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Bendamustine: Mechanism of Action and Clinical Data

Abstract

Bendamustine is a chemotherapeutic agent that displays a unique pattern of cytotoxicity compared with conventional alkylating agents. Bendamustine was originally synthesized in the former East German Democratic Republic in the 1960s. It was designed to have both alkylating and antimetabolite properties. The alkylating agent properties are similar to those seen with cyclophosphamide, chlorambucil, and melphalan, and the benzimidazole ring is similar to cladribine. Molecular analyses have revealed that bendamustine differs from other alkylating agents in its mechanism of action. Differences have been observed in regard to its effects on DNA repair and cell cycle progression. Moreover, bendamustine can induce cell death through both apoptotic and nonapoptotic pathways, thereby retaining activity even in cells without a functional apoptotic pathway. Bendamustine has demonstrated significant efficacy in patients with indolent lymphomas and chronic lymphocytic leukemia (CLL), including in patients with disease refractory to conventional alkylating agents and rituximab. The toxicity profile of bendamustine is also superior to that of conventional alkylating agents. Combination therapy with bendamustine and rituximab has demonstrated superior efficacy to a standard rituximab-containing chemotherapy regimen in patients with previously untreated indolent B-cell non-Hodgkin lymphoma, and it is currently being compared against the standard first-line regimen in CLL: fludarabine, cyclophosphamide, and rituximab. Ongoing and planned studies are evaluating new strategies in which bendamustine is being combined with existing agents and with novel therapies to optimize use in different clinical settings.

Introduction to Bendamustine

Bruce D. Cheson, MD

Bendamustine could be considered either the newest "old" drug or the oldest "new" drug that we have for the treatment of hematologic malignancies. It was initially synthesized in the 1960s at the Institute for Microbiology and Experimental Therapy in Jena in the former East German Democratic Republic. It was intended to be a less expensive form of other effective drugs available at the time, such as cyclophosphamide. Bendamustine was designed to have both alkylating and antimetabolite properties and to have an acceptable toxicity profile. It has demonstrated efficacy in multiple types of non-Hodgkin lymphoma (NHL) and in chronic lymphocytic leukemia (CLL), including in patients whose disease is refractory to conventional alkylator chemotherapeutic agents.

The alkylating properties of bendamustine are similar to those in the nitrogen mustard family alkylators, such as cyclophosphamide, chlorambucil, and melphalan, and the butyric acid side chain is like that found in chlorambucil.[1] Alkylating agents have been in existence for decades, beginning with the use of nitrogen mustard in chemical warfare in World Wars I and II. People exposed to the agent developed skin alterations, blindness, lung damage, nausea, and vomiting. Nitrogen mustard was found to be mutagenic and carcinogenic, and accidental exposure led to lowering of white blood cell counts. This observation suggested that the agent might have a similar effect on cancer cells. The first intravenous treatment of lymphoma with nitrogen mustard, administered in the 1940s, yielded impressive, albeit brief, results.[1] These observations led to the subsequent development of other alkylating agents, which remain a prominent component of chemotherapeutic regimens for lymphoproliferative disorders. The alkylating agents are associated with toxicities including nausea and vomiting, blood count reductions, hair loss, infertility, and secondary malignancies. Thus, the interest in developing another alkylating agent was tepid, at best. However, molecular studies have revealed that bendamustine has a pattern of activity that differs from other DNA alkylating agents. Bendamustine activates the DNA-damage stress response, induces apoptosis, inhibits mitotic checkpoints, and induces mitotic catastrophe. Moreover, bendamustine differs from other alkylators in the type of DNA repair pathways activated. Together, these differences may explain the efficacy of bendamustine observed in a variety of clinical settings.

