CD3	Clinical trials
4G7XH22	Bispecific mAb	Humanized	CD19 and CD30	Clinical trials
TRU-016	SMIP	Humanized	CD37	Clinical trials
TRU-015	SMIP	Humanized	CD20	Clinical trials
Blinatumomab	BiTE	Humanized	CD19 and CD3	Clinical trials

Abbreviations: mAb, monoclonal antibody; SMIP, small-modular immunopharmaceutical; BiTE, bispecific T-cell engager.

received one dose of 100 mg and three doses of 500 mg; three patients received one dose of 300 mg and three of 1,000 mg; and 27 patients received one dose of 500 mg and three doses of 2,000 mg.²⁸ The maximum-tolerated dose (MTD) was not determined. Infection was reported in 51% of patients, but was grade 1 to 2 in 88% of cases. TTP and time to next therapy were 161 and 366 days, respectively, which correlated with the area under the curve of ofatumumab.^{28,29}

Two important clinical trials to define the activity of ofatumumab in CLL are currently ongoing or completed. One of them is the international, phase III pivotal trial for patients with CLL refractory to fludarabine and alemtuzumab (RFA) or with bulky (> 5 cm) fludarabine-refractory (BFR) disease.³⁰ A planned interim analysis on 138 patients (59 with RFA and 79 with BFR), of whom 63% had Rai stage III/IV CLL, has been reported. The main end point was ORR over 24 weeks. Patients received eight weekly infusions of ofatumumab followed by 4 monthly infusions (dose 1, 300 mg; doses 2 through 12, 2,000 mg).³⁰ The ORR was 58% in the RFA group and 47% in the BFR group (one patient achieved CR). The median OS was 13.7 months for the RFA group and 15.4 months for the BFR group. Complete resolution of "B" symptoms, lymphadenopathy, or splenomegaly occurred in 48% and 63%, 16% and 11%, and 47% and 35% of patients in the RFA and BFR groups, respectively.³⁰ The Oncologic Drug Advisory Committee (ODAC) recently recommended approval of ofatumumab for refractory CLL. A decision by the US Food and Drug Administration is expected by the end of 2009. The second trial is a phase II study in which patients with previously untreated CLL received six monthly infusions of ofatumumab in combination with fludarabine and cyclophosphamide. Patients were randomly assigned to two different dose schedules of ofatumumab: (1) first infusion of 300 mg followed by five infusions of 500 mg, or (2) first infusion of 300 mg followed by five infusions of 1,000 mg. A phase III multicenter trial of ofatumumab combined with chlorambucil versus single-agent chlorambucil in patients with untreated CLL is underway.

Lumiliximab

Lumiliximab (IDEC-152) is a chimeric mAb with human IgG1 constant regions and macaque variable regions that targets CD23. Although lumiliximab binds complement and mediates ADCC, its main cell-killing mechanism in primary CLL cells and CD23-expressing B-cell lines seems to be through induction of apoptosis via downregulation of the antiapoptotic proteins Bcl-2, Bcl-x_L, and XIAP, activation of Bax, and release of cytochrome *c* from the mitochondria.³¹ Lumiliximab synergizes with fludarabine and rituximab both in vitro and in a human disseminated CD23⁺ B-cell lymphoma severe combined immunodeficiency (SCID) mouse model.^{31,32}

In a phase I study, 46 patients with heavily pretreated CLL were sequentially assigned to receive lumiliximab at 125, 250, or 375 mg/m² weekly for 4 weeks; 500 mg/m² weekly for 4 weeks; 500 mg/m² three times during week 1 then 500 mg/m² weekly for the next 3 weeks; or 500 mg/m² three times weekly for 4 weeks, respectively.³³ Grade 3 to 4 toxicity occurred in 15% of patients and was unrelated to lumiliximab dose. The MTD was not established. Lumiliximab exhibited a median terminal half-life of 7 days. Lumiliximab accumulated at doses greater than 250 mg/m², being maintained for more than 7 days on completion of therapy at doses greater than 375 mg/m². Lumiliximab decreased peripheral-blood lymphocyte counts in 91% and reduced lymph node size in 52% of patients.

