



## Faculty

### David G. Maloney, MD, PhD

Associate Member  
Clinical Research Division  
Fred Hutchinson Cancer  
Research Center  
Seattle, WA

### Bruce D. Cheson, MD

Head of Hematology  
Lombardi Comprehensive  
Cancer Center  
Georgetown University Hospital  
Washington, DC

### Mathias J. Rummel, MD, PhD

Leiter des Schwerpunktes  
Hämatologie  
Universitätsklinik Giessen  
Giessen, Germany

### Jonathan W. Friedberg, MD

Associate Professor  
Medicine and Oncology  
University of Rochester  
Rochester, NY

### Kanti R. Rai, MD

Chief of Hematology-Oncology  
Long Island Jewish Medical Center  
Co-Director  
Chronic Lymphocytic Leukemia  
Research and Treatment Program  
North Shore Long Island Jewish  
Health System  
The Feinstein Institute for  
Medical Research  
Manhasset, NY

### Michael Hallek, MD

Director  
Department of Internal Medicine  
Klinik I für Innere Medizin  
Universität zu  
Köln, Germany

# The Evolving Role of Multifunctional Agents in Lymphoid Malignancies

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# Factors Leading to Rituximab Resistance and How to Overcome Them

David G. Maloney, MD, PhD

Dr. Maloney is Associate Member in the Clinical Research Division at Fred Hutchinson Cancer Research Center in Seattle, WA.

Address correspondence to:  
David G. Maloney, MD, PhD  
Clinical Research Division  
Fred Hutchinson Cancer  
Research Center  
1100 Fairview Ave N  
Seattle, WA 98109-1024.  
Phone: (206) 667-5616  
Fax: (206) 667-6124  
Email: [dmaloney@fhcrc.org](mailto:dmaloney@fhcrc.org)

**Abstract:** The identification of CD20 as an antigen on malignant B cells provided the opportunity to selectively target these cells for elimination. These studies generated rituximab, an effective antibody used for the therapy of many B-cell malignancies. However, patients may not respond or may develop resistance, and the presence of Fcγ receptor polymorphisms may reduce the effectiveness of antibody dependent cytotoxicity (ADCC). Additionally, CD20 is not an ideal antigen, as it does not appear to have a critical biologic function, thus allowing the possibility of antigen negative selection. Several strategies are currently being employed to treat patients who develop resistance to or are refractory to rituximab. These include combination chemotherapy with rituximab, radiolabeled antibodies with or without chemotherapy, and the inclusion of immunostimulants or cytokines to augment antibody-based killing. Although radiolabeled antibodies have superior response rates even in rituximab-refractory or -resistant patients, several roadblocks exist for their use in the clinic. Next generation anti-CD20 antibodies are being evaluated. These antibodies have higher affinity for CD20, bind different epitopes, and show better affinity for Fc receptor binding. Further clinical trials will evaluate which of these will be most effective for patients with CD20-expressing B-cell malignancies.

## Introduction

Several characteristics are necessary to efficiently target antigens on tumor cells to achieve tumor eradication with the lowest toxicity. The ideal tumor antigen/s would be expressed on tumor cells only and would also be present on all of the cells that come from the malignant clone. If the target tumor antigen is not tumor-specific, then it should not be expressed on critical host cells. The ideal target tumor antigen should have a high density and it should not shed or be secreted. With immunotoxins, the toxin binding the antigen needs to be internalized to destroy the malignant cell. Finally, the ideal target tumor antigen should have biologic activity. A tumor antigen that has a critical biologic function in tumor-cell survival would be less likely to mutate or be lost and would be the best candidate for targeting.

CD20 composed of both extracellular and cytoplasmic components has wide expression on most B-cell malignancies, except with B-cell acute lymphoblastic leukemia and multiple myeloma. Chronic lymphocytic leukemia (CLL) has a characteristically low level of CD20 expression. Antibodies against CD20 generically target B cells and therefore do not have to be personalized for each patient. There is minimal modulation of the surface antigen, but it is thought to be a poor target for an immunotoxin, which

### Key words

CD20, rituximab, B-cell, NHL, CLL, chemotherapy, antibodies, PRO131921, GA101, ofatumumab, velutuzumab (Immu-106), ocrelizumab, AME-133v

requires internalization. The CD20 antigen is not shed into circulation, which could block monoclonal antibody binding, and sequence mutations are infrequent. Toxicities of CD20 antibody therapy are largely related to the depletion of CD20-expressing cells, which can cause prolonged B-cell depletion but has minimal hematologic or other toxicity other than infusion-related symptoms.

Though the CD20 antigen is a good target, it is not ideal. The CD20 antigen does not appear to have a critical biologic function: studies have shown that it may regulate the entry of extracellular calcium ions;<sup>1</sup> however, it is not required for the malignant phenotype. Murine gene knock-out studies showed that it does not have an essential function for B-cell development.<sup>2</sup> Furthermore, some B-cell non-Hodgkin's lymphomas (NHLs) are CD20-negative, and escape mutations have been observed, which may increase in frequency with selective pressure by the increasing use of anti-CD20 monoclonal antibodies. Finally, most monoclonal antibodies binding to CD20 have only modest direct antitumor effects.

There are 2 types of CD20 antibodies. Type I antibodies are rituximab-like, and most monoclonal antibodies are type I. These antibodies cause rapid redistribution of CD20 into lipid rafts, are involved in complement activation, and have relatively fewer direct effects in the absence of cross-linking.<sup>3</sup> Type II antibodies are like the B1 antibody. Type II antibodies do not cause redistribution of CD20 into lipid rafts. Instead, they induce homotypic adhesion resulting in direct antiproliferative effects, cell signaling, and apoptosis-inducing effects.<sup>4</sup> So far, there have not yet been any reported large trials of type II antibodies.

### Antibody Mechanisms of Action

Antibodies have several possible mechanisms of action. Complement-mediated mechanisms include complement-dependent cytotoxicity (CDC) and phagocytosis. Another mechanism is antibody-dependent cell-mediated cytotoxicity (ADCC) in which the target cell is coated with antibodies resulting in recognition and attack by immune cells. Antibodies can also work directly, by biologic effects from binding the target antigen or by inducing apoptosis or growth arrest.<sup>5</sup> Antibodies can also be used to passively target receptors with radioisotopes, drugs, or toxins as the agents inducing cell death.<sup>6</sup> It is also believed that antibodies may be able to induce secondary immune responses against the tumor, associated with antibody-mediated tumor cell destruction. Finally, antibodies may also have some synergy with chemotherapy.

The overall clinical activity of an antibody is related to the combination of multiple activities, which are thought to consist predominantly of immune-mediated effects, such as CDC and ADCC, and direct effects,

which are more obvious when targeting an antigen that is a known biologically active receptor. With anti-CD20 antibodies, it is not clear which activities are most dominant, but it appears that ADCC is the most relevant mechanism of action.

### Monoclonal Antibodies for NHL Therapy

Several characteristics may be advantageous for successful monoclonal antibody therapy of NHL. The ideal antibody should be humanized or chimerized to decrease the immune response against the antibody. It is not clear what degree of complement binding is ideal, but increased complement binding may be responsible for the infusion reactions that are seen with antibodies like rituximab. The optimal degree of Fc $\gamma$  receptor binding is also contentious. There are different forms of Fc receptors: Fc $\gamma$ RIIIa is an activating receptor for ADCC, whereas Fc $\gamma$ RII provides an inhibitory signal for ADCC; and there are polymorphisms of the Fc $\gamma$ RIIIa. There is evidence that high affinity Fc receptors correlate with a higher level of activity using rituximab.<sup>7,8</sup> Changing the binding affinity of antibodies to these receptors may change the amount of ADCC or activity of the antibody. Most newer antibodies are engineered so that they can augment this interaction. Finally, the ideal monoclonal antibody for NHL therapy should have a high-affinity, a long half-life, and a slow off-rate.

### Resistance to Immunotherapy

A variety of mechanisms may cause cells to become resistant to treatment with antibodies. Murine monoclonal antibodies may induce human anti-mouse antibodies (HAMA) that attach to the antibody and thus eliminate it from the circulation. The use of chimeric or humanized antibodies has reduced the frequency of immunogenicity, and immunogenicity is infrequent in patients with B-cell NHL. However, very potent antibodies with structure changes may still induce the development of a human anti-human antibody (HAHA) or an anti-idiotypic response. The modulation of an antigen from the cell surface is another mechanism of resistance. Furthermore, the antigen can be lost by negative selection, or the cell can lose sensitivity or gain resistance to direct effects. In some cases, there is clearly upregulation of complement-resistant proteins which decrease the ability of lysis with complement; and, in some cases, there may be depletion of effector cells.

There are 2 types of resistance to rituximab: innate resistance and acquired resistance. With innate resistance, 20%–50% of patients with relapsed indolent NHL do not respond to rituximab monotherapy. Innate resistance occurs more frequently in patients with disease refrac-

tory to conventional chemotherapy, and it tends to be higher in patients with bulky disease. After first exposure to rituximab, approximately 50%–60% of responding patients will not respond to re-treatment with rituximab. Therefore, in patients with acquired rituximab resistance, there must be a change that occurs between their first and second exposure to rituximab.

Though the causes of acquired resistance to rituximab are not entirely clear, there are several possible explanations. In some rare cases, patients may permanently lose the CD20 antigen.<sup>9</sup> Some patients may also experience a transient loss of CD20 antigen after exposure to rituximab, which may be due to slow modulation of the antigen. This may be a bigger problem with CLL cells, which have lower density of CD20 expression that may be lost through a “shaving” process, suggesting that rituximab resistance may be concentration dependent.<sup>10</sup> However, the most important cause of resistance appears to be a loss of sensitivity or signaling to the direct effects of rituximab binding to CD20, but this finding is largely limited to in vitro cell data.<sup>11</sup>

### Effect of Maintenance Therapy with Rituximab

The use of rituximab for maintenance therapy is increasing, especially in patients with follicular lymphoma. Maintenance is now accepted following single-agent rituximab, chemotherapy, or chemotherapy plus rituximab. However, when patients relapse on maintenance therapy with rituximab, they will likely be refractory to further single-agent rituximab. There is very little information available on the actual mechanism and on the consequences of rituximab-resistance on response to subsequent treatments.

### Strategies to Overcome Rituximab Resistance

There are several strategies to overcome rituximab resistance. The first is to develop next generation anti-CD20 antibodies that have increased antitumor activity or to use radiolabeled CD20 antibodies. Another strategy is to coadminister agents known to boost immune-mediated mechanisms of cell killing. These include agents such as interferon, granulocyte-colony stimulating factor or granulocyte-macrophage colony-stimulating factor, immune stimulatory agents like thalidomide, or interleukin-2 or -12. A final strategy is to coadminister chemotherapeutic agents with synergistic activity, such as corticosteroids, fludarabine, or other chemotherapeutic agents.

### Improving Next Generation Anti-CD20 Antibodies

New anti-CD20 antibodies have a variety of features that may improve upon rituximab. These features include increased antitumor activity through better ADCC;

increased binding to Fc receptors, including Fc receptors with low-affinity polymorphisms; better or similar CDC; and increased direct effects to inhibit growth and induce apoptosis (Table 1). Direct effects can be increased by manipulating epitope specificity or affinity, the on/off rate, or the antibody structure. New generation anti-CD20 antibodies must still lack immunogenicity, lack hematologic or other toxicity, have minimal infusion reactions, and allow shorter infusion times.

To prove that a new generation anti-CD20 antibody is superior to rituximab, the new antibody must either demonstrate activity in rituximab-refractory patients or produce a higher response rate than rituximab in a randomized trial. It may be easier to prove that the new antibody can induce a response in rituximab-refractory patients, but this may be problematic because rituximab-refractory patients may not respond to any new antibody treatment if, for instance, the resistance is due to the absence of the CD20 antigen. It may be difficult to prove superiority of a new antibody in a randomized clinical trial against rituximab. Very good early clinical trial data in some patient population would be necessary to justify the large patient numbers required for a randomized clinical trial. Such a trial would be costly, and it may require rituximab-naïve patients, which is increasingly difficult.

