

Novel Immunotherapeutic Approaches for Lymphomas

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Monoclonal Antibodies for B-Cell Lymphomas: Rituximab and Beyond

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The year 2007 marks the 10th anniversary of approval by the U.S. Food and Drug Administration of the first monoclonal antibody for the treatment of cancer. Rituximab, an anti-CD20 chimeric monoclonal antibody, was approved for the treatment of patients with relapsed/refractory low-grade B-cell non-Hodgkin lymphomas. From an immunologic perspective, this therapeutic indication provided the long-elusive validation of immunotherapy as the fourth modality of treatment for patients with cancer. From a clinical perspective, it was hard to imagine then that this nonchemotherapeutic approach would dramatically impact the management of patients with almost every type of B-cell malignancy and that it would even find a place as a therapeutic option for patients with non-

malignant disorders. Although thousands of patients have been treated worldwide with rituximab, there is still debate regarding its mechanism(s) of action. The demonstration that a number of patients do not benefit with this treatment and that no cures have been achieved with single-agent rituximab prompted several investigators to identify those barriers limiting the efficacy of this monoclonal antibody. Here, we summarize what we have learned in the past 10 years about rituximab efficacy and its mechanisms of action and resistance. We also discuss the new generation of monoclonal antibodies, the development of which has been spurred by the widespread success of anti-CD20 MAb therapy.

Rituximab: The First 10 Years

Rituximab, a chimeric anti-CD20 monoclonal antibody (MAb), induces killing of normal and malignant B cells expressing the cell-surface molecule CD20. Treatment with rituximab as a single agent has resulted in significant responses in patients with almost every subtype of B-cell lymphoma. However, its biggest benefit is seen when it is combined with chemotherapy regimens for patients with indolent as well as aggressive B-cell non-Hodgkin lymphomas (NHLs).¹ In indolent lymphomas, the addition of rituximab to every chemotherapeutic combination (FCM, CVP, CHOP, FND) has resulted in a significant increase in overall response rate (ORR) and complete remission rate (CR), as well as delay of time to progression (TTP).²⁻⁴ In diffuse large B-cell lymphomas, adding rituximab to CHOP chemotherapy not only has positively affected the ORR, CR and TTP, but also has led to an increase in overall survival (OS).⁵ A recently presented 7-year update by the

Groupe d'Etude des Lymphomes de l'Adulte (GELA) group,^a has confirmed the long-term survival benefit of combining rituximab with CHOP for the treatment of elderly patients with diffuse large B-cell lymphomas. However, not always adding rituximab to chemotherapy has led to improved clinical outcomes. For instance, in patients with mantle cell lymphoma (MCL), treatment with CHOP plus rituximab resulted in progression-free survival (PFS) and OS that was similar to the outcomes observed in patients treated with chemotherapy alone.⁶

In addition to its use as a single agent or in combination with chemotherapy, several clinical studies have evaluated the role of rituximab to consolidate or prolong remission after rituximab monotherapy,⁷⁻¹⁰ standard chemotherapy,¹¹⁻¹² or following combined rituximab-chemo-

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Table 1. Schedules of rituximab maintenance.

Reference	Patient population	Maintenance schedule
Hainsworth et al ⁷	Untreated follicular and SLL	Induction: Rituximab monotherapy (375 mg/m ² weekly × 4 weeks) 375 mg/m ² weekly × 4 weeks every 6 months × 3 times
Ghielmini et al ⁸	Untreated and relapsed follicular lymphoma	Induction: Rituximab monotherapy (375 mg/m ² weekly × 4 weeks) 375 mg/m ² at month 2,4,6,8 after end of induction
Gordan et al ⁹	Patients with CD20 ⁺ lymphoproliferative disorders who had received at least 1 prior treatment	375 mg/m ² every time rituximab blood levels fall under 25 mg/mL (375 mg/m ² given every 3-4 months)
Hagenbeek et al EORTC ¹⁰	Relapsed follicular lymphoma	375 mg/m ² every 3 months × 2 years

Abbreviations: SLL, small lymphocytic leukemia; EORTC, European Organization for Research on Treatment of Cancer.

therapy.¹²⁻¹⁴ Although different schedules of maintenance rituximab have been used after rituximab monotherapy (**Table 1**), we still do not know what is the best dose, duration and proper interval between treatments for patients with follicular lymphomas and other B-cell lymphomas. Only one prospective pharmacokinetic study has addressed the optimal interval between rituximab doses.⁹ By defining the serum concentration of rituximab at 25 mg/mL as a target, Gordan et al found that the administration of one dose of the MAb (375 mg/m²) every 3 months was sufficient to maintain active levels of rituximab above this selected threshold. In lieu of these data, the 3-month schedule has been adopted in ongoing clinical studies sponsored by several cooperative groups. As an example, the Eastern Cooperative Oncology Group (ECOG) 4402 (RESORT study) is an important clinical trial that would likely better define the role of maintenance rituximab in the frontline follicular lymphoma setting. Patients with low tumor burden, stage III/IV follicular lymphoma are treated with standard rituximab induction (375 mg/m² weekly for 4 weeks) and those that achieve CR/partial response (PR) are then randomized to either maintenance rituximab (375 mg/m² every 12 weeks until progression or side effects) or retreatment with rituximab at the time of relapse.

Although some studies have shown a significant clinical benefit when using maintenance rituximab after initial standard chemotherapy with either CVP¹¹ or CHOP,¹² there is still debate as to whether maintenance rituximab provides additional benefit to those patients in which this MAb was also used as part of the induction regimen. A recent update of the Intergroup study E4494/C9793^b has further confirmed that patients with diffuse large B-cell lymphoma randomized to receive R-CHOP as induction regimen did not achieve additional clinical benefit with maintenance rituximab (375 mg/m² weekly for 4 weeks) given every 6 months for 2 years. Other studies have shown, however, that in patients with relapsed follicular lymphomas or MCL, the addition of rituximab maintenance (375 mg/m² weekly for 4 weeks on months 3 and 9 after randomization) to

induction chemotherapy with fludarabine, cytoxan, mitoxantrone and rituximab (FCM-R) led to improved response duration compared with the clinical outcome observed in patients that received FCM-R but not maintenance.¹³ Similarly, Van Oers et al have found that, in patients with relapsed/refractory follicular lymphoma who responded to induction treatment with CHOP chemotherapy with or without rituximab, the addition of maintenance rituximab (375 mg/m² every 12 weeks for 2 years) significantly improved PFS and OS.¹⁴ It is important to mention that until recently no studies have addressed the role of rituximab maintenance following combined rituximab-chemotherapy in the frontline follicular lymphoma setting. The European International Primary Rituximab and Maintenance (PRIMA) study is trying to answer this question by assessing the effect of maintenance rituximab (375 mg/m² every 2 months for 2 years) versus observation in patients with follicular lymphoma who achieve a CR/PR after induction treatment with either R-CHOP, R-CVP or R-FCM. Needless to say, in the absence of conclusive data on efficacy and long-term toxicities from prospective randomized trials of maintenance versus no maintenance, the use of rituximab to consolidate or maintain an initial response should not be considered as standard, and its use should be limited to clinical trials.