In a phase I/II study, lumiliximab was given either at 375 mg/m² (n = 3) or 500 mg/m² (n = 28) on day 1 in combination with a 28-day cycle of FCR to 31 patients with relapsed CLL.³⁴ The ORR was 71% (CR, 52%). This compares favorably with historical controls receiving FCR alone (ORR, 73%; CR, 25%).³⁵ Grade 3 to 4 toxicity was similar to that observed with FCR alone.³⁴ An international randomized phase III study comparing the FCR regimen with and without lumiliximab is underway.

INVESTIGATIONAL mAbs IN EARLY PHASES OF DEVELOPMENT

Anti-CD20 mAbs

The high activity shown by anti-CD20–based chemoimmunotherapy has fueled the development of several mAbs that target different epitopes of the CD20 surface antigen with high specificity.

AME-133. A critical aspect of ADCC effectiveness of anti-CD20 mAbs is their ability to engage with and activate effector cells (ie, natural-killer [NK] cells, macrophages) via their IgG Fc receptors (FcγRs).³⁶ AME-133 is an IgG1 with high specificity for CD20 whose Fc region binds CD16 (FcγRIIIa) with high affinity, which makes this mAb more effective at activating NK cells.³⁷ As a consequence, AME-133 is 10-fold more potent than rituximab at inducing ADCC while inducing comparable CDC. Results from a recently finished multicenter phase I/II study of AME-133 for patients with CD20⁺ relapsed/refractory FL should define the clinical potential of this agent.

GA101. GA101 is a mAb designed to rationally increase anti-CD20–induced ADCC activity. Most therapeutic mAbs are produced in Chinese hamster ovary cells, which express high levels of α-1-6 fucosyltransferase, resulting in an antibody with a rich content in fucose. The removal of fucose significantly increases Fc-CD16 binding, which results in 100-fold enhanced ADCC.^{38,39} GA101 is a humanized non–complement-fixing anti-CD20 antibody with poorly fucosylated Fc regions with high affinity for FcγRIII, which results in enhanced ADCC. GA101 induces strong caspase-independent apoptosis on CD20 binding on target cells,⁴⁰ which, in NHL xenograft models, resulted in higher complete tumor remission rates and significantly improved long-term survival compared with rituximab.^{40,41} In a phase I/II study, 24 patients with CD20⁺ B-cell malignancies received IV GA101 at doses from 50 mg to 2,000 mg on days 1, 8, and 22 and subsequently every 3 weeks for a total of nine infusions.⁴² Twelve patients (nine with FL, one with DLBCL, one with CLL, and one with Waldenström macroglobulinemia) treated at doses ranging from 50 mg to 800 mg were evaluable. No dose-limiting toxicities were observed. The most common toxicity was grade 1 to 2 infusion-related reactions. Seven (58%) of 12 patients responded, including three CRs and four PRs.⁴² Dose escalation is ongoing.

Veltuzumab. Veltuzumab (hA20) is a humanized monoclonal IgG1κ constructed recombinantly on the framework regions of the anti-CD22 mAb epratuzumab. Veltuzumab has identical variable kappa light chain (V_k) complementarity-determining regions (CDRs), identical CDR1-variable heavy chain (V_H) and CDR2-V_H, but different CDR3-V_H, compared with rituximab.⁴³ Compared with rituximab, veltuzumab binds CD20 with similar avidity, but significantly reduced off rates and enhanced CDC against several Burkitt's lymphoma cell lines⁴³ due to a single amino acid change (from Asn₁₀₁ to Asp₁₀₁) in CDR3-V_H.⁴³ CDC, ADCC, inhibition of proliferation, and induction of apoptosis have been demonstrated for veltuzumab in

vitro.⁴³ Veltuzumab was significantly more effective than rituximab in rodent and cynomolgus monkey lymphoma models.⁴³ In patients with B-cell NHL, veltuzumab has proven active and safe at lower doses than rituximab.⁴⁴ Interestingly, responses were not correlated with veltuzumab dose, which suggests that ADCC, which is less reliant than CDC on CD20 expression level,⁴⁵ may be the predominant mechanism of action of this mAb. Moreover, increased in vitro and in vivo antitumor activity of veltuzumab was noted when combined with epratuzumab.⁴⁶

In the phase I/II IM-T-hA20-01 trial, patients with refractory/recurrent FL (n = 55) or other B-cell NHL (n = 27) received weekly IV veltuzumab at 80 to 750 mg/m² for 4 weeks.⁴⁷ The ORR was 44% for patients with FL (CR, 27%; median duration, 19.7 months) and 35% for those with other NHL subtypes (CR, 27%).⁴⁷ Preliminary results from a phase I/II study (IM-T-hA20-08) suggest that weekly subcutaneous veltuzumab is safe and active in patients with NHL or CLL.⁴⁸

Monoclonal Antibodies With Other Specificities

Several mAbs targeting alternative B-cell surface antigens are currently being tested in clinical trials.