### New Anti-CD20 Monoclonal Antibodies in Clinical Trials

#### *Ofatumumab*

Ofatumumab is a fully human IgG1 antibody. It binds a distinct epitope, and it is different from type I and type II antibodies. Ofatumumab has more efficient cell lysis mediated by the complement receptor system. Ofatumumab lyses CLL cells, which express a lower density of CD20, more effectively than rituximab, which may be due to the combination of a small and large extracellular loop in ofatumumab's binding epitope. In vitro studies in SCID mouse xenografts show more potent activity than rituximab against low-CD20-expressing cell lines.<sup>12</sup>

#### *PRO131921*

PRO131921 is an engineered, humanized 2H7 antibody. It has been engineered at multiple sites to have increased ADCC through better binding to the poor-affinity FcγRIIIa phenotype, and it is now in clinical trials.<sup>13,14</sup>

#### *GA101*

GA101 is a humanized, type II antibody that has shown greater ADCC and increased direct effects. GA101 also has a glyco-engineered Fc receptor and modified elbow hinge that increases ADCC 10- to 100-fold due to 50-fold better binding to FcγRIIIa. Though GA101 has increased direct induction of apoptosis, it has very low CDC activ-

**Table 1.** New Anti-CD20 Monoclonal Antibodies in Clinical Trials

Antibody name	Description	Clinical trial information
Ofatumumab	Greater complement activity at lower CD20 density	Phase II with CHOP in FL patients Phase II with FC in CLL patients Phase II in relapsed DLBCL patients Phase III in refractory FL patients Phase III in relapsed CLL patients
Veltuzumab (Immu-106)	Humanized, similar activity to rituximab	Phase I/II in NHL and CLL patients
Ocrelizumab	Humanized 2H7	Completed phase I/II trial in R/R FL patients
AME-133v	Better binding to low-affinity Fc receptor	Phase I/II trial in R/R NHL patients
PRO131921	Engineered, humanized 2H7 with greater ADCC, better binding to poor-affinity Fc receptor phenotype	Phase I/II trial in R/R CLL patients Phase I/II trial in R/R NHL patients
GA101	Type II antibody, greater ADCC and direct effects	Preclinical

ADCC=antibody-dependent cell-mediated cytotoxicity; CHOP=cyclophosphamide, doxorubicin, vincristine, and prednisone; CLL=chronic lymphocytic leukemia; DLBCL=diffuse large B-cell lymphoma; FC=fludarabine and cyclophosphamide; FL=follicular lymphoma; NHL=non-Hodgkin's lymphoma; RA=rheumatoid arthritis; R/R=relapsed or refractory.

ity. Preclinical studies have shown that it is very active in xenograft models.<sup>15</sup>

### Others

Other anti-CD20 monoclonal antibodies include veltuzumab (Immu-106), which is a humanized antibody with similar activity to rituximab.<sup>16</sup> Ocrelizumab is a humanized 2H7 antibody that appears to be similar to rituximab.<sup>17</sup> AME-133v has demonstrated better binding to a low-affinity Fc receptor, and it is being evaluated in that setting.<sup>18</sup>

### Radiolabeled Antibodies

Another way to overcome rituximab resistance is to use an antibody to carry a payload. Yttrium-90 ibritumomab tiuxetan is a derivative of the murine parent antibody of rituximab, it has consistent elimination, and it emits beta but not gamma radiation. It is given in conjunction with rituximab. Iodine-131 tositumomab is a murine, type II anti-CD20 antibody, and it emits both gamma and beta radiation. It is given along with unlabeled tositumomab. Both agents have significant clinical activity,<sup>19</sup> even in rituximab-refractory patients.<sup>20,21</sup> Doses of radiolabeled antibodies are based on either millicurie/kg or on actual body clearance. Radiolabeled antibody therapy is generally well tolerated, though it is associated with delayed hematologic toxicity of 4–8 weeks.

Despite significant clinical activity, both agents have had poor penetration into clinical use in the United States due to several challenges. Treatment with radiolabeled

agents requires coordination between oncologists and nuclear medicine physicians. Hematologic toxicity is linked to marrow involvement by the tumor. The treatment has overlapping hematologic toxicity with chemotherapy, which requires the use of staggered rather than simultaneous administration of chemotherapy and radiolabeled antibodies. There are concerns regarding public radiation exposure and the potential long-term effects, such as myelodysplasia or secondary leukemias. Furthermore, there is evidence to suggest that other anti-CD20 antibodies can compete with and block the effects of radiolabeled anti-CD20 antibodies.<sup>22</sup> However, despite these challenges, a phase II trial by the Southwest Oncology Group of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy followed by iodine-131 tositumomab produced outstanding results in previously untreated patients with follicular lymphoma.<sup>23</sup>

### Coadministering Agents

Other strategies to overcome rituximab resistance include coadministering agents known to boost immune-mediated mechanisms of cell killing, such as IFN- $\gamma$ , IL-2, IL-12, or to coadminister chemotherapeutic agents like fludarabine with synergistic activity (Table 2). There is still a need for randomized clinical trials to test the coadministration of many of these combinations, though there are some phase I/II studies that demonstrate the feasibility of these approaches.<sup>24-27</sup>



**Table 2.** Agents used to overcome rituximab resistance

Radiolabeled antibodies
– 90Y-ibritumomab tiuxetan given with rituximab
– 131I-tositumomab given with unlabeled tositumomab
Immunostimulants
– Thalidomide
– CpG motifs/CpG DNA
Cytokines
– Interferon alpha: Similar OR, prolonged TTP
– IL-12
– IL-2
– GM-CSF (CLL)
– G-CSF (FL)
Chemotherapy
– Fludarabine-based (down-regulate complement resistance)
– Corticosteroids (synergistic apoptosis)

The agents used to overcome rituximab resistance have been used in small groups of patients. Randomized trials have yet to be conducted with immunostimulants, cytokines, and alternative chemotherapy regimens.

CLL=chronic lymphocytic leukemia; FL=follicular lymphoma; IL=interleukin; OR=overall response; TTP=thrombotic thrombocytopenic purpura

## Conclusions

The most promising way to overcome rituximab resistance appears to be the next generation anti-CD20 antibodies with higher affinity for different epitopes and better Fc receptor binding. However, while these may be able to replace rituximab and increase activity in rituximab-resistant patients, it is likely that patients will still develop resistance to these antibodies in the future. Radiolabeled antibodies, though some of the most active agents available, are currently not frequently used. Finally, combinations with immunostimulants, cytokines, and synergistic agents like chemotherapy and corticosteroids need to be tested in randomized clinical trials for comparative efficacy.

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# Newer Strategies For Overcoming Rituximab Resistance in Non-Hodgkins Lymphoma and Chronic Lymphocytic Leukemia

Bruce D. Cheson, MD, and Elizabeth Ashforth, PhD

Dr. Cheson is Head of Hematology at the Lombardi Comprehensive Cancer Center of Georgetown University Hospital in Washington, DC. Dr. Ashforth is Principal of Chado Healthcare Consulting, LLC, in Beverly Hills, CA.

Address correspondence to:  
Bruce D. Cheson, MD  
Lombardi Comprehensive  
Cancer Center  
Georgetown University Hospital  
3800 Reservoir Road, NW  
Washington, DC 20007  
E-mail: bdc4@georgetown.edu

**Abstract:** Recent studies have demonstrated a prolongation of survival for patients with follicular and low-grade non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). One major reason is the availability of effective and well-tolerated monoclonal antibodies, such as rituximab, which has reshaped the paradigms for the treatment of these patients. However, many patients are not responsive to rituximab-based regimens, while others become resistant to this drug. Thus, new approaches are needed for such patients. Novel drugs with promising activity include the bifunctional alkylating agent bendamustine, the proteasome inhibitor bortezomib, and, for CLL, flavopiridol. Bendamustine has induced response rates of over 75% in rituximab-relapsed and refractory patients with follicular and low-grade NHL, setting the standard against which other drugs will have to be compared in this setting. Lenalidomide, an immunomodulatory drug (IMiD), has major activity in myelodysplastic syndrome and multiple myeloma. Recent data suggest that about a quarter of patients with relapsed or refractory, indolent or aggressive NHL may respond to this single agent and combination strategies are in development, notably with rituximab and bendamustine. Between 30% and 40% of patients with relapsed and refractory CLL will also respond to this oral agent. Bortezomib appears to be active not only in mantle cell lymphoma, but also in follicular and marginal zone lymphoma, but with minimal activity in diffuse large B-cell NHL, CLL/ small lymphocytic lymphoma (SLL) or Hodgkin's lymphoma. A new generation of monoclonal antibodies are in clinical trials including several human or humanized anti-CD20s and others targeting such antigens as CD80, CD74, CD22, CD23, and CD40. The challenge will be to prioritize the large number of drugs and to develop rational combinations that will be effective in improving the outcome of patients with rituximab relapsed or refractory NHL. Strategies that are effective in relapsed patients may be rapidly moved to the up-front setting and, as a result, may further prolong the survival of patients with indolent NHL and CLL.

## **The Need for New Agents in Chronic Lymphocytic Leukemia (CLL) and Non-Hodgkin's Lymphoma (NHL)**

The clinical course of patients with CLL and indolent NHL is characterized by a pattern of initial response with subsequent repeated relapses and

### **Keywords:**

NHL, CLL, rituximab, lenalidomide, bortezomib, bendamustine, flavopiridol



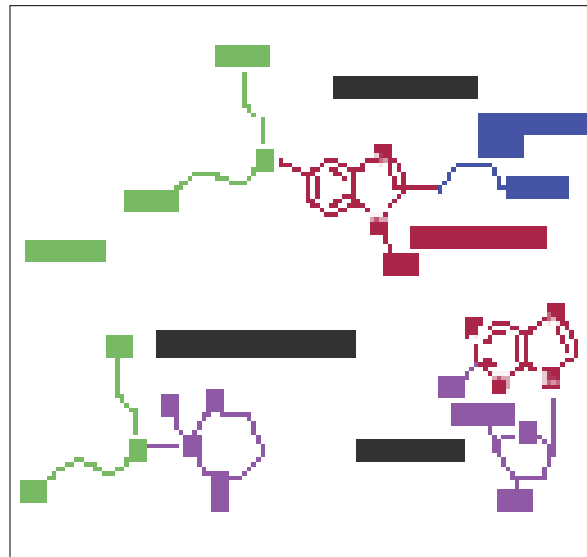
an eventual fatal outcome. The overall goal of improving outcome of patients with CLL and indolent non-Hodgkin's lymphoma (NHL) depends upon increasing the complete remission rate and prolonging progression-free and overall survival (PFS and OS). With an increasing number of effective therapies, patients with relapsed or refractory disease can be treated with a sequence of agents that will induce a succession of responses, prolonging their PFS and OS.

Anti-CD20 therapy with rituximab has played an increasingly important role in the management of indolent NHL over the last two decades. Several studies have demonstrated a survival advantage for patients with follicular and low-grade NHL treated with rituximab-based regimens.<sup>1-3</sup> However, all patients can be considered resistant to the antibody as none are cured with rituximab-containing therapy.

The traditional agents for the treatment of previously untreated patients with CLL included chlorambucil, and subsequently fludarabine. The approach to these patients has undergone a major change with the availability of active and well-tolerated monoclonal antibodies. Fludarabine and rituximab, with or without cyclophosphamide induces responses in more than 90% of patients, including many complete remissions. Importantly, historical comparisons suggest a prolongation of survival.<sup>4,5</sup> A recently reported randomized trial of fludarabine and cyclophosphamide with or without rituximab was terminated early because of a clear advantage in PFS with the antibody-containing arm.<sup>6</sup> Alemtuzumab is also approved for the treatment of patients with relapsed as well as previously untreated CLL. It is very effective, albeit extremely immunosuppressive and of limited efficacy in the setting of bulky lymphadenopathy.<sup>7</sup>

Despite these advances, there is a clear need for new agents in indolent NHL and CLL both in the initial treatment and for relapsed and refractory disease. To continue to prolong PFS and OS, the complete response (CR) rate must be increased. Drugs need to be identified that overcome the primary resistance exhibited by some patients. The result would bring us closer to a curative therapy. However, these patients are currently incurable and additional active agents are needed to improve the response rate in relapsed and refractory patients, resulting in a longer time to next progression with a prolongation of survival.