In the United States, the development program for bendamustine began in the early 2000s, when Salmedix, Inc., acquired the rights to the agent in North America from the German company Ribosepharm. After a series of clinical trials demonstrated the safety and efficacy of bendamustine, the agent was rapidly approved by the US Food and Drug Administration (FDA). In 2008, bendamustine was approved for use in patients with CLL and in patients with indolent B-cell NHL that has progressed during or within 6 months of treatment with rituximab or a rituximab-containing regimen.[2]

In this clinical roundtable monograph, Dr. Lorenzo Leoni will examine the molecular characteristics of bendamustine, with a focus on the ways it does and does not act as a typical alkylating agent. I will examine the clinical trial data of bendamustine in indolent lymphoma and CLL.

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The Alkylating Properties of Bendamustine

Lorenzo Leoni, PhD

Alkylating agents, including the nitrogen mustards cyclophosphamide, chlorambucil, and melphalan, as well as bendamustine, exert cytotoxic effects primarily through their effects on DNA. The first mechanism by which nitrogen mustards induce DNA damage is alkylation, a process in which an alkyl group is added to a DNA residue. Some agents may show some degree of sequence selectivity for the alkylation, although this has not yet been determined for bendamustine. This linkage between the highly reactive nitrogen mustard residue and the DNA is damaging to the DNA, and induces a number of signaling pathways involved in DNA damage repair.

DNA damage is a normal process that occurs on a regular basis in every cell in the body, and it is usually resolved through effective mechanisms of DNA repair. However, DNA repair mechanisms cannot overcome the superpharmacologic damage induced by antitumor agents. Because cells cannot recover from this damage, they initiate programmed cell death through apoptosis. Another mechanism by which alkylating agents induce DNA damage is by cross-linking strands of DNA, creating links both between strands (interstrand cross-linking) and within strands (intrastrand cross-linking). Both alkylation and DNA cross-linking can cause DNA breaks, which also activate DNA repair mechanisms. In addition to their direct effects on DNA, alkylating agents also exert indirect effects on cell division, as cells that have undergone DNA damage exhibit inhibited DNA replication and transcription.

Molecular Characteristics of Bendamustine

Extensive in vitro studies conducted over the past 15 years have revealed several characteristics of bendamustine that are not observed in other alkylating agents. These unique molecular mechanisms, which relate to the agent's effects on DNA repair and cell cycle progression, and the types of cell death induced, may translate into differences in sensitivity of tumors to bendamustine compared with other alkylating agents.

Bendamustine contains 3 elements: a 2-chloroethylamine alkylating group, a benzimidazole ring, and a butyric acid side chain (Figure 1). The 2-chloroethylamine alkylating group is common to multiple nitrogen mustard family alkylators, and the butyric acid side chain is shared by chlorambucil. However, it has been proposed that the benzimidazole ring system, which is unique to bendamustine, may provide enhanced antitumor activity.[1] The heterocyclic ring structure could allow bendamustine to better penetrate and localize within DNA and remain there for a longer period of time. This could explain the unique activity of bendamustine compared with cyclophosphamide, chlorambucil, and melphalan. This hypothesis may soon be verified by ongoing studies quantifying the effects of bendamustine on DNA.

In vitro studies have revealed differences in the nature of DNA strand breaks caused by bendamustine versus conventional alkylators. Studies in human ovarian and breast cancer cell lines have shown that bendamustine induces more DNA double-strand breaks than other alkylating agents, including melphalan, cyclophosphamide, and carmustine.[2] Moreover, the DNA double-strand breaks induced by bendamustine persist longer than those induced by other alkylating agents.[2]

The more extensive and more durable DNA breaks induced by bendamustine may reflect a difference in the way bendamustine acts on the DNA, or they may suggest that bendamustine exerts effects on DNA repair mechanisms that render them ineffective.

Effects of Alkylating Agents on DNA Repair Mechanisms

Multiple DNA repair mechanisms play a role in the detoxification of cells that have undergone DNA damage. These include base excision repair, homologous recombination, the DNA mismatch-repair system, and alkyltransferase-based DNA repair.

In 2008, my colleagues and I reported results from a series of molecular studies comparing the mechanisms of different DNA-alkylating agents.[3] Microarray gene expression profiling, real-time polymerase chain reaction, immunoblot, cell cycle, and functional DNA damage repair analyses revealed several notable differences between bendamustine and other DNA-alkylating agents.