Rituximab, since its initial use in patients with follicular lymphomas, has dramatically changed our approach toward patients with B-cell malignancies. Ongoing and future clinical trials will define how to maximize the efficacy of this treatment that, although in use for 10 years, is still endowed with a therapeutic potential that remains to be fully unveiled.

Rituximab's Mechanism of Action

Rituximab works by binding to CD20, a cell-surface antigen expressed on almost all B-cell lymphomas and in normal B cells. How this binding results in cytotoxicity is not entirely known, but likely includes several mechanisms.¹⁵ Among the proposed mechanisms of action of rituximab are (1) elicitation of antibody-dependent cellular cytotoxicity (ADCC), (2) induction of lymphoma cell death through complement-dependent cytotoxicity (CDC) and/or comple-

^b ASCO Meeting, June 2007

ment-dependent cellular cytotoxicity, and (3) direct induction of apoptosis following engagement of CD20 by rituximab. In addition, a mechanism that is gaining particular attention relates to the potential “vaccinal effect” of rituximab. Killing of malignant B cells by rituximab has been proposed, might promote cross-presentation of lymphoma antigens by antigen-presenting cells (APCs) and priming of lymphoma antigen-specific T cells.¹⁶

Antibody-dependent cellular cytotoxicity

This mechanism involves binding of the antibody’s Fc portion to Fcγ receptors expressed in immune cells with cytotoxic capabilities such as monocytes, natural killer cells and granulocytes, which would then lead to destruction of rituximab-bound B cells either by phagocytosis or by the release of cytotoxic granules contained in immune effector cells. Several lines of evidence have led many investigators to consider ADCC as the major mechanism of action of rituximab. First, studies *in vitro* have shown that depletion of malignant B cells by rituximab requires the presence of functional mononuclear cells.¹⁷ Second, elegant studies using mice genetically devoid of inhibitory Fc receptors demonstrated that the *in vivo* antitumor effect of rituximab requires interaction of its Fc portion with Fc receptors expressed in the host’s immune cells.¹⁸ Perhaps the strongest evidence favoring ADCC as the main mechanism of action of rituximab was provided by three independent groups that found that properties of the host’s FcγR to which the antibody binds on leukocyte effector cells influences the efficacy of rituximab therapy.¹⁹⁻²¹

Complement-mediated cytotoxicity

Given the ability of the Fc portion of rituximab to bind complement, it was proposed that this MAb might induce lymphoma cell death through complement-dependent cytotoxicity (CDC) and/or complement-dependent cellular cytotoxicity. Support for this mechanism of action was provided by *in vitro* studies demonstrating that rituximab can trigger complement-dependent killing of a variety of human lymphoma cell lines.¹⁵ The additional observation that complement activation occurred during rituximab treatment *in vivo* and that modulation of two complement inhibitors, CD55 and CD59, led to enhanced CDC in rituximab-treated cells, provided further support for the role of complement activation as a mechanism by which rituximab might kill B cells.^{22,23} However, the enthusiasm for this mechanism was tempered by the findings that B-cell depletion induced by rituximab still occurred in mice genetically deficient of some complement factors.²⁴ Therefore, the exact role played by complement activation in rituximab-induced tumor killing remains to be elucidated.

Direct induction of apoptosis

The B-cell surface phosphoprotein CD20 has been postulated to play a role in several cellular functions such as proliferation, activation, differentiation and cell survival.²⁵

In vitro studies have shown that engagement of CD20 by rituximab triggers a cascade of intracellular signaling events and selective down-regulation of antiapoptotic factors. For instance, Bonavida’s group has shown that rituximab diminishes the activity of the p38MAPK signaling pathway, resulting in inhibition of the IL-10/IL-10R cytokine loop, leading to the inhibition of constitutive STAT3 activity and subsequent down-regulation of the antiapoptotic molecule bcl-2.²⁶ Rituximab also up-regulates Raf-1 kinase inhibitor protein (RKIP) expression in some malignant B cells. RKIP is a negative regulator of ERK1/2 as well as NF-κB pathways, two major survival pathways in B cells. Rituximab-induced increased expression of RKIP therefore led to diminished levels of ERK1/2 and NF-κB, both of which contribute to the down-regulation of Bcl-xL and subsequent sensitization to drug-induced apoptosis.²⁵ An important recent observation relates to the ability of rituximab to translocate CD20 into lipid rafts *in vitro* and to induce caspase activation via increased calcium mobilization.²⁷ Needless to say, the relative contribution of these different intracellular events to the overall antitumor efficacy of rituximab *in vivo* remains to be fully elucidated. Byrd and colleagues provided what is considered one of the strongest pieces of evidence supporting a direct apoptotic effect of rituximab in the clinical setting. Analysis of circulating malignant B cells immediately after *in vivo* treatment with rituximab demonstrated that these cells display activation of several caspases and poly (ADP-ribose) polymerase (PARP) cleavage. This apoptotic effect was observed long before other potential mechanism such as ADCC could be triggered *in vivo*, suggesting that direct apoptosis induced by rituximab might play a role in depletion of circulating malignant B cells.²⁸

Cross-presentation of lymphoma-derived antigens and “vaccinal” effect of rituximab