We are fortunate to have an increasing number of promising new agents for the treatment of patients with indolent NHL and CLL. These include chemotherapy drugs such as bendamustine, bortezomib and flavopiridol, monoclonal antibody-based therapy, immunomodulatory agents, and drugs that promote apoptosis.



**Figure 1.** Bendamustine has structural similarities to the alkylating agent cyclophosphamide and the purine analog cladribine.

### ***Indolent NHL***

**Bendamustine** Bendamustine was first synthesized in the early 1960's at the Institute for Microbiology and Experimental Therapy in Jena, in the former East German Democratic Republic (GDR). The intent was to develop a nitrogen mustard compound that was less toxic but at least as effective as other alkylating agents. By relocating the nitrogen mustard group to position 5 on a benzimidazole ring, they developed a compound, with the chemical name *g*-[1-methyl-5-bis-(*b*-chloroethyl)-amino-benzimidazolyl-(2)]-butyric acid hydrochloride. Bendamustine has structural similarities to both alkylating agents and purine analogs (Figure 1). The benzimidazole ring system may confer nucleoside-like properties and provides stability, allowing for longer-lasting DNA damage. Initially, the compound was identified by the code IMET3393, but later called bendamustine.

Bendamustine exhibits incomplete cross-resistance with other alkylating agents due to a substance-specific interaction between bendamustine and DNA. Bendamustine may also be associated with a relatively slower repair of DNA damage than with other alkylating agents, and may be more stable than cyclophosphamide and chlorambucil.

Bendamustine is non-cross-resistant with a variety of other cytotoxic drugs, and is active in primary NHL cells refractory to conventional chemotherapeutic agents such as cyclophosphamide, doxorubicin, and etoposide. In vitro data demonstrate that bendamustine has a

Investigator	Pts	NHL Grade	CR (%)	ORR (%)	Dose/schedule (mg/m <sup>2</sup> )
Bremer ('02)	62	Indolent	15	82	50–60, d1–5
Heider ('01)	52	Indolent	11	73	120, d1–2
Weidmann ('02)	18	Aggressive	16	44	120, d1–2
Friedberg ('08)	77	Indolent, MCL, Trans	34	77	120, d1–2
Kahl ('08)	100	Indolent, MCL	17	75	120, d1–2

**Table 1.** Bendamustine in Refractory/relapsed NHL

CR=complete response; MCL=mantle cell lymphoma; NHL=non-Hodgkin's lymphoma; ORR=overall response rate; Pts=patients.

different mechanistic profile than other alkylating agents. In primary NHL lymphocytes from patients refractory to cyclophosphamide, bendamustine still induces growth inhibition. Most recently, bendamustine has been shown to differ from other alkylating agents by activating DNA-damage stress responses and apoptosis, inhibition of mitotic checkpoints, induction of mitotic catastrophe, and activation of a base excisions DNA repair pathway rather than an alkyltransferase DNA repair mechanism. Treatment of lymphoma cells with bendamustine initiates a p53-dependent stress pathway resulting in activation of intrinsic apoptosis, with an increase in Bax protein expression. Bendamustine also down-regulates genes important in mitotic check-point regulation. The unique structure of bendamustine, and these observations suggest that bendamustine has the potential for activity in patients with lymphoma relapsing following or resistant to alkylating agent-based therapy.

Synergism between bendamustine and rituximab was demonstrated in severe combined immunodeficiency mice with Daudi xenografts. While adding rituximab reduces the dose of bendamustine required to induce apoptosis in DOHH-2 and WSU-NHL cell lines and ex-vivo B-CLL cells. These preclinical observations supported clinical studies combining the two agents.

Interest in pursuing bendamustine in NHL was stimulated by several trials conducted in Germany in patients with relapsed or refractory indolent NHL (Table 1). Overall response rates were reported as being 51%–73%, including 9%–11% complete remissions.<sup>8–10</sup> Activity was also suggested against aggressive NHL by a single study in which there was an overall response rate of 44% including 16% complete remissions.<sup>11</sup> These data were confirmed by 2 phase II trials conducted in the US in rituximab-refractory follicular and low-grade NHL with overall response rates of 75%–77% including 17%–34% complete remissions.<sup>12,13</sup> Rummel and co-workers first combined bendamustine with rituximab in a variety of histologies of relapsed and refractory NHL, and reported a

response rate of 90% including 60% complete remissions.<sup>14</sup> A U.S. and Canadian trial,<sup>15</sup> recently reported by Robinson and colleagues, confirmed those findings in patients with indolent and mantle cell lymphoma. Response rates of over 90% were achieved half of which were complete remissions, with a median PFS of almost 2 years. Based on these results, bendamustine was recently approved by the FDA for the treatment of rituximab-refractory follicular and low-grade NHL.

**Bortezomib** Bortezomib was the first of a class of agents of proteasome inhibitors to be evaluated in the clinic. Proteasomes are enzymes that comprise about 3% of the weight of normal human cells. They function as a sort of waste disposal in that they are responsible for the degradation and destruction of abnormal cellular proteins. Proteasome inhibitors block the proteasome from functioning, resulting in an intracellular accumulation of abnormal proteins that can induce cell death.

Bortezomib was initially approved for use in patients with myelodysplastic syndrome and subsequently multiple myeloma. It was later shown to have considerable single agent activity in mantle cell lymphoma (MCL) with response rates ranging from around 30%–55%.<sup>16–18</sup> Bortezomib is also active in follicular lymphoma (FL) and marginal zone lymphoma (MZL),<sup>20</sup> but has limited activity in diffuse large B-cell NHL, small lymphocytic lymphoma (SLL)/CLL,<sup>20</sup> and no activity in Hodgkin's lymphoma.<sup>19,20</sup>

Bortezomib has been used in combination with rituximab in patients with follicular NHL. In a randomized phase II trial,<sup>81</sup> patients with relapsed indolent NHL were randomized to receive standard-dose rituximab with either standard-dose bortezomib (1.3 mg/m<sup>2</sup>) twice weekly, or a higher dose of 1.6 mg/m<sup>2</sup> given weekly. Both schedules had similar rates of overall and complete responses, over 50% and ~12%, respectively, and the PFS curves for both schedules are almost identical. However, the standard regimen was associated with a higher overall frequency of grade 3 and 4 toxicities, especially

thrombocytopenia, peripheral neuropathy, nausea, and vomiting. The weekly regimen appeared to be less toxic and equally effective, but it remains to be seen how this regimen will be integrated into standard patient care.

**Lenalidomide** Lenalidomide, a second generation immunomodulatory drug, is a more potent analog of thalidomide which effects cell kill through a number of mechanisms including targeting the tumor microenvironment.<sup>22</sup> Lenalidomide is being widely used in clinical trials in various relapsed or refractory NHL histologies, such as MCL,<sup>23</sup> diffuse large B-cell lymphoma (DLBCL),<sup>24</sup> indolent lymphoma,<sup>25</sup> and in patients who have had a prior stem cell transplant.<sup>26</sup> Although these trials include small numbers of patients, around a quarter of those with indolent lymphoma respond. Lenalidomide is given orally once a day for 3 weeks of a 4-week cycle. Adverse effects primarily include neutropenia and thrombocytopenia in NHL and episodes of tumor lysis syndrome and tumor flare reaction in CLL.<sup>27</sup> Current studies are combining this drug with other active agents, such as bendamustine and bortezomib.

**Monoclonal Antibodies** A number of monoclonal antibodies are undergoing clinical evaluation targeting a variety of cell surface proteins, including CD52, CD23, CD80, CD22, CD74 and CD40. Several second and third generation anti-CD20 antibodies are being developed with the goal of improving on the efficacy and safety profile of rituximab. The mechanisms of action for each antibody differ from rituximab in their relative antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity (CDC), apoptosis, and binding. Trials have yet to clarify which properties or combinations of properties are important clinically.

**Ofatumumab** Ofatumumab has received the most attention to date of the new generation of anti-CD20 antibodies. It has a high binding affinity, potent ADCC and CDC, and binds to an epitope distinct from rituximab. In a phase I/II dose-escalation trial in 40 patients with relapsed or refractory follicular lymphoma, all patients achieved a rapid and sustained decrease in peripheral blood B cells.<sup>28</sup> There were no dose-limiting toxicities reported, but there were some hematologic toxicities (6 grade 1–2 neutropenia, 1 grade 3 neutropenia, and 1 grade 2 thrombocytopenia) and 2 grade 3 infections. Patients started at 300 mg of ofatumumab and the dose was escalated to a gram, but there was no dose-response effect. The overall response rate (ORR) was 43%, and 64% of patients who had been previously exposed to rituximab responded, including 3 of 4 patients who were rituximab-refractory. The median duration of response was approximately

2.5 years, but time to progression for all patients was 9 months. A trial focusing on rituximab-refractory patients would be of interest.

**Galiximab** Galiximab, an anti-CD80 antibody, has been studied as a single agent and in combination with rituximab, demonstrating modest activity. In a phase I/II study, single-agent galiximab showed only modest activity, with an ORR of 11%.<sup>29</sup> In vitro data suggest synergy with rituximab. In a phase I/II trial of galiximab in combination with rituximab in relapsed or refractory FL patients, 64% of patients treated with the highest dose of galiximab plus rituximab experienced a response.<sup>30</sup> The combination was well-tolerated with one transient grade 4 neutropenia. A randomized clinical trial is ongoing to determine whether the combination of galiximab with rituximab is better than single-agent rituximab for relapsed and refractory FL patients. However, when used as initial treatment it induces a response rate of 92% with 75% complete remission in patients with low risk by the Follicular Lymphoma International Prognostic Index (FLIPI), and 80% responses with 48% complete remissions in those with an intermediate FLIPI score.<sup>31</sup>

**Epratuzumab** Studies with the anti-CD22 antibody epratuzumab have also shown activity as a single agent or in combination with rituximab. Response rates in a phase I/II trial of single-agent epratuzumab in indolent NHL histologies, with an overall 18% response rate; however, 43% of FL patients treated with a 360 mg/m<sup>2</sup>/week dose achieved a response.<sup>32</sup> Furthermore, the median time to progression for responders was 23.7 months, and 3 patients achieved a complete remission. In a study of the combination of epratuzumab and rituximab in relapsed or refractory NHL patients, 54% of FL patients achieved an objective response, including the 24% of FL patients who achieved a complete response, half of whom were still in remission at 44.3 months.<sup>33</sup> Objective responses were also observed in 57% of patients with SLL. A randomized trial is needed to compare this combination against single-agent rituximab.

**SGN40** SGN40 is a humanized anti-CD40 monoclonal antibody that triggers a pro-apoptotic signal and mediates antibody dependent cellular phagocytosis and cytotoxicity. A recently reported phase I, open-label, dose-escalation study demonstrated preliminary antitumor activity in patients with relapsed NHL.<sup>34</sup> Treatment with SGN-40 was well-tolerated, and a maximum tolerated dose was not reached. 12% of patients achieved an objective response (partial response [PR] or CR) and 26% achieved stable disease (SD). The median OS was 10.5 months (range 0.1–16.9). SGN40 is now being studied in 3 clinical trials: in

combination with RICE chemotherapy (rituximab, ifosfamide, carboplatin, and etoposide) in DLBCL patients; in combination with rituximab in FL and MZL patients; and in combination with rituximab and gemcitabine in patients with relapsed or refractory DLBCL. A recent report by Advani and colleagues provided an update in patients with DLBCL where treatment with SGN40 was very well tolerated and an ORR of 10% was observed (5% CR, 5% PR from preliminary data in 38 patients).<sup>35</sup>

**Radioimmunotherapy** Radioimmunotherapy is one of the most effective, least used treatments for patient with NHL. A radioimmunoconjugate involves binding of a radioisotope to a monoclonal antibody providing local radiation therapy. Whereas typical monoclonal antibodies are able to kill only those cells to which they are bound, radioimmunotherapeutics, through a cross-fire effect, are also able to kill neighboring tumor cells as well. Two radioimmunoconjugates are approved for the treatment of NHL, 90Y-ibritumomab tiuxetan (Zevalin®) and 131I tositumomab (Bexxar®). Response rates with Y-90 ibritumomab tiuxetan range from 73%–83% with complete remissions from 15%–51%, varying with the extent of prior therapy, previous rituximab responsiveness, tumor bulk, and other features.<sup>36</sup> Horning et al reported a series of 40 patients with follicular NHL who had progressed after rituximab therapy and were treated with 131I tositumomab.<sup>37</sup> Although 59% were considered refractory to their prior chemotherapy, the ORR was 65% with 38% CRs and an overall progression-free survival of about a year. Nevertheless, durable responses were observed.