Epratuzumab. CD22 is a 135-kDa type I transmembrane sialoglycoprotein of the immunoglobulin superfamily developmentally expressed at low levels in the cytoplasm of pro-B and pre-B cells and at high levels on the surface of mature IgM+, IgD+ B cells.⁴⁹ CD22 participates in cell adhesion, regulation of B-cell survival, CD19 and B-cell antigen receptor (BCR) signal transduction, and BCR-induced cell death.^{50,51} Epratuzumab is an anti-CD22 humanized monoclonal IgG_{1(k)} that contains the original murine sequence only at the antigen-binding sites, comprising only 10% of the molecule. Epratuzumab is active both as a single agent⁵² and in combination with rituximab in patients with relapsed/refractory indolent B-cell NHL.⁵³ A weekly dose of 360 mg/m² was recommended for further development.⁵² Based on preclinical work suggesting that epratuzumab can enhance antitumor effects of anti-CD20 antibodies,⁴⁶ epratuzumab has been shown to be safe when administered in combination with rituximab in patients with B-cell NHL. However, the activity of epratuzumab in CLL has been modest, with most responses reported in patients with FL. Results from an international, multicenter trial evaluating the activity of the combination of epratuzumab and rituximab in 49 patients with relapsed/refractory FL (n = 41) or SLL (n = 7) have been recently reported.⁵⁴ Patients received IV epratuzumab 360 mg/m² followed by a 375 mg/m² IV dose of rituximab given weekly for 4 weeks. No patient discontinued therapy because of adverse events. The ORR was 54% in the FL group, including 24% with CR/unconfirmed CR, and 57% in the SLL group, including 43% with CR/unconfirmed CR. The median response duration was 13.4 months for patients with FL (29.1 months in those who achieved CR) and 20 months in those with SLL, thus confirming the tolerability and activity of this combination in patients with recurrent indolent NHL. Epratuzumab and rituximab in combination with standard R-CHOP have been recently reported to be safe in patients with previously untreated DLBCL. The combination produced an ORR of 95%; the CR/CR unconfirmed rate was 72%. Event-free survival (80%), PFS (82%), and OS (88%) rates at 12 months compared favorably with historical controls receiving R-CHOP, particularly in patients with intermediate/high-risk disease.⁵⁵

Apolizumab. Apolizumab (Hu1D10; Remitogen; Protein Design Labs, Fremont, CA) is a humanized IgG1 mAb that targets a

polymorphic epitope on the HLA DR β chain,⁵⁶ typically present on normal B cells and in 80% to 90% of CLL cases.⁵⁷ In vitro, apolizumab has been shown to induce ADCC, CDC, and apoptosis of B cells.⁵⁶ Despite an acceptable toxicity profile demonstrated in patients with previously treated CLL/SLL,⁵⁸ apolizumab affords modest benefit in the treatment of relapsed CLL.^{59,60} An increased incidence of hemolytic-uremic syndrome was observed among patients with B-cell malignancies treated with apolizumab and rituximab.⁶¹ Although this complication was solved by administering apolizumab on day 1 followed by rituximab on day 2,⁶¹ these results may prevent the development of apolizumab in B-cell malignancies.

Milatumuzumab. CD74 is a type-II transmembrane chaperone molecule that associates with HLA-DR, thus inhibiting binding of antigenic peptides to the class-II antigen presentation structure.⁶² In addition, CD74 has been shown to be directly involved in B-cell maturation and activates several signaling pathways such as SYK, PI3K, AKT, and NF- κ B^{63,64} and is expressed on B cells, monocytes, and histiocytes, as well as CLL and NHL cells.⁶⁵ Milatumuzumab (hLL1, IMMU-115), an anti-CD74 mAb, does not cause direct cytotoxicity in vitro; in the presence of an appropriate cross-linking agent, this antibody induces inhibition of cell proliferation and apoptosis but very modest ADCC or CDC.⁶⁶ In vivo, milatumuzumab therapy resulted in significant prolongation of survival in SCID mice using the human Burkitt's, Daudi, and Raji lymphoma cell lines.⁶⁷ Phase I trials of milatumuzumab in B-cell NHL and CLL are underway. Preliminary results suggest that milatumuzumab is safe in multiple myeloma at doses up to 8 mg/kg twice weekly for 4 weeks.⁶⁸