One of the most intriguing clinical observations in patients with follicular lymphomas was the finding that retreatment with rituximab was associated with a median response duration that was longer than the one observed following first treatment with this MAb.²⁹ Furthermore, in those patients that responded to retreatment, the antitumor effect of rituximab persisted long after the antibody was cleared from circulation. These findings led some investigators to propose that rituximab-induced killing of malignant B cells might result in priming of lymphoma antigen-specific T-cell responses *in vivo*. Generation of these T-cell responses or “vaccinal effect” of rituximab might in turn be responsible for an antilymphoma immunity that persists far beyond the initial cytotoxic effect of the antibody itself. Supporting this mechanism, Selenko et al have shown that *in vitro* treatment of lymphoma cells with rituximab led to cell destruction and generation of apoptotic bodies that are taken up and processed by APCs and subsequent cross-presentation of tumor-derived antigens to T cells.¹⁶

Rituximab Resistance

Despite being an effective agent in the treatment of B-cell lymphomas, approximately 50% of patients with relapsed/refractory CD20⁺ follicular lymphomas do not respond to initial therapy with rituximab (innate resistance)³⁰ and close to 60% of prior rituximab responding patients will no longer benefit with retreatment with this monoclonal antibody (acquired resistance).²⁹ Whether these forms of rituximab-resistance are due to an adaptive property of the malignant B cell or to an impaired host's immune effector mechanisms remains unclear. Below, we describe the studies that have increased our understanding of the mechanisms of rituximab-resistance and fostered the rational development of therapeutic strategies aimed to overcome these barriers to anti-CD20 therapy.

Tumor-related mechanisms

It was initially thought that the degree of CD20 expression in malignant B cells would influence the magnitude of response to rituximab treatment. However, several studies have demonstrated that the intensity of CD20 expression does not predict response to treatment and/or represents a major resistance mechanism to rituximab therapy.¹⁵ An alternative explanation was that perhaps malignant B cells undergo mutations that render them less susceptible to rituximab. Support for this mechanism has been provided by extensive studies of rituximab-resistant (RR) cell lines displaying altered cell-signaling pathways. For instance, it has been observed that cross-linking of the rituximab molecule bound to the surface of RR cells do not lead to their apoptosis.³¹ RR cell lines have been shown to display changes in the intracellular domain of the CD20 antigen that affect the pro-apoptotic proteins SERCA3 and Bax/Bak resulting in a decrease in intracellular Ca⁺⁺ mobilization and inhibition of apoptosis.³² Other studies have shown that RR cells display constitutive hyperactivation of the survival pathways NF- κ B and ERK1/2, leading to overexpression of Bcl-2, Bcl-2-related gene, and myeloid cell differentiation-1 and increased drug resistance.³¹ Unlike parental cells, treatment of RR cells with rituximab does not diminish the expression of resistant factors. In contrast, specific pharmacologic inhibition of NF- κ B, ERK1/2 pathways or Bcl-2 was able to sensitize the RR clones, providing support for a rational combination of pharmacologic inhibitors targeting survival/antiapoptotic pathways with rituximab as a strategy to overcome intrinsic mechanisms or resistance in malignant B cells.

Host-related mechanisms

Evidence accumulated in recent years is pointing to host-related mechanisms rather than intrinsic properties of the malignant B cell as playing a dominant role in rituximab-resistance. Indeed, the host's Fc γ R to which the antibody binds on leukocyte effector cells seems to greatly influence rituximab efficacy.¹⁹⁻²¹ Three classes of Fc γ R (Fc γ RI,

Fc γ RII and Fc γ RIII) have been described in immune effector cells. Eight genes located on chromosome 1 in humans encode these receptors and their subclasses. Some of these genes display allelic polymorphism that results in allotypes with different receptor properties.¹⁹ Among those genes, one that is particularly important is the *FCGR3A* gene that, given its dimorphism, can encode Fc γ RIIIa (CD16) with either the amino acid phenylalanine (F) or valine (V) at amino acid position 158. It turns out that such a change in a single amino acid significantly affects the affinity of the Fc γ RIIIa for human immunoglobulin G1 and the subsequent efficacy of ADCC. Indeed, it is now well established that human IgG1 binds more strongly to homozygous Fc γ RIIIa-158 valine/valine (V/V) expressed in natural killer (NK) cells than homozygous Fc γ RIIIa-158 phenylalanine/phenylalanine (F/F) or heterozygous Fc γ RIIIa-158F carriers. Given that rituximab is an IgG1 MAb, Cartron and colleagues asked whether FCGR3A polymorphism might influence the therapeutic response to rituximab. In 49 patients with follicular lymphoma who received this antibody as a single agent, these investigators found that patients with homozygous Fc γ RIIIa-158V/V, who account for approximately 20% of the healthy population, had an objective response rate of 90% at 12 months compared with the 51% response rate observed in patients with the Fc γ RIIIa-158F/F genotype.¹⁹ Weng and Levy confirmed these findings with the demonstration that patients with follicular lymphomas homozygous for Fc γ RIIIa-158V/V had a higher response rate and freedom from progression (FFP) following rituximab treatment.²⁰ Furthermore, these same investigators found a favorable clinical outcome in patients with follicular lymphoma treated with an idiotype vaccine that correlated with the induction of specific anti-idiotype antibody response and the Fc γ RIIIa 158 V/V genotype.³³ Finally, in a larger series of patients with follicular lymphoma, the Swiss Group of Clinical Cancer Research (SAKK) observed similar outcomes, with the Fc γ RIIIa-158V/V genotype being associated with a median event-free survival (EFS) of 41 months relative to an EFS of 9 months in patients with the Fc γ RIIIa-158F/F genotype.²¹ It should be pointed out, however, that such a positive correlation between Fc γ RIIIa 158V/V polymorphism and responses to rituximab has not been observed in patients with chronic lymphocytic leukemia (CLL).³⁴ The above findings prompted several groups to engineer the Fc portion of MABs as a strategy to increase their binding to the low-affinity receptors Fc γ RIIIa-158F/F or V/F (which accounts for the large majority of the population) that augments ADCC and ultimately try to improve the response to antibody therapy.