### Chronic Lymphocytic Leukemia

**Bendamustine** Bendamustine has been extensively studied in CLL as a single agent and in combination regimens (Table 2). ORR have ranged from 56%–75% including 15%–20% complete remissions.

The German CLL study group (GCLLSG) conducted a phase I/II study in 16 patients with relapsed or refractory CLL.<sup>38</sup> Patients had received a median of 3 prior regimens and 50% had previously received fludarabine. Dose escalation started at 100 mg/m<sup>2</sup> intravenously (i.v.) on days 1 and 2 every 3–4 weeks. Six patients experienced dose-limiting toxicities (DLTs) which led to dose de-escalation; 2 at 100 mg/m<sup>2</sup>, 1 at 90 mg/m<sup>2</sup>, 2 at 80 mg/m<sup>2</sup> and 1 out of 7 at 70 mg/m<sup>2</sup>. Major toxicities included grade 3/4 leukopenia in 50%, and grade 3/4 infection in 43% of patients; 2 patients died from atypical pneumonia. The maximum tolerated dose in this study was 70 mg/m<sup>2</sup> every 4 weeks. The ORR was 58% (9/16), including 7 of 12 patients treated at doses of ≤80 mg/m<sup>2</sup>. The median duration of response in patients evaluable for response was 42.7 months and

**Table 2.** Bendamustine Clinical Studies in CLL

Investigator	Regimen	N	CR (%)	ORR (%)
Kath 2001	B 60 mg/m <sup>2</sup> (<70 yr) or 50 mg/m <sup>2</sup> (>70 yr) D1–5 q 4 weeks	20	30	75
Bergmann 2005	B 100 mg/m <sup>2</sup> D1, 2 q 3–4 wk	16	12	56
Lissitchkov 2006	B 100 mg/m <sup>2</sup> D1, 2 q 4 wk	15	27	60
Koppler 2007	B 80–240 mg/m <sup>2</sup> over D1–3, M 8–10 mg/m <sup>2</sup> D1	22	27	86

B=bendamustine; CR=complete response; M=mitoxantrone; ORR=overall response rate.

43.6 months for the responders. Responses were not observed in fludarabine-refractory patients.

Lissitchkov and colleagues conducted a phase I/II study of bendamustine in fludarabine naïve patients with CLL.<sup>39</sup> Dose escalation started at 100 mg/m<sup>2</sup> every 3 weeks and in the absence of DLT during the first cycle the dose was to be increased by 10 mg/m<sup>2</sup> increments. The treatment interval had to be prolonged to every 28 days to allow for bone marrow recovery. DLTs included grade 3/4 hyperbilirubinemia, grade 4 anemia and grade 4 thrombocytopenia. Rate of grade 3/4 leukopenia was 20%. All 6 patients treated at 100 mg/m<sup>2</sup> responded, including 4 CRs and 2 PRs. After a follow up of 15 months only 1 patient had relapsed and the median duration of response of patients with CR was 22 months (range, 18–27 months). The recommended dose from this study was 100 mg/m<sup>2</sup> on days 1 and 2 every 28 days.

Bendamustine has been directly compared with fludarabine in patients with CLL who had relapsed after one prior therapy.<sup>40</sup> Fludarabine naïve patients were randomized to either bendamustine (100 mg/m<sup>2</sup> d 1–2 q 28 days) or fludarabine (25 mg/m<sup>2</sup> d1–5 q 28 days) until best response or to a maximum of 8 cycles. The primary objective of the study was to determine if PFS was comparable between the 2 treatment arms. Out of a total of 96 patients 89 were eligible for an interim analysis. Ninety-five percent of patients had received a chlorambucil-based regimen as their initial therapy. About half of the patients received 6 or more cycles in either treatment arm. The ORR was 78% (29% CR) in the bendamustine arm versus 65% (10% CR) in the fludarabine arm. After a median follow up of two years, median PFS was 83 weeks versus 63 weeks (hazard ratio [HR], 0.93; CI 0.59–1.47)

with bendamustine and fludarabine, respectively. The bendamustine arm was associated with a slightly higher incidence of hematologic toxicity, but the rate of grade 3/4 infections was similar, occurring in 15% of the patients in both the arms. This study suggested that bendamustine can be considered a reasonable alternate to fludarabine.

The GCLLSG conducted a multicenter phase II study (CLL2M) in patients with relapsed or refractory disease.<sup>41</sup> Patients were treated with bendamustine at 70 mg/m<sup>2</sup> on days 1 and 2, with rituximab at a dose of 375 mg/m<sup>2</sup> for the first cycle and 500 mg/m<sup>2</sup> for subsequent cycles, treatment was administered every 28 days for a maximum of 6 cycles. In the 81 enrolled patients, the median age was 66 years and they had received a median 2 (range, 1–3) prior therapies. After 328 cycles in the 81 evaluable patients, 123 > grade 3 toxic events were seen, including leukopenia/neutropenia (11.9%), thrombocytopenia (9.1%), and anemia (6.1%). Sixteen episodes of at least grade 3 infections were observed, most were managed successfully. However, treatment related mortality occurred in 3 (3.7%) patients, all related to infections during neutropenia. The reported ORR was 77.4%, with 14.5% CRs in 62 evaluable patients. Only 3 (4.8%) patients had progressive disease. No molecular remissions were achieved in the bone marrow, as assessed by 4-color flow cytometry. Further development of this combination will be of interest.

**Monoclonal Antibodies** Alemtuzumab is an anti-CD23 monoclonal antibody originally approved for use in fludarabine and alkylating failed CLL. Approval was based on an ORR of 33% with 2% CRs.<sup>42</sup> However, this agent is also associated with a high risk of opportunistic infections. It also has marginal activity in the setting of bulky lymphadenopathy.<sup>43</sup> Moreover, response rates in currently treated patients would likely be lower than previously reported because initial therapy now includes not only fludarabine with or without cyclophosphamide, but also rituximab.

**Lenalidomide** Two studies have clearly demonstrated activity for lenalidomide in patients with relapsed or refractory CLL. Chanan-Khan first reported on 45 patients who had received at least 1 prior therapy; among these, 51% had fludarabine-refractory disease.<sup>44</sup> Lenalidomide was given at a dose of 25 mg per day for 21 days out of a 28-day cycle. Rituximab was added in patients with progressive disease. The ORR was 47%, with 9% of patients achieving a complete remission. The median PFS was 19.4 months. In patients with fludarabine-refractory disease the ORR was 41%, with 12% CR. The most commonly reported adverse event was grade 1 or 2 fatigue (73%). Myelosuppression was common, with neutropenic infections in 15% of patients.

Ferrajoli and colleagues used a 10 mg daily starting dose, and attempted to escalate to 25 mg daily. In their 44 patients with relapsed or refractory CLL, including 27% of patients with fludarabine-refractory disease.<sup>45</sup> The ORR recorded was 32% including 25% for those with fludarabine-refractory disease. The differences in activity between the 2 trials may reflect the lower starting dose of lenalidomide in the Ferrajoli study or differences in patient selection.

Lenalidomide has 2 additional and important adverse effects seen in patients with CLL, but typically not in NHL. A tumor flare reaction is characterized by the sudden onset of tender swelling of the CLL-involved lymph nodes accompanied by overlying erythema of the skin, enlargement of the liver and/or spleen, low-grade fever, rash, and in some cases with a rise in peripheral blood white cell counts. The other effect is tumor lysis syndrome which does not appear to be dose-related.

**Lumiliximab** CD23 is highly expressed on the surface of B-CLL cells. Lumiliximab is an IgG1 chimeric, primate-derived, anti-CD23 monoclonal antibody that is structurally indistinguishable from human antibodies. In a phase I trial in patients with relapsed or refractory CLL, more than half of the patients had fludarabine-refractory disease, and the median number of prior regimens was 4.<sup>46</sup> Almost all (89%) of the patients experienced an adverse event, but most were grade 1 or 2, although there were 2 incidences each of grade 3/4 autoimmune hemolytic anemia and neutropenia. The dose-limiting toxicities were neutropenia and headache. Most (91%) of the patients had a decrease in the absolute lymphocyte count, and 63% had a decrease in lymph node bulk, but these responses were transient. Furthermore, there were no CRs or PRs by National Cancer Institute-Working Group criteria.<sup>47</sup>

In preclinical studies, lumiliximab was shown to be synergistic with fludarabine and rituximab.<sup>48</sup> Byrd and coworkers piloted the combination of FCR (fludarabine, cyclophosphamide, rituximab) plus lumiliximab.<sup>49</sup> When they compared their results with the historical control FCR data from the MD Anderson Cancer Center<sup>50</sup> the overall response rates were similar (73% vs 71% for FCR and FCR-L, respectively), but there was a suggestion of improvement in complete response rates in the FCR plus lumiliximab study (48% vs 25%).<sup>51</sup> FCR plus lumiliximab is safe, and the tolerability is similar to FCR alone. Currently, a large phase II/III randomized trial is comparing FCR with and without lumiliximab for patients with relapsed CLL.

**Ofatumumab** Coiffier and colleagues recently published the first phase I experience with ofatumumab in CLL.<sup>52</sup> Using 4 weekly infusions in 33 patients evaluable for efficacy, the ORR was 44% with no complete remissions



and a median PFS of 106 days. Activity in patients who had previously received rituximab was minimal. Infusion reactions were comparable what would be expected with rituximab; however, there were 51% infections and 9 severe adverse events including a patient who died with interstitial lung disease. Additional data in patients treated with prior rituximab will be of interest.

### ***Apoptosis as a Therapeutic Target***

The combination of dysregulated cellular proliferation and dysregulated cell death is the main cellular characteristic in the development of malignant disease. The apoptotic pathway presents multiple targets for anticancer therapy by virtue of the plethora of proteins involved in control and signaling of programmable cell death.

The indolent lymphomas and CLL are lymphoacumulative disorders, not lymphoproliferative diseases, and these diseases result from a defect in programmed cell death.<sup>53</sup> There are two distinct but interconnected apoptotic pathways: the cell-extrinsic pathway, which involves the tumor necrosis family (TNF) of proteins and death receptor domains; and the intrinsic pathway, which is mitochondrial-based and is mediated by members of the BCL2 superfamily. The activation of effector caspases 3, 6, and 7 is integral to both pathways, as the caspases initiate methodical degradation of critical proteins and DNA, leading to programmed cell death. Other proteins, such as survivin, inhibit apoptosis.

A number of small molecules are in development that target the apoptotic pathways. Some of the most widely studied agents are those that target BCL2 and the BCL2 family, survivin, and antibodies that target TRAIL (TNF-related apoptosis-inducing ligand) death receptors 4 and 5.

Oblimersen sodium is an antisense oligonucleotide compound designed to specifically bind to *bcl-2* mRNA. This results in degradation of the *bcl-2* mRNA and decreased *bcl-2* protein translation leading to increased levels of apoptosis. Oblimersen sodium has been studied in a variety of histologies in combination and as a single agent. Most recently, 5-year follow-up data was published from an earlier randomized phase III trial of patients with relapsed or refractory CLL who were treated with fludarabine and cyclophosphamide with or without oblimersen.<sup>54</sup> The regimen with oblimersen significantly increased the major response (CR plus nodular PR) rate versus fludarabine and cyclophosphamide without oblimersen, 17% (11 CRs and 9 nodular PRs) vs 7% (3 CRs and 5 nodular PRs), respectively. The follow-up data also showed that 60% (12/20) of patients who received oblimersen were alive after 5 years, compared to 38% (3/8) who did not. Furthermore, 5 patients in the oblimersen arm continued in remission and 2 patients

who relapsed did not require further therapy. In the arm that did not receive oblimersen, no patients remained in remission and 3 patients who relapsed required further therapy. Fludarabine-sensitive patients treated with oblimersen and achieved a CR or nodular PR had a significant survival advantage over those patients who did not receive oblimersen.