HCD122 and dacetuzumab (SGN-40). CD40, a member of the tumor necrosis factor receptor superfamily, is another surface glycoprotein highly expressed on the surface of malignant B cells.⁶⁹ Engagement of CD40 by its ligand (CD40L) promotes proliferation and antagonizes spontaneous and chemotherapy-induced apoptosis.^{70,71} HCD122 (formerly CHIR-12.12) is a fully-human anti-CD40 antagonist IgG₁ that blocks CD40/CD40L-mediated signaling, resulting in clearance of malignant B cells and enhanced ADCC activity as compared with that of rituximab.⁷² Ongoing phase I studies are evaluating the tolerability of HCD122 in NHL or Hodgkin's lymphoma, multiple myeloma, and relapsed CLL.

Dacetuzumab (SGN-40) is a humanized IgG1 mAb that, on binding to CD40, activates proapoptotic signal transduction pathways and mediates effector cell functions such as ADCC, but not CDC.⁷³ Dacetuzumab was administered at doses between 1 and 8 mg/kg/wk to patients with CLL after failure of fludarabine-containing therapy in a phase I/II study. Accrual to this trial has been recently completed, but results have not been yet been disclosed. A phase I study using 2 to 8 mg/kg/wk IV $\times$ 6 was completed in patients with relapsed NHL. Six (12%) of 50 patients achieved response, and at 2 years of completion of therapy, three responses were ongoing.⁷⁴ A phase II trial in patients with heavily pretreated DLBCL reported an ORR of 10%.⁷⁴ Ongoing studies are exploring the activity of dacetuzumab in combination with conventional therapies in multiple myeloma, DLBCL, and low-grade NHL.

MDX-1411. MDX-1411 is a fully-human mAb that targets with high specificity the CD70 receptor. CD70, also known as CD27 ligand, is a member of the tumor necrosis factor gene family and is normally expressed only on the surface of highly activated B cells, T cells, and dendritic cells.⁷⁵ CD70 is expressed on CLL cells and in other B-cell malignancies such as Burkitt's lymphoma, but rarely on normal B cells or T cells.⁷⁶ In vitro studies have shown MDX-1411 to induce ADCC.

An ongoing phase I study is evaluating the safety and highest tolerated dose of MDX-1411 in patients with relapsed/refractory CLL and mantle-cell lymphoma.

Galiximab. The presence of the costimulatory molecule CD80 on the surface of various B-cell malignancies makes it an attractive therapeutic target. Galiximab is a chimeric (human IgG1 constant regions with cynomolgus macaque variable regions) anti-CD80 mAb with excellent tolerability in relapsed/refractory FL.⁷⁷ Galiximab (500 mg/m² weekly for 4 weeks) given concurrently with a standard course of rituximab to patients with relapsed/refractory FL yielded an ORR of 64% (17% CR) with a median PFS of 12.2 months.⁷⁸ When given on an extended induction schedule (galiximab plus rituximab weekly $\times$ 4, then every 2 months $\times$ 4) to patients with previously untreated FL, this combination resulted in an ORR of 70% and PFS of 67%.⁷⁹ A randomized phase III trial comparing rituximab plus galiximab versus rituximab plus placebo in patients with relapsed/refractory FL is ongoing.

NOVEL CLASSES OF TARGETED IMMUNOTHERAPEUTICS

mAbs are large, complex molecules whose design requires significant molecular engineering to be effective. In addition, mAbs are very expensive therapeutics to develop and manufacture. The need for more cost-effective immunotherapeutics has led to the development of novel scaffold strategies, many of them based on the use of mAb fragments or single-domain antibodies. Some of the advantages of these novel approaches include (1) the generation of smaller molecules, which may allow them to bind cryptic epitopes more efficiently than full-size antibodies and to access tissues more efficiently; (2) the possibility of generating multispecific or multifunctional molecules; (3) the potential to recruit effector cells more efficiently; and (4) a lower manufacturing cost. The development of these agents has given rise to novel classes of immunotherapeutics, some of which have already shown promising clinical activity.