Our first observation related to the type of DNA repair mechanisms induced by the agents. Whereas the conventional alkylators cyclophosphamide and mel-
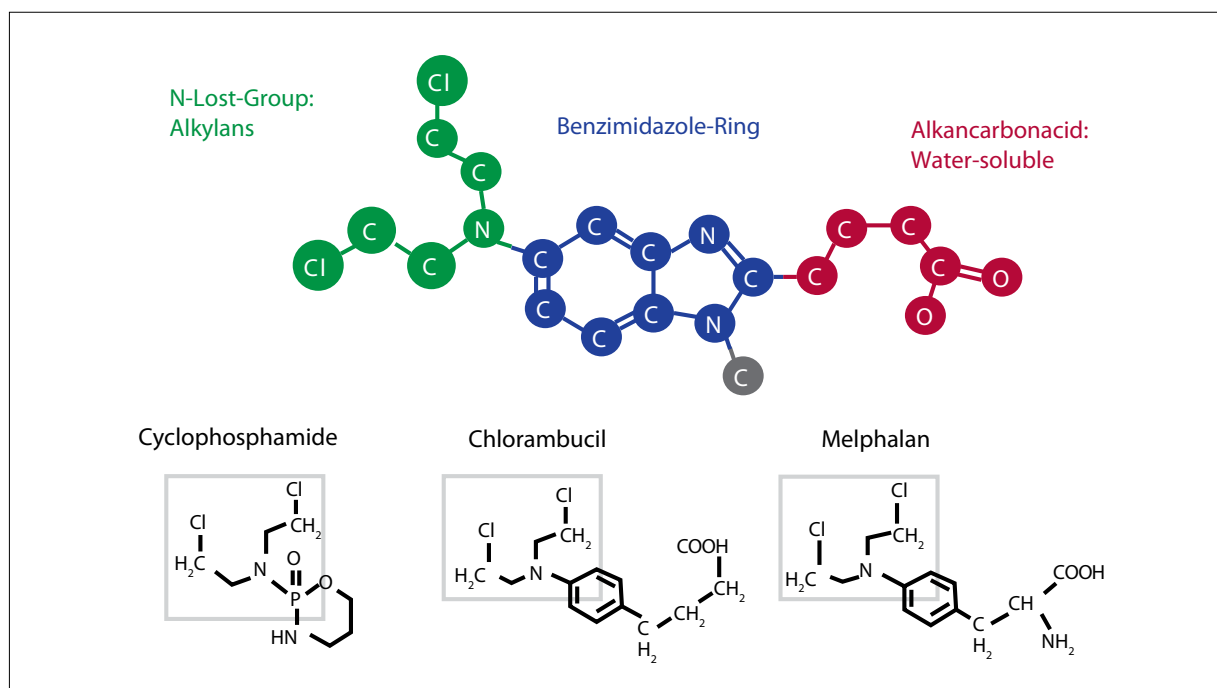
Figure 1. The chemical structure of bendamustine shares similarities with other alkylating agents.

phalan induce alkyltransferase DNA repair mediated by methylguanine methyltransferase, bendamustine activates a base excision DNA repair pathway. This was a rather surprising finding, and it appears to be important to the unique activity of bendamustine. By inducing a more complex DNA repair mechanism, bendamustine may slow the cells' capacity to efficiently repair the damage. This could also lead to the activation of signaling pathways downstream of DNA repair, and could delay cell replication. Such a delay could negatively affect cell cycle division or passage into mitosis in cells with a sufficiently high mitotic index. Our studies indicated that bendamustine does indeed induce changes in genes associated with DNA replication and cell cycle progression.[4]

Mechanisms of Cell Death Induced by Alkylating Agents

A failure to effectively and efficiently repair DNA damage can result in apoptosis. The DNA-alkylating agents are very efficient in inducing apoptosis via the conventional pathway mediated by p53 and caspases. However, cancer cells can have impaired apoptosis, or they can develop mechanisms to overcome apoptosis, rendering them resistant to many alkylating agents. Both preclinical studies[2,4] and clinical trials[5] have shown that bendamustine retains activity in cancer cells that are resistant to conventional alkylating agents.