Several BH3 mimetics are being studied in a variety of histologies. Obatoclox mesylate, a pan-BCL2 family inhibitor, was active and well tolerated as a single agent in patients with CLL.<sup>55</sup> When used in combination with bortezomib, obatoclox has shown some activity in patients with relapsed MCL and in CLL cell lines *ex vivo* and in MCL lines *in vitro* and *ex vivo*. AT-101 is an orally available small molecule that targets BCL2, BCL-XL, and MCL-1. In a phase II study, AT-101 was used in combination with rituximab in relapsed or refractory CLL patients, and some partial responses have been observed. Responses have also been reported with ABT-263 in patients with bulky CLL.<sup>56</sup> YM155, a small molecule that targets survivin, induced partial responses in 3 of 5 (60%) patients with NHL (2 DLBCL and 1 FL) in a phase I dose-escalation study.<sup>55</sup> A phase II study in DLBCL patients is ongoing.<sup>56</sup>

Antibodies against TRAIL receptors are also being studied for patients with NHL and CLL. Apomab, a recombinant human APO2L/TRAIL antibody, is currently being studied in combination with rituximab in patients with NHL who have relapsed following therapy with rituximab.<sup>57</sup>

**Flavopiridol** Flavopiridol is a semisynthetic flavone derivative that has demonstrated efficacy in fludarabine-refractory, high-risk CLL patients when administered with a pharmacologically derived schedule.<sup>58</sup> Updated results showed an overall response rate of 46%.<sup>59</sup> Response rates were around 45% in patients with unfavorable features such as *del*(17p13), a complex karyotype, or bulky adenopathy; and 59% of patients *del*(11q22) responded. However, flavopiridol's major toxicities include severe neutropenia, thrombocytopenia, diarrhea, and abnormal liver chemistries. Tumor lysis syndrome was a life-threatening and fatal complication until patient selection improved by excluding patients with circulating lymphocyte counts greater than 200,000/mm<sup>3</sup>.

### **Conclusion**

A wealth of new agents is emerging with potential to treat NHL and CLL effectively. These new drugs may enable physicians to overcome the clinical problems presented by refractory or resistant disease, but several key challenges must be addressed to optimize therapy. Firstly,



how should these agents be prioritized once available for general use? Secondly, what are the optimal study designs for these agents, particularly if the aim of the studies is approval by the FDA or other agencies? Specifically, how can rational combinations be developed? Finally, to get new and effective drugs to market, it is essential to accrue patients to well-designed clinical research trials.

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# German Experience with Bendamustine in B-cell Lymphoid Malignancies

Mathias J. Rummel, MD, PhD

Dr. Rummel is Chief, Department of Hematology at Klinik der Justus-Liebig-Universität Giessen in Giessen, Germany.

Address correspondence to:  
Mathias J. Rummel, MD, PhD,  
Leiter des Schwerpunktes  
Hämatologie, Universitätsklinik  
Giessen, Klinikstrasse 36, D-35392  
Giessen, Germany  
E-mail: mathias.rummel@innere.  
med.uni-giessen.de

**Abstract:** Non-Hodgkin's lymphoma (NHL) is a challenging disease to treat, due to an inability to achieve a cure with conventional therapeutic regimens and a high likelihood of relapse after treatment. There are many subtypes of lymphoma; some are indolent, such as follicular lymphoma (FL), while others are aggressive and require aggressive therapies. Bendamustine, a novel agent that is a hybrid of nitrogen mustard with a purine antimetabolite, has shown promise in relapsed/refractory patients with FL and other indolent lymphomas. In a multicenter phase II study, bendamustine as a single agent has induced responses even in heavily pretreated patients. Several German clinical trials have explored and are continuing to explore the effects of bendamustine in combination with other agents for lymphomas. Bendamustine has been combined with rituximab in several trials with promising results. Other trials have combined bendamustine with fludarabine, vincristine and prednisolone, or mitoxantrone with or without rituximab. These studies by the German groups should offer new insights into the utility of bendamustine in the treatment of B-cell lymphomas.

## Introduction

Bendamustine was developed in 1963 in East Germany, and hence, most of the experience with bendamustine comes from Germany. Bendamustine is a hybrid of nitrogen mustard alkylator with a purine analog antimetabolite, combining the pharmacologic properties of several agents into one compound. Bendamustine has a dual mechanism of action: damaging cancer cell DNA, which leads to apoptosis, and causing cell death through mitotic catastrophe. The U.S. Food and Drug Administration recently approved bendamustine for the treatment of chronic lymphocytic leukemia (CLL), but the indication has not yet been widened for lymphoma.

In low-grade and mantle cell lymphomas, bendamustine is widely used in Germany both in ongoing studies and in the clinic. It has been used in combination with rituximab, an anti-CD20 monoclonal antibody, for a large variety of B-cell neoplasms with great success. Bendamustine is also widely used in patients with multiple myeloma, where the combination of bendamustine and prednisone has been shown to

### Keywords:

follicular lymphoma, bendamustine, purine antimetabolite, nitrogen mustard, combination therapy, fludarabine, vincristine, prednisolone, mitoxantrone, rituximab

be superior to melphalan plus prednisone in a large randomized study.<sup>1</sup> Bendamustine is also active in CLL, both as a single agent and in combination with other agents, such as rituximab and mitoxantrone.<sup>2-5</sup> Although bendamustine is given in relapsed and refractory Hodgkin's disease, its efficacy has not yet been established in clinical trials.

Bendamustine has also been administered in the treatment of some solid tumors: a phase III trial in untreated breast cancer showed that a combination of bendamustine, methotrexate, and 5-fluorouracil prolongs progression-free survival when compared to a combination of cyclophosphamide, methotrexate, and 5-fluorouracil;<sup>6</sup> and another study showed that bendamustine has some efficacy in small cell lung cancer, where it has been used with palliative intent in first- and second-line treatment.<sup>7</sup>

### Single-agent Bendamustine

Bendamustine as a single agent was well-tolerated and effective in German trials with patients with relapsed low-grade non-Hodgkin's lymphoma (NHL) and aggressive relapsed or refractory NHL. Bendamustine induced a response ranging from 76.5%<sup>8</sup>–83%<sup>9</sup> in relapsed low-grade disease, and a 44% response rate in patients with aggressive relapsed or refractory NHL.<sup>10</sup> The results of these trials led to the initiation of several trials of bendamustine in combination with various agents for NHL.

### Bendamustine, Vincristine, and Prednisolone

German trials with a combination of bendamustine, vincristine, and prednisolone (BOP) showed high response rates in patients with advanced low-grade NHL and mantle cell lymphomas. In heavily or pretreated relapsed or refractory indolent lymphoma patients, 86% of patients in a phase II study achieved a response to therapy, with 45% complete remissions (CR) and 41% partial remissions (PR).<sup>11</sup> The mean duration of remission was 16.1 months, and grade 3 or 4 leukopenia occurred after 8% of treatment cycles. Thrombocytopenia and anemia were rare, occurring after 3% and 4% of cycles, respectively.

In a subsequent randomized phase III trial in patients with previously untreated advanced indolent NHL and mantle cell lymphoma, BOP compared positively to a standard regimen of cyclophosphamide, vincristine, and prednisone (COP).<sup>12</sup> The CR rates for both arms were similar, with 22% of patients randomized to BOP achieving CR vs 20% of patients randomized to COP. Safety was also comparable, but alopecia and leukopenia were more prevalent in patients treated with COP.

### Bendamustine and Fludarabine

A multicenter phase I/II study by the East German Society of Hematology and Oncology examined the efficacy and toxicity of the combination of bendamustine and fludarabine in 29 patients with relapsed or refractory indolent lymphoma.<sup>13</sup> All mantle cell lymphoma patients (n=9) experienced a response to treatment, and the overall response rate (ORR) across all histologies was 77% (45% CR and 32% PR). However, more than half of the responders (8 of 15) relapsed after a median follow-up time of 14 months. Hematotoxicity was common, with 47% of patients experiencing grade III adverse events and 26% experiencing grade 4 adverse events. Four patients (21%) experienced neutropenic fever.

### Bendamustine and Rituximab

In vitro studies of bendamustine and rituximab in lymphoma cell lines showed that the combination is synergistic and rational.<sup>14,15</sup> In the low-grade lymphoma cell line DOHH-2, 10 mg/mL of bendamustine induces a 30% apoptosis rate, but when rituximab is added, a significantly lower dose of bendamustine is needed to induce the same rate of apoptosis.<sup>15</sup> Single-agent rituximab did not saturate the CD20 surface antigens on the cells and could not induce apoptosis. These results suggest that there is a synergistic effect between rituximab and bendamustine.

These in vitro studies led to a phase II study of bendamustine and rituximab in relapsed and refractory indolent and mantle cell lymphoma.<sup>16</sup> The trial included 63 patients, 33% of whom were refractory to their last treatment, though none of the patients was pretreated with rituximab. Twenty-four patients had follicular lymphoma, 16 had mantle cell lymphoma, 17 had small lymphocytic lymphoma, and 6 had marginal zone lymphoma. The drug was well-tolerated with a good toxicity profile. Only 16% of patients experienced grade 3 and 4 leukocytopenia, and thrombocytopenia and anemia were rarely seen (3% and 1%, respectively). There was no observed cardiotoxicity or neurotoxicity, but 43% of patients experienced grade 1 nausea and vomiting lasting for 1 week after treatment, in spite of receiving effective antiemetic drugs.

For all histologies, a 90% ORR was observed, including a 60% CR rate and a 30% PR rate. Of the 16 mantle cell lymphoma patients, 7 of whom were refractory to a previous regimen of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), 12 (75%) experienced a response. All but one follicular lymphoma patient experienced a response (23/24, 96%); 83% (5/6) marginal zone lymphoma patients achieved a response; and all 17 small lymphocytic lymphoma patients had a response.

A subsequent German phase III study in indolent NHL and mantle cell lymphoma compared combination bendamustine and rituximab to a regimen of CHOP plus rituximab (CHOP-R),<sup>17</sup> because CHOP-R is very widely used and has been proven superior to other regimens in German studies.<sup>18-21</sup> The combination of bendamustine (90 mg/m<sup>2</sup> for 2 days) and rituximab (375 mg/m<sup>2</sup>) was given every 4 weeks for 6 courses, and CHOP-R was given on a 21-day schedule. To be included in the study, patients had to be treatment-naïve with CD20-positive low-grade lymphoma. Patients also had to have stage III or IV disease and a histology younger than 6 months.

The primary objective of the trial was to prove the noninferiority of combination bendamustine and rituximab vs CHOP-R, defined as a difference of less than a 15% progression-free survival after 3 years. The secondary objectives were response rates, a safety profile, survival, and the capacity of mobilizing stem cells after the end of treatment for a later relapse. The study was expected to enroll 214 patients in 4 years, but after 2 years, accrual had already reached 200 patients. Therefore, the primary objective was amended to reflect a difference in progression-free survival of less than 10% after 3 years.

The first interim analysis included data from 315 patients: 166 patients had follicular lymphoma; 44 had immunocytoma; 62 had mantle cell lymphoma; and 43 had marginal zone lymphoma. The overall median age was 64 years, though the median age of the mantle cell lymphoma patients was 70 years. One hundred sixty-six patients were randomized to receive bendamustine and rituximab, and 149 patients received CHOP-R. Many of the patients had unfavorable prognosis: more than half of the patients had a Follicular Lymphoma International Prognostic Index (FLIPI) score of 3 or more; more than 70% had stage IV disease; and more than 70% had bone marrow involvement.

The toxicity profile of bendamustine and rituximab was lower than the toxicity profile of CHOP-R, with less alopecia (0% vs 94%), less leukocytopenia of grade 3 or 4 (16% vs 41%), and a lower infection rate (23% vs 41%). With 315 patients evaluable, the response rate in both treatment arms was 93%. Similarly, there was not a significant difference between bendamustine and rituximab vs CHOP-R in CR rates (47% vs 42%), stable disease rates (3% vs 4%), and primary refractory patients (4% vs 3%). During the first 18-month observation period, 33 patients in the bendamustine and rituximab treatment arm had relapsed, compared to 43 patients in the CHOP-R arm, although the number of deaths was the same for both regimens (13 vs 14).