An additional mechanism of rituximab resistance involves overexpression on the malignant cells of CD55 and CD59, molecules that have been shown to inhibit CDC.²³ A recent Japanese study has demonstrated that repeated exposure of B-cell lymphoma cell lines to rituximab in

vitro resulted in selection of RR cells displaying an increased expression of these complement-inhibitory molecules.³⁵ Highlighting further the role of complement in the clinical response to rituximab, Weiner's group have recently found that polymorphism in the C1qA component of complement correlates with prolonged complete remission following rituximab therapy in patients with follicular lymphomas.³⁶

Beyond Rituximab Therapy

Regardless of the mechanisms, rituximab resistance represents a significant barrier to immunotherapy of B-cell lymphomas. Several approaches to overcome this resistance are under evaluation, such as drugs that interfere with intrinsic tumor-related mechanisms of resistance or the development of a newer generation of MAb (Table 2). Among the latter, two general strategies are being pursued: (1) engineering of novel anti-CD20 MAb, and (2) development of MAb that target antigens other than CD20.

Novel anti-CD20 monoclonal antibodies

Several new anti-CD20 MAb are currently undergoing pre-clinical and clinical evaluation. These antibodies fall under two categories: (1) anti-CD20 MAb displaying higher affinity than rituximab for FcγRIIIa, and (2) anti-CD20 antibodies with lower immunogenicity (humanized).

Anti-CD20 MAb with improved binding to FcγRIIIa. Three novel engineered anti-CD20 antibodies, AME-133v, rhuMAb v114, and GA-101, are currently in early phases of clinical development. AME-133v binds with an increased affinity to the FcγRIIIa (CD16) and has been shown to display higher affinity than rituximab for this receptor expressed in NK cells *in vitro*.³⁷ Furthermore, when compared with rituximab, AME-133v has a 10-fold improved killing of human B cells. This novel MAb is being evaluated in a phase 1/2 dose escalation study using weekly intravenous doses for 4 consecutive weeks in patients with relapsed/refractory follicular B-cell NHLs.

RhuMAb v114 is another MAb that, because of the engineering of its Fc portion, displays a 30-fold greater

Table 2. New monoclonal antibodies and their targets in B-cell non-Hodgkin lymphoma (NHL).

Name	Target	Studies/comments
AME-133v	CD20	Binds with an increased affinity to FcγRIIIa (CD16). 10-fold increase in cytotoxicity relative to rituximab <i>in vitro</i> . Phase 1/2 study ongoing for patients with relapsed/refractory follicular lymphoma
GA-101	CD20	Glycol-engineered Fc portion and a modified elbow hinge. 50-fold higher binding affinity to the FcγRIIIa resulting in a 10 to 100-fold increase in ADCC <i>in vitro</i> . Apoptosis-inducer.
rhuMAb v114	CD20	30-fold greater binding to the low-affinity variant of FcγRIIIα than to rituximab. 2 to 10 times improved ADCC relative to rituximab <i>in vitro</i> .
Ofatumumab (HuMaxCD20)	CD20	Humanized antibody that binds to a different CD20 epitope than rituximab. Phase 1/2 trials demonstrated activity in patients with follicular lymphoma and CLL.
IMMU-106 (hA20)	CD20	Humanized antibody. Phase 1/2 studies showed a 53% ORR in patients with recurrent B-cell NHL, including 6 patients that achieved CR.
Lumiliximab	CD23	Active in CLL when used in combination with fludarabine-based chemotherapy. Phase 3 clinical trial comparing lumiliximab plus FCR to FCR alone currently ongoing.
Epratuzumab	CD22	Epratuzumab in combination with rituximab for patients with refractory/recurrent B-cell NHL was associated with a 47% ORR and 24% CR.
SGN-40	CD40	In animal models, combination of SGN-40 with CHOP was more active than either CHOP or SGN-40 alone. Phase 1 study demonstrated activity in heavily pretreated patients with aggressive NHL.
HCD122	CD40	<i>In vitro</i> studies have shown that HCD122 is a more potent mediator of ADCC than rituximab. Phase 1 trial ongoing.
Galiximab	CD80	Active in relapsed/refractory follicular NHL either as a single agent or in combination with rituximab. Phase 3 trial comparing galiximab plus rituximab versus rituximab plus placebo ongoing.
Apolizumab (Hu1D10)	HLA-DR	Active when used in combination with rituximab or G-CSF in patients with relapsed/refractory NHL.
CMC-544 (immunoconjugate)	CD22	Conjugated to calicheamicin. Clinical responses in patients with relapsed/refractory follicular lymphoma and large B-cell lymphoma.
BL22	CD22	Recombinant anti-CD22 antibody combined with <i>Pseudomonas</i> exotoxin. Clinical responses in hairy cell leukemia and to a lesser extent in patients with CLL. Hemolytic-uremic syndrome.

Abbreviations: FCR, ADCC, antibody-dependent cell-mediated cytotoxicity; CLL, chronic lymphocytic leukemia; ORR, overall response rate; CR, complete response; CHOP, cyclophosphamide, adriamycin, vincristine, prednisone; G-CSF, granulocyte colony-stimulating factor

binding to the low-affinity variant of FcγRIIIa (FF or FV) than rituximab. This has resulted in 2 to 10 times improved ADCC relative to rituximab in *in vitro* models. Preclinical studies in cynomolgus macaques have shown, however, that treatment with rhuMAB v114 is associated with a dose-dependent reversible neutropenia and thrombocytopenia. Given these hematologic toxicities, a phase 1/2 clinical study has been recently opened in the U.S. to assess the safety of administration of escalating doses of rhuMAB v114 in patients with relapsed or refractory follicular B-cell NHL who have been treated with a prior rituximab-containing regimen.

GA-101 is a third-generation humanized anti-CD20 anti body with a glycol-engineered Fc portion and a modified elbow hinge. The glycol-engineered Fc gives it a 50-fold higher binding affinity to the FcγRIII, resulting in a 10- to 100-fold increase in ADCC against CD20-expressing NHL cell lines. In addition, the modified elbow hinge area resulted in stronger induction of apoptosis of several NHL cell lines and primary malignant B cells. Such a dual modification confers GA-101 with an increased therapeutic efficacy, leading to complete responses and long-term survival in xenograft models of diffuse large B-cell lymphoma and MCL.³⁸

In spite of the excitement generated by the new generation of anti-CD20 MAb, their potential place in the therapeutic armamentarium against B-cell malignancies will mainly depend not only on their safety profile but more importantly on the demonstration of a therapeutic efficacy far superior than the provided by rituximab. An additional barrier faced by novel anti-CD20 MAb is the increasing use of maintenance rituximab, especially in patients with follicular lymphomas. In this patient population, it is likely that the extended use of rituximab would make the interpretation of clinical results with novel anti-CD20 MAb a challenge. Well-designed clinical trials targeting patients in whom rituximab treatment is associated with minimal or no efficacy, e.g., patients with MCL, will help to define the role of novel engineered anti-CD20 MAb in the treatment of B-cell malignancies.