Our molecular analyses revealed a potential mechanism for this activity: in addition to inducing apoptosis, bendamustine also appears to induce cell death through an alternative mechanism called mitotic catastrophe.[3] This necrotic form of cell death, which is morphologically distinct from apoptosis, occurs in cells that enter mitosis with significant DNA damage and has been observed in cells lacking functional p53 or caspases.[6,7] Thus, bendamustine retains activity even in the absence of a functional apoptotic pathway.[3]

Bendamustine also appears to induce a potent, rapid adenosine triphosphate (ATP) depletion. Although the actual mechanism of this effect is unknown, it occurs independently from apoptosis. ATP depletion may serve to weaken cells, making them more susceptible to nonapoptotic cell death through a metabolic shutdown. Bendamustine has also been shown to induce reactive oxygen species stress pathways.[8] Many of these pathways appear to converge around mitochondria, which are critical in maintaining energy levels within the cell. Thus, metabolic equilibrium may also be a target of bendamustine, either directly or indirectly.

Our gene expression profiling studies showed that bendamustine specifically inhibits the expression of genes involved in DNA repair and mitotic checkpoints. Modulation of DNA repair genes could have an amplifying effect. To some extent, this finding is complementary to our other observations about the activity of bendamus-

tine. If bendamustine can specifically intercalate into DNA and inhibit mitotic checkpoints, this could explain why cells treated with bendamustine appear to proceed through mitosis and then exit mitosis to mitotic catastrophe, essentially committing a type of suicide. At the same time, the ability of bendamustine to modulate the expression of DNA repair genes could explain why the DNA damage induced by the agent is so durable and cannot be efficiently repaired, as was observed 15 years ago by Strumberg and colleagues.[2] Additional studies are needed to further investigate these hypotheses.

The contribution of other nonapoptotic pathways of cell death to the activity of bendamustine cannot be excluded. Overall, bendamustine may affect multiple cellular events, which can contribute to the agent's demonstrated activity in drug-resistant tumors. This activity is supported by clinical evidence and in vitro studies showing a lack of cross-resistance with other alkylating agents.

Bendamustine Exhibits a Unique Cytotoxicity Profile

In our 2008 study, my colleagues and I compared the anticancer activity of various alkylating agents by querying the National Cancer Institute (NCI) antitumor screen, a program that tests the activity of thousands of different agents against 60 human tumor cell lines.[3] The conventional alkylating agents melphalan, chlorambucil, and cyclophosphamide showed similar sensitivity patterns to other agents in the database and correlated highly with each other in regard to their antitumor activity. Conversely, there was little correlation between the cytotoxicity profiles of bendamustine and the other compounds tested. The only agent showing a sensitivity agreement above 50% was dacarbazine.

Other studies have confirmed the unique cytotoxicity profile of bendamustine. In one study, my coworkers and I used the differential staining cytotoxicity assay to evaluate the activity of different alkylating agents in primary lymphoma samples from patients refractory to conventional alkylator chemotherapy.[4] A ranking of specimens based on sensitivity to these agents showed no significant correlations between bendamustine and agents such as cyclophosphamide and doxorubicin, supporting the NCI data.

To further evaluate the activity of bendamustine, my colleagues and I generated bendamustine-resistant cells by continuously exposing lymphoma cells to increasing concentrations of bendamustine. After approximately 1 year, the cells were 10-fold more resistant to bendamustine. However, this acquired resistance appeared to be reversible, as removal of bendamustine from the media resulted in restoration of bendamustine sensitivity after 1 month.[4] This is a rather unique resistance profile, as drug resistance is generally more stable. Moreover, bendamustine-resistant cells remained sensitive to doxorubicin, taxanes, and other drugs, including cyclophosphamide. Finally, using bortezomib-resistant cell lines, we confirmed the expression of genes involved in DNA repair originally identified by gene array analysis. These results confirm the unique expression pattern induced by bendamustine at the DNA repair level.[4]