Response rates were comparable when divided by subtype, but progression rates differed somewhat. A smaller proportion of mantle cell lymphoma patients progressed

in this first interim analysis during the median observation period of 18 months in the bendamustine and rituximab arm when compared to the mantle cell patients on the CHOP-R arm (24% vs 41%). Marginal zone lymphoma and small lymphocytic lymphoma patients also progressed less on the bendamustine and rituximab arm (9% vs 29% and 13% vs 28%).

Progression rates were comparable between the 2 arms for follicular lymphoma patients (23% vs 24%). Therefore, interim results show that the progression-free survival associated with the bendamustine and rituximab regimen is not inferior to progression-free survival after treatment with CHOP-R. Additionally, the overall survival rates for both arms are almost identical.

In conclusion, in this first and premature interim analysis, the combination of bendamustine and rituximab appeared to be not inferior to CHOP-R in regard to efficacy. Furthermore, the combination of bendamustine and rituximab is associated with less toxicity. The final results with evaluation of all 549 randomized patients with a longer observation period will further define the role of bendamustine and rituximab in the treatment algorithms for patients with indolent and mantle cell lymphoma.

There is also an ongoing phase II trial in Germany examining the combination of bendamustine and rituximab in patients with relapsed or refractory aggressive B-cell NHL.<sup>22</sup>

## Bendamustine, Mitoxantrone, and Rituximab

Several German studies have explored the use of a combination regimen of bendamustine, mitoxantrone, and rituximab (BMR) in indolent lymphomas. In the pilot study of BMR in 20 patients (4 with high-grade lymphoma, 12 with indolent lymphoma, and 4 with CLL), the ORR was 95% (CR 35% and PR 60%).<sup>23</sup> Symptomatic hematotoxicity of grade 3 or 4 occurred in 20% of patients, unsymptomatic grade 3 or 4 hematotoxicity occurred in 45% of patients, and there was no observed major nonhematologic toxicities.

The final results of the pilot study included 52 patients (21 with B-CLL, 1 with B-PLL, 8 with lymphoplasmacytic lymphoma, 14 with follicular lymphoma, 2 with mantle cell lymphoma, 2 with marginal zone lymphoma, and 6 with secondary high-grade lymphoma) who achieved an ORR of 96% (41% CR and 55% PR).<sup>24</sup> The median time to progression for lymphoma patients had not been reached at a median of 27 months, and the regimen was well-tolerated.

A subsequent phase II study conducted by the German Low Grade Lymphoma Study Group explored BMR in 57 rituximab-pretreated, relapsed, or refractory indolent or mantle cell lymphoma patients.<sup>25</sup> The ORR was



**Table 1.** Summary of Clinical Trials with Bendamustine in Lymphoma

Regimen	Source	Phase trial	Patients n	Histology	ORR (%)
Single-agent bendamustine	Heider 2001 <sup>9</sup>	II	52	Relapsed low-grade NHL	83
	Bremer 2002 <sup>8</sup>	II	102	R/R low-grade NHL	76.5
	Weidmann 2002 <sup>10</sup>	II	18	Aggressive R/R NHL	44
BOP	Kath 2001 <sup>11</sup>	II	22	Pretreated R/R indolent lymphoma	86
	Herold 2006 <sup>12</sup>	III	164	Untreated advanced indolent NHL and MCL	22 (CR)
BF	Koenigsmann 2004 <sup>13</sup>	I/II	29	R/R indolent lymphoma	77
			9	MCL	100
BR	Rummel 2005 <sup>16</sup>	II	63	R/R indolent lymphoma and MCL	90
	Rummel 2007 <sup>17</sup>	III	273	Untreated indolent NHL and MCL	93
BMR	Weide 2002 <sup>23</sup>	I	20	HG lymphoma, indolent lymphoma, CLL	95
	Weide 2004 <sup>24</sup>	I	52	CLL, PLL, lymphoplasmacytic lymphoma, FL, MCL, MZL, secondary HG lymphoma	96
	Weide 2007 <sup>25</sup>	II	57	Rituximab-pretreated R/R indolent lymphoma and MCL	89

BF=bendamustine and fludarabine; BMR=bendamustine, mitoxantrone, rituximab; BOP=bendamustine, vincristine, and prednisolone; BR=bendamustine and rituximab; CLL= chronic lymphocytic leukemia; CR=complete response; FL=follicular lymphoma; HG=high-grade; MCL=mantle cell lymphoma; MZL=marginal zone lymphoma; NHL=non-Hodgkin's lymphoma; ORR=overall response rate; PLL=prolymphocytic leukemia; R/R=relapsed/refractory.

89% (35% CR and 54% PR), and, among those who had been pretreated with rituximab-containing regimens, the ORR was 76% (38% CR and 38% PR). Patients with follicular lymphoma and mantle cell lymphoma had a 2-year overall survival rate of 60%, and the median progression-free survival was 19 months after a median observation time of 27 months. The frequency of myelosuppressive grade 3/4 toxicities was high (78% leukocytopenia, 46% granulocytopenia, 16% thrombocytopenia, 10% anemia), but reversible. The addition of mitoxantrone does not improve on the expected efficacy of the combination of bendamustine and rituximab but worsened the toxicity profile. Combinations of bendamustine and mitoxantrone appear therefore discouraged for future perspectives.

## Conclusions

The combination of bendamustine with other chemotherapeutic agents has produced great results in several histological subtypes of lymphoma (Table 1). Addition-

ally, several trials with bendamustine are still ongoing. These new bendamustine-containing regimens promise to improve outcomes for patients with untreated or relapsed or refractory lymphoma, as bendamustine has improved outcome for patients with CLL.

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# The US Clinical Experience with Bendamustine

Jonathan W. Friedberg, MD

Dr. Friedberg is Associate Professor of Medicine and Oncology at University of Rochester in Rochester, NY.

Address correspondence to:  
Jonathan W. Friedberg, MD  
University of Rochester  
601 Elmwood Avenue, Box 704  
Rochester, NY 14612  
Email: jonathan\_friedberg@URMC.rochester.edu

**Abstract:** Bendamustine is a promising new agent for the therapy of patients with non-Hodgkin's lymphoma (NHL) who are resistant or refractory to therapy with rituximab, a widely used anti-CD20 monoclonal antibody. Bendamustine is an alkylating agent with a unique chemical structure. It has been used successfully in a variety of malignancies in Germany. Bendamustine has received approval in the United States to treat chronic lymphocytic leukemia (CLL). Recent US clinical trials with bendamustine have produced promising results for patients with indolent NHL and mantle cell lymphoma (MCL). Three trials of bendamustine have been completed in the United States: two single-agent studies in the rituximab-refractory population, and one trial of bendamustine plus rituximab in the rituximab-sensitive population. Ongoing studies are also examining combinations of bendamustine with rituximab and the proteasome inhibitor bortezomib.

## Introduction

Bendamustine is an alkylating agent that also has a benzimidazole ring found on purine analogs. Bendamustine induces durable DNA damage in vitro, resulting in rapid cell death through apoptosis and mitotic catastrophe.<sup>1</sup> Historically, European studies have reported that bendamustine has single-agent activity in patients with non-Hodgkin's lymphoma (NHL),<sup>2</sup> chronic lymphocytic leukemia (CLL),<sup>3</sup> multiple myeloma,<sup>4</sup> and breast cancer.<sup>5</sup> Bendamustine was initially developed in the United States by Salmedix, Inc., which was later acquired by Cephalon, Inc., and bendamustine was approved by the US Food and Drug Administration for the treatment of CLL in March 2008.

## Bendamustine: US Development

Several considerations were taken into account during the development program for bendamustine in NHL in the United States. First, despite more than 40 years of experience with the drug, there is no modern phase I trial, and multiple doses and schedules have been reported. Although a Japanese phase I study has recently been completed, when

**Keywords:** NHL, rituximab resistant, alkylating agent, bendamustine, combination therapy, bortezomib

work started with bendamustine in the US, no sound data were available. In addition, very little data existed on the single-agent activity of bendamustine in the rituximab era. From the earlier German studies, there was a lack of rigorous follow-up and adequate toxicity experience. Furthermore, according to the US medical community at the time, a new group of patients with rituximab-refractory indolent lymphoma had an unmet medical need for an effective, durable treatment.

### ***Clinical Studies on Bendamustine***

Development of the drug in the US has been based largely on 3 clinical trials: a phase II single-agent study for rituximab-refractory patients with indolent lymphoma;<sup>6</sup> a subsequent phase III or pivotal, single-arm trial to confirm the results of the phase II study;<sup>7</sup> and a phase II study that combined bendamustine and rituximab for patients with indolent lymphoma.<sup>8</sup> The plan for CLL registration was to use European data. Therefore, despite FDA approval in CLL, there are no published US studies of bendamustine in CLL.

**Single-agent Phase II Study** This multicenter, single-agent study of bendamustine enrolled patients with relapsed, indolent, or transformed B-cell NHL refractory to rituximab.<sup>6</sup> Rituximab refractoriness was defined by the lack of a response to a rituximab-containing regimen or a response that endured less than 6 months. Bendamustine was given in 120 mg/m<sup>2</sup> doses intravenously over 30–60 minutes on days 1 and 2 every 21 days for a planned 6 cycles of treatment, although patients could receive more cycles at the discretion of the treating physician.

The median age of the patients was 63 years (range: 38–84). As was expected, the vast majority of patients had advanced stages of disease (stage III or IV, 30% and 58%, respectively). Within the indolent lymphomas, the majority of patients (61%) had follicular lymphoma; and there were smaller numbers of patients with other indolent histologies. However, 20% of patients had transformed indolent lymphoma.

The patient population was relatively heavily pretreated. As defined in the entry criteria, all patients had been treated with prior rituximab, and most patients (52%) had been treated with 2 or more prior chemotherapy regimens. This was also one of the first prospective trials that had an appreciable number of patients previously treated with radioimmunotherapy (12%); in this trial all prior radioimmunotherapy was ibritumomab tiuxetan. Furthermore, the majority of patients (62%) had received at least 3 prior systemic regimens.

Overall, the response rate to single-agent bendamustine was high (77%), and more than one-third of patients

(34%) achieved a complete response. Though the majority of responses were of relatively short duration, there was a group of patients that seemed to derive particular benefit from this drug, with responses lasting more than 2 years. Patients with transformed disease had a shorter median progression-free survival (4.2 months) than those with indolent disease (8.3 months).

Of the 23 enrolled patients who were refractory to prior alkylating agent-containing regimens, the majority were refractory to rituximab in combination with cyclophosphamide, vincristine, and prednisone (CVP) or cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). A few patients were refractory to chlorambucil along with rituximab. More than 61% of the patients refractory to prior alkylators achieved an overall response, with an equal number achieving complete and partial responses. The complete response rate for this population was similar to the complete response rates for the entire group of patients. These results suggest that there is a difference between bendamustine and the other alkylating agents currently used in the US.

The trial led to the conclusion that, in the US, rituximab-refractory patients are often refractory to rituximab-containing combinations. Furthermore, many of these patients have been treated aggressively, including prior autologous stem cell transplant or radioimmunotherapy. However, single-agent bendamustine was generally well-tolerated and showed a high response rate in this group of patients, including patients who were refractory to alkylator therapies.

Though single-agent bendamustine was well-tolerated overall, the median number of cycles completed was 5. Only 45% of the patients completed the original goal of 6 cycles. The main reason for termination of therapy was adverse events, of which most (13/20) were cytopenias. There were 3 cases of myelodysplasia that occurred post-therapy, but given extensive prior therapy with radiation, radioimmunotherapy, and multiple cycles of chemotherapy, it is difficult to determine the contribution of bendamustine to the development of myelodysplastic syndromes in these patients.

Of note, the dose and schedule were more intense than the regimen used in German studies of bendamustine plus rituximab (120 mg/m<sup>2</sup> for 2 days every 3 weeks vs 90 mg/m<sup>2</sup> for 2 days every 4 weeks).<sup>9</sup> Many patients who delayed treatment for 1 week were able to receive treatment, and several patients taken off the study might have tolerated dosing at a longer interval.