Anti-CD20 antibodies with lower immunogenicity. Approximately 25% of patients receiving rituximab for the first time will experience infusion-related side effects such as fever, chills, rigors, pruritus and/or rash. Although the mechanism(s) involved in these adverse reactions are not completely understood, it is thought that they are the result of the rapid lysis of circulating B cells that accompanies the first rituximab infusion.³⁹ By the time of the second infusion, the large majority of circulating B cells have already disappeared, and this kinetic of B-cell depletion might explain why infusional reactions are less common during the second and subsequent rituximab treatment.³⁰ Nowadays, treatment with rituximab and especially management of its side effects is a routine practice in oncology. However, its use outside this field, such as in the treatment of rheumatologic and autoimmune diseases, still represents a challenge. It is in these settings where novel humanized

anti-CD20 MAb displaying lower immunogenicity and perhaps better side-effect profile might find a therapeutic niche. In contrast, humanized anti-CD20 MAb will find a place in the therapeutic armamentarium of B-cell malignancies only if ongoing clinical trials demonstrate that they are far better than rituximab, if they are active against B-cell lymphomas in which rituximab has minimal activity (i.e., CLL, MCL), or if they are shown to have clinical activity in rituximab-refractory patients. The recent demonstration that rituximab infusion can be safely administered over 90 minutes in patients with B-cell NHL⁴⁰ has raised the bar further for humanized anti-CD20 MAb since one of the potential advantages of these novel antibodies was precisely to allow shorter infusion time relative to the slow and gradually increasing speed used in rituximab treatment.

There are three humanized monoclonal anti-CD20 antibodies undergoing clinical evaluation. One of these MAb is ofatumumab (HuMaxCD20), an IgG1κ human MAb that binds to a novel CD20 epitope and elicits stronger CDC but is less of an apoptosis-inducer than rituximab.⁴¹ Earlier phase 1/2 studies evaluated the efficacy of ofatumumab in patients with relapsed or refractory follicular NHL, patients with CLL and patients with rheumatoid arthritis (RA). In the first trial, 40 patients with follicular lymphoma were treated with four-weekly infusions of ofatumumab given at different doses (300-1000 mg). Out of 37 patients, 5 CR, 2 CR unconfirmed (CRu), and 9 PR were observed. The median duration of response and median time to disease progression in responding patients was not reached after 12 months of follow-up.⁴¹ In view of these results, ofatumumab is being evaluated in a phase 3 clinical trial for patients with follicular lymphoma that are refractory to rituximab in combination with chemotherapy or to rituximab given as maintenance treatment. A separate phase 3 trial is also evaluating ofatumumab as a single agent in patients with refractory CLL, a B-cell disorder characterized by lower CD20 expression and minimal susceptibility to rituximab treatment, at least at standard doses. The rationale for this phase 3 clinical trial was provided by *in vitro* studies showing that this MAb induced effective killing of target cells expressing low levels of CD20 and by encouraging results obtained by Coiffier et al in earlier phase 1/2 clinical studies. These investigators showed that 11 of 21 evaluable patients (52%) treated at the highest dose level of ofatumumab (1st week, 500 mg; weeks 2 through 4, 2000 mg) achieved objective responses (4 clinical CR and 7 PR) lasting at least 8 weeks.⁴²

The second humanized anti-CD20 MAb ongoing clinical evaluation is IMMU-106 (hA20), an engineered antibody with complementarity-determining regions (CDR) of murine origin and with 90% of the human framework regions identical to epratuzumab, a humanized anti-CD22 IgG1 antibody. Preclinical studies showed that IMMU-106 displays mechanisms of cytotoxicity similar to rituximab. Furthermore, phase 1/2 dose escalation studies for patients with recurrent B-cell lymphomas have demonstrated a 53%

ORR, including 6 patients that achieved CR at a median follow up of 12 weeks.⁴³ Although preliminary, these results demonstrating CR, tolerability, and short infusion times are promising. Finally, the use of the third humanized anti-CD20 MAb, PR070769, is currently being limited to the treatment of patients with rheumatoid arthritis.

Targets other than CD20

Monoclonal antibodies targeting surface molecules other than CD20 in B-cell NHL have shown promise in early clinical trials and might represent an additional strategy to overcoming rituximab resistance. Below, we describe a selected list (not intended to be all inclusive) of MAbs against B-cell malignancies.

Anti-idiotypic antibodies. B-cell lymphomas represent clonal expansion of lymphoid cells with rearranged immunoglobulin genes. The V-D-J recombination sequence results in a unique hypervariable region characteristic of each individual tumor. This sequence is known as the idiotype (Id). Although not the product of a gene mutation, the idiotype represents a truly tumor-specific antigen that has been the target for both passive immunotherapy by using anti-Id antibodies⁴⁴ as well as active immunization with Id vaccines (discussed by Dr. L. Kwak in this volume). Levy's group at Stanford was able not only to generate monoclonal anti-Id antibodies against human B-cell lymphomas, but also to demonstrate tumor regression in patients treated with these custom-made, Id-specific MAbs.⁴⁵ However, a major drawback of this strategy has been the difficulty and labor intensity of tailor-made antibodies as well as the emergence of Id variants with no reactivity to the MAb.

Anti-CD23 MAb. CD23 is a low-affinity IgE receptor found to be highly expressed on CLL cells. Lumiliximab is a primatized anti-CD23 MAb with a human IgG1 constant region and macaque variable regions. Phase 1 clinical trials demonstrated that as a single agent this MAb has an excellent safety profile but minimal activity in patients with CLL. Given that preclinical studies showed that lumiliximab enhances the antitumor effect of fludarabine or rituximab *in vitro* and *in vivo*, Byrd and colleagues recently conducted a phase 1/2 trial of lumiliximab in combination with fludarabine, cyclophosphamide and rituximab (FCR) for patients with recurring CLL. In this study, an ORR of 72% (52% CR, 10% PR, and 10% unconfirmed PR) was observed. Furthermore, when these outcomes were compared with published results using FCR, it seems that the addition of lumiliximab to FCR was better than FCR alone.⁴⁶ As such, a phase 3 clinical trial comparing lumiliximab plus FCR with FCR alone has been recently initiated.