Bispecific mAbs

The use of bispecific mAbs affords a means to target autochthonous T cells to tumor cells.⁸⁰⁻⁸² Constructs with specificity against CD19 and CD3 demonstrated high antitumor activity against primary B-cell NHL as well as CLL cells.^{82,83} However, these agents have been largely ineffective in clinical trials.^{84,85} Recently, the new trifunctional antibody Bi20 (FBTA05, anti-CD20 $\times$ anti-CD3), which engages B cells and T cells through its variable regions and recruits Fc γ RI/RIII+ accessory immune cells via its Fc region, has proven more effective than rituximab in killing low CD20 antigen-expressing B cells derived from patients with CLL.⁸⁶ This suggests that Bi20 may have clinical

activity in patients with B-cell malignancies. In a pilot trial, six patients with recurrent B-cell malignancies after stem-cell transplantation received escalating doses of Bi20 followed by DLI or mobilized peripheral-blood stem-cell transplantation. All three patients with CLL had a prompt but transient response; one patient with a high-grade NHL had stable disease for 4 months. Side effects (fever, chills, bone pain) were tolerable and appeared at doses between 40 and 200 μ g.⁸⁷ An ongoing phase I/II clinical trial conducted in Germany is addressing the safety and activity of Bi20 in patients with CLL.

Given the central role acquired by anti-CD20 mAbs in the treatment of B-cell malignancies, and the promising activity shown by anti-CD22 mAbs such as epratuzumab in CLL, mAbs with dual specificity have been generated through recombinant engineering from veltuzumab (humanized anti-CD20) and epratuzumab (humanized anti-CD22).^{88,89} These bispecific mAbs have shown higher ADCC activity than the single parental mAbs, but not CDC.^{88,89} Therapy with anti-CD20/anti-CD22 bispecific mAbs prolonged the survival of SCID mice in a Burkitt's lymphoma model over that seen with parental anti-CD20 mAb.⁸⁸ The antitumor efficacy of this bispecific mAb was abolished when ADCC potential was diminished by depletion of neutrophils and NK cells, thus suggesting that ADCC plays a critical role in the killing of malignant cells in these murine models.⁹⁰ In light of this strong antitumor activity, the testing of this bispecific mAb in clinical trials is warranted. A phase I trial is currently evaluating the safety and tolerability of the bispecific antibody 4G7XH22 (targeting CD19 and CD30) in patients with relapsed/refractory NHL or CLL.

Small-Modular Immunopharmaceuticals

Small-modular immunopharmaceuticals (SMIPs) are single-chain polypeptides that consist of a target-binding domain attached to an effector domain through a flexible hinge domain, the latter allowing the association of multiple SMIP units, which can be crucial to biologic activity.⁹¹ Effector domains can be designed in a way that they govern the engagement of receptors found on immune cells for enhanced ADCC activity.⁹¹ A critical advantage of SMIPs is that typically their size is approximately two thirds that of mAbs while retaining full-binding capacity and effector function.⁹¹ CD37, a glycoprotein of the tetraspan transmembrane family of proteins, is strongly expressed on the surface of B cells and CLL cells.⁹² CD37, however, is minimally expressed on T cells⁹³ and is absent on NK cells, platelets, and erythrocytes.⁹⁴ A CD37-SMIP (TRU-016) has been developed using V_L and V_H from G28-1 hybridoma and engineered constant regions encoding human IgG₁ domains (Fig 1).⁹⁵ TRU-016 induced potent apoptosis and ADCC against B-cell leukemia/lymphoma cell lines and showed therapeutic activity in a SCID mouse xenograft leukemia/lymphoma model, which was enhanced in the presence of NK cells.⁹⁵ In a phase

Fig 1. Structure of the CD37–small-modular immunopharmaceutical (SMIP). The CD37-SMIP (G28-1 scFv-Ig) was generated using variable regions (V_L and V_H) from the G28-1 hybridoma and engineered constant regions encoding human IgG₁ domains (hinge, CH₂, and CH₃).⁹⁵ CD37 is commonly expressed in chronic lymphocytic leukemia and other mature B-cell malignancies. CD37-SMIP mediated apoptosis and antibody-dependent cellular cytotoxicity, but not complement-dependent cytotoxicity, against CD37⁺ B-cell lymphoma cell lines. IgG1, immunoglobulin G1.