**Phase III, Single-agent, Pivotal Trial** To confirm these results, a pivotal trial<sup>7</sup> was designed with a very similar patient population (median age, 60 years), though patients with transformed disease were excluded because

they did so poorly on the phase II trial. A larger percentage of patients had received prior radioimmunotherapy (24% vs 12%), and most (71%) had intermediate- or high-risk follicular lymphoma, according to the Follicular Lymphoma International Prognostic Index (FLIPI). Patients had received a median of 2 prior therapies, and 36% of patients enrolled were refractory to their last chemotherapy regimen. In order to confirm the results of the phase II study, the dose and schedule remained the same (120 mg/m<sup>2</sup> for 2 days every 3 weeks).

The results were very similar to the results observed in the phase II trial. As of this writing, these results are only available in abstract form. The median progression-free survival was 9.3 months, which was virtually identical to the progression-free survival of patients with indolent lymphoma from the phase II trial (median 9.0 months). The overall response rates were also similar (75% vs 77%), though the CR rate (including unconfirmed) was lower (17% vs 34%). There were some hematologic toxicities, especially reversible myelosuppression and grade 3–4 cytopenias. In addition, there were 8 deaths, most due to infections, but some may potentially have been related to disease progression. Further interpretation of these results will require a peer-reviewed publication.

**Combination Bendamustine and Rituximab** In vitro work performed in Germany suggests synergy when bendamustine is combined with rituximab, enhancing antilymphoma activity.<sup>10</sup> Given survival benefits observed when rituximab is combined with cytotoxic therapy for follicular lymphoma in the de novo<sup>11</sup> and relapsed setting,<sup>12</sup> it is clear that development of a novel cytotoxic drug must include evaluation in combination with rituximab. A German phase II trial combining bendamustine and rituximab has been published, suggesting very high response rates, and long progression-free survival in both indolent and mantle cell histologies.<sup>9</sup> Therefore, a United States trial was designed using the same dose and schedule as the German experience, 90 mg/m<sup>2</sup> intravenously for 2 days consecutively with rituximab for 4 to 6 cycles. The trial enrolled 66 patients, 56 with indolent histology and 10 with small lymphocytic lymphoma (SLL). The patients had to be sensitive to rituximab, making them a less heavily pretreated group when compared to the patients enrolled on the single-agent bendamustine studies described above.

The results of the US study<sup>8</sup> were very similar to the results of the German study. There was a very high overall response rate (92%) and a significantly longer median progression-free survival (23 months vs ~10 months), which may be due to the patient population being less heavily pretreated. Of the 12 patients with mantle cell

lymphoma, 42% achieved a CR, and the median duration of response was 19 months. The results compare favorably to any other drug that has been studied in a relapsed mantle cell population. The toxicity profile in combination was similar to the German study, with grade 3–4 neutropenia occurring in 36% of patients. Less thrombocytopenia occurred than in the single-agent studies, perhaps due to the cycle duration of 4 weeks rather than 3.

These results confirmed the synergy observed in the laboratory, and the German and US studies have had nearly identical results. When the data on mantle cell patients from the German and US studies are combined, the results are promising, and de novo mantle cell lymphoma may be an appealing population for future studies with bendamustine.

### *New US Trials with Bendamustine*

Two phase II studies are ongoing in the US using bendamustine in combination with rituximab and the proteasome inhibitor bortezomib,<sup>13,14</sup> though the schedules vary slightly. In a study developed at the University of Rochester, in conjunction with the University of Nebraska and Cornell University,<sup>14</sup> patients with relapsed or refractory indolent or mantle cell NHL are given 1.3 mg/m<sup>2</sup> bortezomib on days 1, 4, 8, and 11; 90 mg/m<sup>2</sup> bendamustine on days 1 and 4; and 375 mg/m<sup>2</sup> rituximab on day 1. In the multicenter study,<sup>13</sup> patients with relapsed or refractory follicular lymphoma who have previously received 4 or more doses of rituximab receive bortezomib intravenously on days 1, 8, 15, and 21 of a 5-week cycle; 50 mg/m<sup>2</sup> bendamustine on days 1 and 2 of each cycle; and rituximab at 375 mg/m<sup>2</sup> on days 1, 8, 15, and 22 of cycle 1 and on day 1 of cycles 2, 3, 4, and 5. These trials may create opportunities to use bendamustine in combination with some other newer targeted agents.

### **Conclusions**

Bendamustine has significant potential as a therapeutic agent in NHL. It has significant single-agent activity in a highly relapsed population. Bendamustine has already been approved by the US Food and Drug Administration for treatment of CLL, and, based on the results of the aforementioned studies (Table 1), approval was granted for NHL in October 2008. Results are promising, particularly in mantle cell lymphoma, when used in combination with rituximab. Results from the German study using bendamustine with rituximab upfront in patients with indolent and mantle cell lymphoma may define a new role for this agent,<sup>15</sup> though the results for progression-free survival are still too immature to make decisions. Furthermore, bendamustine seems to have significant

**Table 1.** Summary of U.S. Clinical Trials with Bendamustine

Regimen	Source	Phase	Evaluable patients (n)	Histology	ORR (%)
Single-agent bendamustine	Friedberg 2008 <sup>6</sup>	II	74	Rituximab-refractory indolent NHL	77
	Kahl 2007 <sup>7</sup>	III	100	Rituximab-refractory indolent NHL	75
BR	Robinson 2008 <sup>8</sup>	II	67	Relapsed, indolent, rituximab-sensitive B-cell NHL or MCL	92

BR=bendamustine and rituximab; MCL=mantle cell lymphoma; NHL=non-Hodgkin's lymphoma; ORR=overall response rate; R/R=relapsed/refractory.

potential to incorporate into novel combination regimens in a variety of histologies.

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# Biology and New Diagnostic Tools in the Treatment of Chronic Lymphocytic Leukemia

Kanti R. Rai, MD

Dr. Rai is Chief of Hematology-Oncology at the Long Island Jewish Medical Center and Co-Director of Chronic Lymphocytic Leukemia Research and Treatment Program at the North Shore Long Island Jewish Health System of The Feinstein Institute for Medical Research in Manhasset, NY.

Send correspondence to:  
Kanti R. Rai, MD  
The Feinstein Institute for  
Medical Research  
350 Community Drive  
Manhasset, NY 11030  
Phone: (516) 470-4050  
Fax: (516) 470-4250  
Email: rai@lij.edu

## Key words:

Chronic lymphocytic leukemia (CLL),  
IgVH, ZAP-70, CD30, B cells, NFkB

**Abstract:** Increased knowledge of the biology of chronic lymphocytic leukemia (CLL), specifically on the importance of the mutation status of IgV<sub>H</sub> genes and expression of ZAP-70 and CD38, has led to debate over whether CLL should be classified as one or two distinct diseases. These characteristics are not among the criteria for diagnosis for CLL, but they each have an important prognostic impact. New techniques, such as fluorescence in situ hybridization (FISH) to determine cytogenetic status, also have further improved clinicians' ability to predict outcome. Additionally, new guidelines have been developed to assist in more correctly diagnosing and stratifying patients with CLL. It is now a challenge for us to integrate these new advances in prognostic features with decision-making for when and how to treat an individual patient with CLL.

## Biology of CLL

In light of the number of very interesting and fascinating developments recently published on the molecular biology of chronic lymphocytic leukemia (CLL), it has become unclear whether or not it should be classified as a single disease. Forty years ago, William Dameshek<sup>1</sup> and David Galton<sup>2</sup> suggested that CLL cells are immunologically naïve, functionally inert B lymphocytes. However, recent knowledge suggests that CLL is a heterogeneous disease that can have two different presentations: one in which the immunoglobulin heavy-chain variable-region (IgV<sub>H</sub>) genes have somatic mutations, called the mutated CLL cells, and the other, a presentation of unmutated IgV<sub>H</sub> CLL. Expression of unmutated IgV<sub>H</sub> confers more aggressive disease and a worse prognosis.<sup>3,4</sup>

There has also been debate over whether memory B cells are the leukemic cells in mutated and unmutated CLL. If the immunologically naïve B cells were the leukemic cells in unmutated CLL, it would be possible to note that CLL is made up of two separate diseases. Recent reports have clearly demonstrated that all CLL lymphocytes have tell-tale markers that show that the cells have previously traversed through



**Table 1.** Prognostic Markers for Outcome Prediction in CLL

Marker	Prognosis	
	Good	Poor
Lymphocyte doubling time	Slow	Rapid
Pattern of lymphocyte infiltration (BM Rx)	Non-diffuse	Diffuse
Cytogenetic analyses (FISH)	Normal, del 13q	del 11q, del 17p, Trisomy 12*
IgV <sub>H</sub> gene	Mutated	Unmutated
ZAP-70 expression	Negative	Positive
CD38 expression	Negative	Positive
MYC rearrangement <sup>25</sup>	Absent	Present

\* Present in only 15% of the patients tested<sup>16</sup>

This table outlines the prognosis based on physical and cytogenetic analyses. Combination cytogenetic abnormalities are also seen, such as ZAP-70 and CD38 expression or IgV<sub>H</sub> and chromosomal translocations/deletions. These also confer worse prognosis.

the germinal center,<sup>5,6</sup> suggesting that CLL lymphocytes are not immunologically naïve. CLL cells may be best described as splenic marginal zone B cells or the equivalent. Therefore, CLL might be derived from memory-type B cells, regardless of the mutation status of IgV<sub>H</sub>. This interpretation suggests that CLL is a single disease and that CLL lymphocytes are not immunologically naïve B cells.

### Prognostic Indicators

The prognosis of patients with CLL is affected by numerous factors, including the mutation status of the IgV<sub>H</sub> genes, mentioned above, expression of zeta-associated protein (ZAP-70), expression of CD38, and cytogenetic abnormalities determined through fluorescence in situ hybridization (FISH).

ZAP-70 is a protein tyrosine kinase (PTK) classically present on T cells and monocytes. Following T-cell receptor (TCR) ligation, ZAP-70 phosphorylates ITAMs (immunoreceptor tyrosine-based activation motifs) and initiates downstream signaling. In B cells, Syk is the PTK that initiates BCR signaling. The role of Syk in BCR signaling is analogous to the role of ZAP-70 in TCR signaling. Though ZAP-70 is not highly expressed on B cells, it plays an important role in BCR signaling by activating kinases such as Akt and ERK, which are required to induce antiapoptotic proteins.<sup>7,8</sup> In some CLL patients, ZAP-70 is expressed in CLL B cells, mostly of the unmutated variety. These ZAP-70-positive CLL cells have enhanced signaling in response to BCR ligation,<sup>9</sup> leading to enhanced response to survival signals, resistance to apoptosis, and activation of NFκB. Patients who are ZAP-70-positive are generally refractory to conventional chemotherapy and have a poorer risk of overall survival.<sup>10</sup>

Expression of CD38, a 45kDa transmembrane glycoprotein found on B cells and natural killer cells, has been found to confer worse prognosis.<sup>11</sup> A 30% expression level was confirmed to be the cutoff to determine CD38 positivity or negativity,<sup>3,12</sup> but several groups have proposed a lower cutoff level. Though early reports showed a correlation between IgV<sub>H</sub> mutation status and CD38 expression,<sup>3</sup> later studies showed that CD38 was an independent predictor of prognosis.<sup>12-14</sup>

Several cytogenetic abnormalities with prognostic significance can be determined through FISH. Based on a prognostic model by Döhner and colleagues,<sup>15</sup> deletion of 11q and deletion of 17p confer poor prognosis and shorter overall survival, and deletion of 13q was associated with the longest overall survival and median progression-free survival. The prognostic significance of trisomy 12 is somewhat unclear, as another study by Döhner and colleagues showed that patients with trisomy 12 had a prognosis between those with deletion 13q (good prognosis) and those with deletion 17p or deletion 11q.<sup>16</sup> Based on follow-up studies, deletion 17p is associated with resistance to chemotherapy, deletion 11q is associated with extensive lymphadenopathy, and deletion of 13q was associated with better prognosis (Table 1).<sup>11</sup>

Table 1 outlines the prognosis based on physical and cytogenetic analyses. Combination cytogenetic abnormalities are also seen, such as ZAP-70 and CD38 expression or IgV<sub>H</sub> and chromosomal translocations/deletions. These also confer worse prognosis.