Anti-CD22 MAb. CD22 is a glycoprotein expressed on B cells in the mature stages of differentiation. The function of CD22 is unclear, although some studies suggest it plays a role in the B-cell activation complex and as an adhesion molecule. Epratuzumab is a humanized anti-CD22 antibody that has been evaluated as a single agent or in combination with rituximab in phase 1/2 studies for patients with

refractory/relapsed B-cell NHL. Leonard et al have demonstrated that epratuzumab is well tolerated with no dose-limiting toxicity. Furthermore, in patients with aggressive lymphomas, treatment with this MAb resulted in 10% ORR, including 3 CR.⁴⁷ In patients with refractory or recurrent B-cell NHL, the combination of rituximab with epratuzumab was associated with a higher ORR and CR (47% and 24%, respectively).⁴⁸

Anti-CD40 MAb. CD40 is a member of the tumor necrosis factor receptor (TNFR) family that is expressed primarily on B cells and other APCs such as dendritic cells and macrophages. Binding of CD40 leads to subsequent activation of multiple downstream signaling pathways involved in cellular proliferation and survival. SGN-40 is a humanized anti-CD40 MAb that has shown promise in the treatment of B-cell lymphomas. In human NHL cell lines expressing CD40, SGN-40 induced apoptosis and mediated ADCC. In addition, in a xenograft animal model the combination of SGN-40 with CHOP chemotherapy was significantly more active than either CHOP or SGN-40 used alone. Furthermore, a recently completed phase 1 clinical study has shown that SGN-40 monotherapy has a favorable safety profile and induces durable objective responses in heavily pretreated patients with recurring aggressive NHL.⁴⁹

HCD122 is another humanized anti-CD40 MAb in development. *In vitro* studies have shown that HCD122 blocks CD40-mediated cell-cycle proliferation and is a more potent mediator of ADCC than rituximab.⁵⁰ This antibody is currently undergoing evaluation in phase 1 clinical trials for patients with B-cell malignancies.

Anti-CD80 MAb. Galiximab is a primatized IgG1 monoclonal antibody that targets CD80, a costimulatory molecule constitutively expressed on malignant B cells and Reed-Sternberg cells. CD80 is also transiently expressed on activated normal B cells and APCs. Preclinical studies demonstrated that crosslinking of CD80 inhibits cell-cycle proliferation, up-regulates pro-apoptotic molecules, and induces ADCC. In a phase 1/2 escalation study of galiximab for patients with relapsed/refractory follicular NHL, Czuczman et al found that this MAb therapy was safe and resulted in an ORR of 11% (4 of 37 patients: 2 CR and 2 PR).⁵¹ Encouraging clinical outcomes were obtained by combining galiximab with rituximab. Friedberg et al have shown that this combination is associated with an ORR of 66% (19% CR, 14% uCR and 33% PR). In addition, the observed PFS of 12.1 months in the galiximab-plus-rituximab group was superior to historic rituximab single-agent studies.⁵² These promising results prompted the initiation of a phase 3 clinical trial comparing galiximab plus rituximab with rituximab plus placebo for patients with recurring follicular B-cell NHL.

Anti-HLA-DR antibodies. The human leukocyte antigen (HLA)-DR is selectively expressed on immune cells such as B lymphocytes, activated T lymphocytes, monocytes, and dendritic cells. Studies using human cell lines have shown that binding of class II HLA molecules in-

duces cell death in B-cell tumors.⁵³ The exact mechanism for this cell death is unknown but could be related to induction of apoptosis through direct signaling, ADCC and/or CDC. Some studies also suggest the formation of reactive oxygen species as the cause of cell death.⁵⁴ There are several humanized monoclonal anti-HLA-DR antibodies undergoing clinical evaluation, including apolizumab (Hu1D10), KRN848 and 1D09C3. Rech et al evaluated apolizumab combined with G-CSF in 6 patients with relapsed or refractory 1D10⁺ B-cell NHL. In this study, 2 of the 6 patients obtained a prolonged stabilization of their disease of 12 months or more.⁵⁵ Another study is evaluating apolizumab in combination with rituximab for patients with relapsed/refractory NHL. Preliminary results of this phase 1 clinical trial have shown a response rate of 42% and a CR rate of 21%.⁵⁶

Immunoconjugates. The development of MAb conjugated with radioisotopes (radioimmunotherapy), chemotherapeutic drugs or toxins represents a very active field of research aimed to improve the efficacy of MAb therapy. Radioimmunotherapy (RIT) is already an accepted treatment modality for patients with B-cell malignancies. Two anti-CD20 IgG-radioconjugates, ⁹⁰Y-ibritumomab tiuxetan (Zevalin) and ¹³¹I-tositumomab (Bexxar), have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of relapsed/refractory, indolent or transformed B-cell NHL. Several studies have shown that RIT is well tolerated, has the highest single-agent activity observed in lymphoma therapy and can provide durable responses even in patients who had failed previous treatments, including rituximab therapy.⁵⁷ The mechanism of action of RIT involves both binding of CD20 as well as induction of DNA damage in tumor cells by radioactive emissions delivered by the radionuclides. This is considered a major attribute for radioimmunoconjugates relative to other immunoconjugates, since RIT can be therapeutically active even if there are factors that impede targeting of every single cell.

As for immunoconjugates involving drugs bound to MAbs, promising results have been seen in early clinical studies. One such agent is CMC-544, an anti-CD22 MAb combined with calicheamicin, a potent cytotoxic antitumor antibiotic that binds DNA. In a phase 1 trial using this conjugated antibody, an overall response rate of 69% and 33% was observed in patients with relapsed/refractory follicular lymphoma and diffuse large B-cell lymphoma, respectively.⁵⁸ Another antibody-drug conjugate in development is rituximab-vcMMAE, which combines the anti-CD20 properties of rituximab with the antimitotic agent monomethyl auristatin E (MMAE). Rituximab-vcMMAE has been shown to be selectively cytotoxic against CD20⁺ B-cell lymphoma cell lines *in vitro* and *in vivo* xenograft models.⁵⁹

Toxins conjugated to MAbs represent a third area of development in immunoconjugate therapy of B-cell malignancies. Most of the toxins being evaluated are natural proteins derived from plants, bacteria, or fungi that by in-

activating ribosomal proteins disrupt protein synthesis in the target cells. Immunotoxin BL22, a recombinant anti-CD22 antibody combined with *Pseudomonas* exotoxin, has been shown to be effective in patients with hairy cell leukemia and, to a lesser extent, in patients with CLL. In a phase 1 trial, 86% of patients with hairy cell leukemia achieved a CR with a median duration of 36 months. However, 5 patients developed hemolytic-uremic syndrome (HUS) that responded to plasmapheresis.⁶⁰ Phase 2 studies with this immunoconjugate are now under way.