I/II study in patients with relapsed CLL, 10 patients have been treated with IV TRU-016 at doses ranging from 0.03 mg/kg to 3.0 mg/kg given weekly for 4 weeks. No dose-limiting toxicity has been established. Beginning with the dose of 0.3 mg/kg, all eight patients demonstrated evidence of biologic activity, including patients with del17p.⁹⁶ TRU-015, a CD20-SMIP with more potent ADCC than rituximab, is also being evaluated in patients with B-cell NHL.

T-Cell Engaging Antibodies

Cytotoxic T cells are key in tumor growth control.⁹⁷ However, therapeutic induction of specific antitumoral T-cell responses, including vaccines and CTLA-4–blocking antibodies, has thus far rendered disappointing results. An alternative approach to harness the cytotoxic potential of T cells is the use of T-cell–engaging antibodies. Bispecific T-cell engager (BiTE) molecules are recombinant protein constructs consisting of two linked single-chain antibodies capable of redirecting previously unstimulated CD8⁺ and CD4⁺ T cells toward target cells for elimination^{98,99} at very low effector to target cell ratios.¹⁰⁰ These molecules transiently tether T cells to tumor cells, resulting in costimulation-independent T-cell activation and lysis of tumor cells (Fig 2).¹⁰⁰ Importantly, T cells can rapidly adopt a serial lysis mode, recharge granzymes on activation, and vigorously proliferate when engaged in target cell lysis, which makes them more efficacious at eliminating tumor cells than Fcγ receptor-expressing immune cells and complement.⁹⁹ Blinatumomab (MT103/MEDI-538; bscC19XCD3) is a BiTE with specificity for CD19 and CD3 capable of inducing lysis of normal and malignant B cells at low picomolar concentrations without causing sustained global T-cell activation. Rather, this agent selectively activates only those T cells that engage in redirected lysis of CD19-positive target cells.¹⁰⁰ The cytotoxic activity induced by blinatumomab with T cells was 150- to 450-fold higher than that mediated by rituximab-induced ADCC.⁹⁹ The cytotoxic activities of both blinatumomab and rituximab were additive. The latter, along with the fact that blinatumomab and rituximab bind different targets, suggests that combining these agents may be effective against immune escape mechanisms in patients with B-cell malignancies.

In an ongoing phase I study in patients with relapsed B-cell malignancies, blinatumomab induced tumor regression in all seven

patients treated at a dose level of 0.06 mg/m²/d.¹⁰¹ Tumor regression was observed in patients with FL, mantle-cell lymphoma, and CLL. Permanent treatment discontinuation as a result of toxicity occurred in eight patients, six of whom had fully reversible CNS events.¹⁰² Engagement of only a few CD3 receptor subunits resulted in activation and local proliferation that caused serial lysis and clearance of tumor cells from bone marrow and liver. Ongoing phase II studies will clarify the role of blinatumomab in the treatment of B-cell malignancies.

In conclusion, rituximab-based chemoimmunotherapeutic regimens are currently the standard of care for the management of B-cell malignancies. However, the use of mAbs in this setting is inextricably linked to the phenomenon of resistance. Because salvage options for these patients are limited, novel agents with different mechanisms of action are highly sought after. A series of novel mAbs targeting a variety of antigens on the surface of malignant B cells has been developed. Given the high cost of mAb therapy and its increasing usage, it is desirable to develop more affordable immunotherapeutics. In this regard, the investigation of diverse scaffolds based on the structure of existing mAbs has yielded agents with lower-molecular weight that are easier to manufacture while preserving high activity against malignant B cells. The rational integration of this ever-expanding therapeutic repertoire with patient characteristics and disease-specific prognostic factors will hopefully optimize current treatment algorithms and improve clinical outcomes.

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Fig 2. Schema of the mechanism of action of blinatumomab (MT103/MEDI-538), a bispecific T-cell engager (BiTE). BiTE antibodies, unlike conventional monoclonal antibodies, are capable of directly activating T cells to seek out and destroy cancer cells by transiently tethering resting T cells to cancer cells. Blinatumomab has dual specificity for CD19, expressed on chronic lymphocytic leukemia cells and non-Hodgkin's lymphoma cells, and CD3, present on the surface of all T cells.

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