### New Diagnostic Tools

A report from the International Workshop on CLL<sup>17</sup> recently updated the 1996 National Cancer Institute-

Working Group guidelines<sup>18</sup> for the diagnosis and treatment of CLL by resolving some of the subsequent developments on diagnostic and response evaluation criteria. In the 1996 guidelines on blood lymphocytosis, a minimum of 5,000 lymphocytes had to be present in order to make a diagnosis of CLL, though less than 5,000 lymphocytes was sufficient to make a diagnosis of small lymphocytic lymphoma (SLL), a very similar disease, if other criteria were met. The new guidelines on blood lymphocytosis suggest that there must be a minimum of 5,000 B cells, rather than just lymphocytes. Therefore, even if T cells are present, the minimum number of B cells (5,000/mm<sup>3</sup>) must be reached before a diagnosis of CLL can be confirmed.

The new guidelines require FISH cytogenetics for patients on clinical trials and also strongly recommend FISH to make distinctions in clinical practice before starting therapy for patients not participating in research protocols. However, the new guidelines say that evaluation of IgV<sub>H</sub> mutation status, ZAP-70, and CD38 expression are not required for diagnosis or treatment decisions if the patient is not participating in a research protocol, but these are always advised if the patient is participating in a clinical trial. Furthermore, these tests can be given to patients not participating in a clinical trial who would like more information on their prognosis, but physicians should stress that treatment decisions will not be based on the results of these prognostic markers, but rather on the patient's clinical stage and disease activity.

Imaging techniques, like positron emission tomography (PET) scanning, ultrasound, and computed tomography (CT) scanning, though popular in lymphoma, are not required in clinical practice in CLL, according to the new guidelines. CT scans do not affect clinical staging, though they do predict more aggressive disease in Rai stage 0 patients with detectable abdominal disease.<sup>19</sup> CT scans may still be useful in clinical trials if there is a clinical question or to evaluate response to therapy, but PET scans will probably only be justified to determine whether the patient is going into Richter transformation.<sup>20</sup>

Treatment of CLL occurring once patients become symptomatic or develop signs of rapid progression is non-curative and is directed at reducing the symptoms and the disease burden. Treatment regimens incorporating purine nucleoside analogs result in an increased rate of successful remission. More recently, combination chemoimmunotherapy regimens have produced frequent complete molecular remissions, and early evidence suggests this may result in an improved long-term survival.<sup>21</sup> Active immunotherapy strategies, such as vaccines and administration of expanded and activated T cells, are currently being explored.<sup>22,23</sup> For the refractory patient, allogeneic hematopoietic cell transplantation is the only curative

option but is not suitable for a patient of older age. Reduced intensity conditioning regimens have improved the toxicity levels, but transplant is only possible with the availability of a viable donor.<sup>24</sup> There is a paucity of randomized studies determining the efficacy and tolerability of several new agents. As these studies are conducted and the results become available, we will be able to interpret and compare clinical trials of the new therapeutic agents and evaluate them for risk-assessed treatment of CLL patients.

## Conclusions

CLL, once considered a homogeneous disease, is now recognized to be a heterogeneous malignancy with patients separated into low, intermediate, and high-risk disease based on traditional and novel prognostic factors. Treatment of CLL is in the process of rapid evolution, with a flurry of new agents being initiated into clinical trials. The recommendations of the International Workshop on Chronic Lymphocytic Leukemia, which updated the 1996 guidelines of the National Cancer Institute-sponsored Working Group (NCI-WG), will help to improve response rate and overall survival for CLL patients in clinical trials and general practice.

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# Advances in Chemotherapy: Bendamustine for Chronic Lymphocytic Leukemia

Michael Hallek, MD

Dr. Hallek is Director of the Department of Internal Medicine at Klinik I für Innere Medizin, Universität zu Köln, Germany.

Address correspondence to:  
Michael Hallek, MD  
Klinik I für Innere Medizin  
Universität zu Köln  
Joseph-Stelzmann Str. 9  
50924 Köln, Germany  
Phone: +49 (0221) 478-4400  
Fax: +49 (0221) 478-5455  
E-mail: michael.hallek@uni-koeln.de

**Abstract:** Bendamustine is promising both as a single agent and in combination for treatment-naïve and relapsed or refractory chronic lymphocytic leukemia (CLL) patients. In vitro studies have demonstrated that bendamustine can induce apoptosis in B-CLL cells as a single agent and in combination with fludarabine. The German CLL Study Group (GCLLSG) has conducted several successful trials with bendamustine, including single-agent trials as a first-line option and for heavily pretreated, relapsed/refractory CLL patients and combination trials with bendamustine and rituximab with or without mitoxantrone. Overall, bendamustine appears generally well-tolerated as both a single agent and in combination, and it has minimal cross-reaction with other therapeutics. The development of new regimens that include bendamustine promises to improve outcomes for CLL patients.

## Introduction

B-cell chronic lymphocytic leukemia (CLL) is a clonal hematopoietic disorder characterized by proliferation and accumulation of aberrant lymphocytes. The management of CLL is determined by the stage and activity of the disease. Chlorambucil, with or without steroids, has been the standard treatment for newly diagnosed CLL patients. Purine nucleoside analogs, such as fludarabine and cladribine, used in randomized studies have indicated a higher overall response and longer response duration than with chlorambucil or cyclophosphamide combination regimens. Monoclonal antibodies have also been used in combination with purine analogs for relapsed or refractory patients.

Bendamustine, a drug currently approved by the US Food and Drug Administration for the treatment of CLL, has several chemical components that confer different pharmacologic properties: it has a purine-like benzimidazole ring, which confers an antimetabolite property; a nitrogen mustard, which enables DNA alkylation; and a carboxylic acid group, which aids in water solubility. The combination of properties of bendamustine is interesting because other combina-

### Keywords:

CLL, bendamustine, combination therapy, rituximab, mitoxantrone, German CLL Study Group

**Table 1.** Early Phase I/II Trials of Single-agent Bendamustine in CLL

Reference	Phase	Patients (n)	Prior therapy	ORR (%)	CR (%)	PR (%)
Anger 1975 <sup>4</sup>	III	39	No	82	28	5
Kath 2001 <sup>5</sup>	II	23	17/23	75	30	45
Bremer 2002 <sup>6</sup>	II	15	Yes	94	7	87
Henze 2002 <sup>7</sup>	II	25	Yes	56	16	40
Aivado 2002 <sup>8</sup>	II	23	Yes	67	29	38
Köppler 2004 <sup>9</sup>	I/II	22	Yes	86	27	59
Bergmann 2005 <sup>10</sup>	I/II	16	Yes	56	12.5	31
Lissitchkov 2006 <sup>11</sup>	I/II	15	Yes	60	27	13

CR=complete response; CLL=chronic lymphocytic leukemia; ORR=overall response rate; PR=partial response.

tions of protein analogs with similar properties, such as fludarabine plus cyclophosphamide, have been shown to be beneficial in CLL. Furthermore, studies have shown that, when compared to single-agent fludarabine, progression-free survival is longer after treatment with the combination of fludarabine and cyclophosphamide.<sup>1-3</sup>

### Single-agent Bendamustine in CLL

Though bendamustine has been in use since the 1960s and it has been proved to have a high potency, there is a scarcity of phase I and phase II data on bendamustine as a single agent. The early phase I/II trials had small patient numbers (median n=23) but high response rates, with overall response rates (ORR) ranging between 56%–94% (Table 1).<sup>4-11</sup> In the German CLL Study Group phase I/II study in heavily pretreated patients, the 100 mg/m<sup>2</sup> dose of bendamustine on days 1 and 2 was determined to be too high to be tolerated and was subsequently de-escalated to 70 mg/m<sup>2</sup>.<sup>10</sup> That study shows that, though bendamustine has high activity in pretreated patients, it is myelosuppressive and, therefore, needs to be given at a lower dose or in a reduced number of courses.

A recent phase III trial compared bendamustine to chlorambucil in patients with untreated CLL.<sup>12</sup> Chlorambucil, the standard first-line treatment for CLL, was given at a very high dose, but only 39% of patients achieved a response, whereas 68% of patients treated with bendamustine achieved a response. Furthermore, progression-free survival (PFS) was much longer with bendamustine treatment (21.7 months) when compared to chlorambucil (9.3 months). A higher percentage of patients treated with bendamustine experienced grade 3

or 4 infections (5.8% vs 3.5%), but the difference was not statistically significant.

### *Bendamustine in Combination*

A few small trials have been conducted using bendamustine in combination with various agents. A German trial on the triple combination of bendamustine, mitoxantrone, and rituximab in relapsed or refractory lymphomas included 22 patients with B-cell CLL or prolymphocytic leukemia (PLL).<sup>13</sup> The ORR in CLL patients was 95%, with a complete response (CR) rate of 23%.

The same group studied the combination of bendamustine plus mitoxantrone in a phase I/II trial.<sup>9</sup> The dose of bendamustine was very high (80–240 mg/m<sup>2</sup> on days 1–3) and was given with mitoxantrone at 10 mg/m<sup>2</sup> on day 1 for up to 6 cycles. All patients had been pretreated and had advanced CLL of Binet stage B or C. Most patients (37%) were administered 150 mg/m<sup>2</sup> of bendamustine, but a significant proportion (27%) received 240 mg/m<sup>2</sup>. However, toxicity was too high for the highest-dose group. The ORR across all doses was 86%, and responses were not found to be dose-dependent. Therefore, keeping the dose of bendamustine lower for safety reasons should not affect efficacy.

The German CLL Study Group conducted a phase II trial on the combination of bendamustine and rituximab. Bendamustine was given on days 1 and 2 at 70 mg/m<sup>2</sup> for pretreated patients and 90 mg/m<sup>2</sup> for first-line treatment, and rituximab was given at 375 mg/m<sup>2</sup> in the first cycle and at 500 mg/m<sup>2</sup> for the second through sixth cycles. Preliminary results on the combination as first-line treatment have not yet been published, but interim staging and assessment for 31 pretreated patients



has been completed.<sup>14</sup> Most patients (60%) were Binet stage B. The ORR was 66.7%, which is anticipated to increase once more patients are examined; 12% achieved a CR, 54% achieved a partial response (PR), and the regimen was not associated with major toxicity.

This trial also addressed the question of how patients who had received prior fludarabine would perform when given the combination of rituximab and bendamustine. Two of 4 fludarabine-refractory patients achieved a response, and 10 of 15 fludarabine-sensitive patients achieved a response. This suggests that the bendamustine and rituximab combination may have a mechanism similar to that of the combination of fludarabine and cyclophosphamide, which is also effective in some fludarabine-refractory patients, due to the presence of similar pharmacologic components.

Additionally, the study addressed how patients with adverse cytogenetics responded to treatment. The 4 most difficult patients to treat, those with deletion of 17p, had no responses. However, responses were achieved in patients with trisomy 12, deletion of 11q, or unmutated IgV<sub>H</sub>.

A new German CLL Study Group follow-up phase III trial for first-line therapy of B-CLL will compare the German standard regimen of fludarabine, cyclophosphamide, and rituximab to a regimen of bendamustine plus rituximab (CLL10 protocol). The trial will examine whether the two regimens have similar efficacy and whether the bendamustine plus rituximab regimen is associated with lower toxicity. Accrual began in September of 2008 and is projected to be finished within 2 years. Results will not be available for 3 to 4 years.

### Dose and Schedule of Bendamustine

With all of the trials of bendamustine in CLL, it has become obvious that there is no consensus on the best schedule or dose of bendamustine. There is little pharmacokinetic data to suggest one regimen will be better than another, and currently-used schedules range from daily administration at lower doses to a once weekly schedule, all of which are well tolerated. There are similar problems with chlorambucil, another well-studied drug in CLL, and with rituximab used in combination. It is possible that the best regimens for all of these agents will never be determined because future studies will continue to use the schedules that have been used in the previously published larger trials. However, it is important to conduct systematic trials to determine the best administration schedule to optimize efficacy and minimize toxicity.

### Conclusions

The treatment of CLL has improved with the addition of bendamustine to the therapeutic armamentarium.

Bendamustine can be myelosuppressive and myelotoxic, but while the toxicities can be major, they are usually manageable. Caution must be used, especially for those patients who have been heavily pretreated. Treatment of advanced CLL with bendamustine and mitoxantrone results in a significant response rate (ORR of 73%). However, this combination is probably too toxic and will be difficult to pursue in the future, as there are less toxic treatment options available. Other bendamustine combinations, particularly bendamustine and rituximab, are generally well-tolerated and effective for treatment of CLL.

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