Concluding Remarks

As can be gathered from above, the last 10 years have witnessed an impressive improvement in the management of patients with B-cell malignancies. Rituximab, the first MAb ever approved for the treatment of patients with cancer, has been at the forefront of this remarkable journey. Either alone or more effectively in combination with chemotherapy, rituximab has dramatically changed our therapeutic approach to patients with almost every type of B-cell lymphomas. With these successes, however, also came the realization of the limitations associated with MAb treatment. A better understanding of the barriers limiting the efficacy of rituximab prompted the rational design of novel MAbs that are already providing encouraging results in preclinical as well as in early clinical studies. In the next 10 years, ongoing and future clinical trials will not only fully unveil the remaining therapeutic potential of rituximab, but they will also better define the therapeutic role of the emerging generation of novel MAbs against B-cell lymphomas.

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References

1. Coiffier B. Treatment of non-Hodgkin's lymphoma: a look over the past decade. *Clin Lymphoma Myeloma*. 2006;7:S7-S13.
2. Forstpointner R, Dreyling M, Repp R, et al. The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood*. 2004;104:3064-3071.
3. Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood*. 2005;105:1417-1423.
4. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood*. 2005;106:3725-3732.

5. 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;346:235-242.
6. Lenz G, Dreyling M, Hoster E, et al. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). *J Clin Oncol*. 2005;23:1984-1992.
7. Hainsworth JD, Litchy S, Burris HA 3rd, et al. Rituximab as first-line and maintenance therapy for patients with indolent non-hodgkin's lymphoma. *J Clin Oncol*. 2002;20:4261-4267.
8. Ghielmini M, Schmitz SF, 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;103:4416-4423.
9. Gordan LN, Grow WB, Pusateri A, Douglas V, Mendenhall NP, Lynch JW. Phase II trial of individualized rituximab dosing for patients with CD20-positive lymphoproliferative disorders. *J Clin Oncol*. 2005;23:1096-1102.
10. Hagenbeek A, Van Glabbeke M, Teodorovic I. The role of rituximab maintenance treatment in relapsed follicular NHL: an interim analysis of the EORTC randomized intergroup trial [abstract]. *Ann Oncol*. 2005;16:v52.
11. Hochster HS, Weller E, Gascoyne R, 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]. *Blood*. 2005;106: Abstract no. 349.
12. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J Clin Oncol*. 2006;24:3121-3127.
13. Forstpointner R, Unterhalt M, Dreyling M, et al. Maintenance therapy with rituximab leads to a significant prolongation of response duration after salvage therapy with a combination of rituximab, fludarabine, cyclophosphamide, and mitoxantrone (R-FCM) in patients with recurring and refractory follicular and mantle cell lymphomas: Results of a prospective randomized study of the German Low Grade Lymphoma Study Group (GLSG). *Blood*. 2006;108:4003-4008.
14. van Oers MH, Klasa R, Marcus RE, et al. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. *Blood*. 2006;108:3295-3301.
15. Smith MR. Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene*. 2003;22:7359-7368.
16. Selenko N, Maidic O, Draxier S, et al. CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross-priming of CD8+ cytotoxic T cells. *Leukemia*. 2001;15:1619-1626.
17. Flieger D, Renoth S, Beier I, Sauerbruch T, Schmidt-Wolf I. Mechanism of cytotoxicity induced by chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20-expressing lymphoma cell lines. *Cell Immunol*. 2000;204:55-63.
18. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets [see comments]. *Nat Med*. 2000;6:443-446.
19. Cartron G, Dacheux L, Salles G, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. *Blood*. 2002;99:754-758.
20. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol*. 2003;21:3940-3947.
21. Ghielmini M, Rufibach K, Salles G, et al. Single agent rituximab in patients with follicular or mantle cell lymphoma: clinical and biological factors that are predictive of response and event-free survival as well as the effect of rituximab on the immune system: a study of the Swiss Group for Clinical Cancer Research (SAKK). *Ann Oncol*. 2005;16:1675-1682.
22. van der Kolk LE, Grillo-Lopez AJ, Baars JW, Hack CE, van Oers MH. Complement activation plays a key role in the side-effects of rituximab treatment. *Br J Haematol*. 2001;115:807-811.
23. Golay J, Zaffaroni L, Vaccari T, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. *Blood*. 2000;95:3900-3908.
24. Uchida J, Hamaguchi Y, Oliver JA, et al. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. *J Exp Med*. 2004;199:1659-1669.
25. Jazirehi AR, Bonavida B. Cellular and molecular signal transduction pathways modulated by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin's lymphoma: implications in chemosensitization and therapeutic intervention. *Oncogene*. 2005;24:2121-2143.
26. Alas S, Emmanouilides C, Bonavida B. Inhibition of interleukin 10 by rituximab results in down-regulation of bcl-2 and sensitization of B-cell non-Hodgkin's lymphoma to apoptosis. *Clin Cancer Res*. 2001;7:709-723.
27. Janas E, Priest R, Wilde JI, White JH, Malhotra R. Rituxan (anti-CD20 antibody)-induced translocation of CD20 into lipid rafts is crucial for calcium influx and apoptosis. *Clin Exp Immunol*. 2005;139:439-446.
28. Byrd JC, Kitada S, Flinn IW, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. *Blood*. 2002;99:1038-1043.
29. 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;18:3135-3143.
30. 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;16:2825-2833.
31. Jazirehi AR, Vega MI, Bonavida B. Development of rituximab-resistant lymphoma clones with altered cell signaling and cross-resistance to chemotherapy. *Cancer Res*. 2007;67:1270-1281.
32. Hernandez-Ilizaliturri FJ. Impaired calcium mobilization in rituximab-resistant cells is associated with changes in the structure of CD20 antigen. *J Clin Oncol*. 2006;24:2516.
33. Weng WK, Czerwinski D, Timmerman J, Hsu FJ, Levy R. Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. *J Clin Oncol*. 2004;22:4717-4724.
34. Farag SS, Flinn IW, Modali R, Lehman TA, Young D, Byrd JC. Fc gamma RIIIa and Fc gamma RIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia. *Blood*. 2004;103:1472-1474.
35. Takei K, Yamazaki T, Sawada U, Ishizuka H, Aizawa S. Analysis of changes in CD20, CD55, and CD59 expression on established rituximab-resistant B-lymphoma cell lines. *Leuk Res*. 2006;30:625-631.
36. Racila E, Weng W-K, Wooldridge J, Link BK, Levy R, Weiner GJ. A polymorphism in the C1qA component of complement correlates with prolonged complete remission following

- rituximab therapy of follicular lymphoma [abstract]. *Blood*. 2005;106: Abstract no. 778.
37. Weiner GJ, Bowles JA, Link BK, Campbell MA, Wooldridge JE, Breitmeyer JB. An anti-CD20 monoclonal antibody (mAb) with enhanced affinity for CD16 activates NK cells at a lower concentrations and more effectively than rituximab [abstract]. *Blood*. 2005;106: Abstract no. 348.
 38. Umana P, Moessner E, Bruenker P, et al. Novel 3rd generation humanized Type II CD20 antibody with glycoengineered Fc and modified elbow hinge for enhanced ADCC and superior apoptosis induction [abstract]. *Blood*. 2006;108: Abstract no. 229.
 39. Ghilmini M. Multimodality therapies and optimal schedule of antibodies: rituximab in lymphoma as an example. *Hematology Am Soc Hematol Educ Program*. 2005:321-328.
 40. Sehn LH, Donaldson J, Filewich A, et al. Rapid infusion rituximab in combination with corticosteroid-containing chemotherapy or as maintenance therapy is well tolerated and can safely be delivered in the community setting. *Blood*. 2007;109:4171-4173.
 41. Hagenbeek A, Plesner T, Johnson P, et al. HuMax-Cd20, a novel fully human anti-Cd20 monoclonal antibody: Results of a Phase I/II trial in relapsed or refractory follicular non-Hodgkin's lymphoma [abstract]. *Blood*. 2005;106: Abstract no. 4760.
 42. Coiffier B, Tilly H, Pedersen LM, et al. HuMax CD20 fully human monoclonal antibody in chronic lymphocytic leukemia: early results from an ongoing Phase I/II clinical trial [abstract]. *Blood*. 2005;106: Abstract no. 448.
 43. Morschhauser F, Leonard JE, 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]. *Blood*. 2005;106: Abstract no. 2428.
 44. Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotypic antibody. *N Engl J Med*. 1982;306:517-522.
 45. Davis TA, Maloney DG, Czerwinski DK, Liles TM, Levy R. Anti-idiotypic antibodies can induce long-term complete remissions in non-Hodgkin's lymphoma without eradicating the malignant clone. *Blood*. 1998;92:1184-1190.
 46. Byrd JC, Castro J, O'Brien SG, et al. Comparison of results from a phase I/II study of Lumiliximab (anti-CD23) in combination with FCR for patients with relapsed CLL with published FCR results [abstract]. *Blood*. 2006;108: Abstract no. 32.
 47. 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;10:5327-5334.
 48. Strauss SJ, Morschhauser F, Rech J, et al. Multicenter phase II trial of immunotherapy with the humanized anti-CD22 antibody, epratuzumab, in combination with rituximab, in refractory or recurrent non-Hodgkin's lymphoma. *J Clin Oncol*. 2006;24:3880-3886.
 49. Advani A, Forero-Torres A, Furman R, et al. SGN-40 monotherapy induces durable objective responses in patients with relapsed aggressive Non-Hodgkin's lymphoma: Evidence of antitumor activity from a Phase I study [abstract]. *Blood*. 2006;108: Abstract no. 695.
 50. Long L, Patawaran M, Tong X, et al. Efficacy of an antagonist anti-CD40 monoclonal antibody, HCD122 (CHIR-12.12), in preclinical models of human Non-Hodgkin's lymphoma and Hodgkin's disease [abstract]. *Blood*. 2006;108: Abstract no. 230.
 51. 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;23:4390-4398.
 52. Leonard J, Friedberg J, Younes A, et al. A phase I/II study of galiximab (an anti-CD80 monoclonal antibody) in combination with rituximab for relapsed or refractory, follicular lymphoma. *Ann Oncol*. 2007;18:1216-1223.
 53. Longo DL. DR's orders: human antibody kills tumors by direct signaling. *Nat Med*. 2002;8:781-783.
 54. Mone AP, Huang P, Pelicano H, et al. Hu1D10 induces apoptosis concurrent with activation of the AKT survival pathway in human chronic lymphocytic leukemia cells. *Blood*. 2004;103:1846-1854.
 55. Rech J, Repp R, Rech D, et al. A humanized HLA-DR antibody (hu1D10, apolizumab) in combination with granulocyte colony-stimulating factor (filgrastim) for the treatment of non-Hodgkin's lymphoma: a pilot study. *Leuk Lymphoma*. 2006;47:2147-2154.
 56. Dunleavy K. Phase I study of combination of rituximab with apolizumab in relapsed/refractory B-cell lymphoma and chronic lymphocytic leukemia [abstract]. *J Clin Oncol*. 2005; 23:6607.
 57. Schaefer-Cuttillo J, Friedberg JW, Fisher RI. Novel concepts in radioimmunotherapy for non-Hodgkin's lymphoma. *Oncology*. 2007;21:203-212.
 58. Fayad L, Patel H, Verhoef G, et al. Clinical activity of the immunoconjugate CMC-544 in B-cell malignancies: Preliminary report of the expanded maximum tolerated dose (MTD) cohort of a phase I study [abstract]. *Blood*. 2006;108: Abstract no. 2711.
 59. Law CL, Cervený CG, Gordon KA, et al. Efficient elimination of B-lineage lymphomas by anti-CD20-auristatin conjugates. *Clin Cancer Res*. 2004;10:7842-7851.
 60. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al. Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. *J Clin Oncol*. 2005;23:6719-6729.