In summary, bendamustine acts on DNA in a unique manner, causing significant damage that cannot efficiently be repaired and results in apoptosis. Bendamustine can also induce a nonapoptotic pathway, which is particularly important for cells unable to undergo apoptosis. The combination of these mechanisms probably explains the agent's unique cytotoxic activity. Bendamustine will likely play a critical role in the treatment of lymphomas and other hematologic malignancies, both in the frontline setting, in which direct induction of apoptosis is important, and also in patients with refractory disease, in which the nonapoptotic pathway may play a larger role.

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Bendamustine in Clinical Trials

Bruce D. Cheson, MD

When bendamustine was first being studied in the United States, there was little interest in the drug, which was considered just another alkylating agent. However, after clinical trials demonstrated the significant efficacy of bendamustine, there is now a greater appreciation for the unique activity of this agent.

Early studies demonstrated significant activity with single-agent bendamustine in patients with previously treated hematologic malignancies, including relapsed/refractory CLL, relapsed/refractory indolent B-cell NHL, and relapsed/refractory aggressive NHL.[1-4] However, these older data were published in journals that were not widely read, and the quality of the data was uncertain. It was incumbent upon other investigators to demonstrate the efficacy of the agent in rigorous clinical trials.

Bendamustine in Indolent Lymphoma

A series of multicenter, phase II trials was undertaken to evaluate the safety and efficacy of bendamustine in patients with previously treated lymphoma. Friedberg and colleagues evaluated single-agent bendamustine in 76 patients with rituximab-refractory indolent and transformed NHL.[5] In these heavily pretreated patients, 32% of whom were refractory to chemotherapy, bendamustine was associated with a remarkable overall response rate (ORR) of 77%, including 15% complete responses (CRs), 19% unconfirmed CR, and 43% partial responses (PRs). The median duration of response (DOR) was 6.7 months. In another multicenter study of 100 patients with rituximab-refractory, indolent B-cell NHL, single-agent bendamustine was associated with a similarly high ORR of 75%, including 14% CR, 3% unconfirmed CR, and 58% PR.[6] The median DOR in these patients was 9.2 months, and the median progression-free survival (PFS) was 9.3 months. These results demonstrate superior efficacy with bendamustine versus any other drug in comparable patients and have provided a foundation on which to build new and more effective regimens.

In 2010, my colleagues and I conducted a pooled analysis of data from these 2 trials to further characterize the activity of bendamustine in this patient population.[7] Together, the 2 trials enrolled 161 patients with indolent NHL that had progressed within 6 months of rituximab therapy. The histology of enrolled patients included follicular lymphoma (68%), small lymphocytic leukemia (20%), marginal zone lymphoma (11%), and lymphoplasmacytic lymphoma (1%). There was an even distribution among patients with low-risk, intermediate-risk, and high-risk disease, as measured by the Follicular Lymphoma International Prognostic Index (FLIPI). Prior therapies included single-agent rituximab; cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)-like regimens, with or without rituximab; cyclophosphamide, vincristine, and prednisone with or without rituximab, purine analogs with or without rituximab, and radioimmunotherapy. Patients had received a median of 2 prior therapies. More than a third of patients (34.1%) were refractory to their last chemotherapy, and 30.1% were refractory to alkylating agents.

In this pretreated, rituximab-refractory population, single-agent bendamustine was associated with an ORR of 76%, with 23% CR and CRu. These responses were observed irrespective of FLIPI category. Notably, the response rate to bendamustine in patients with alkylator-refractory disease was 59%, including 12% CR and CRu. The DOR was 10 months, and did not differ according to sensitivity to alkylating agents. The fact that bendamustine induced responses in nearly 60% of patients with alkylator-refractory disease highlights that bendamustine is not just another alkylating agent.

In 2005, Rummel and colleagues published results of a multicenter, single-arm trial evaluating combination therapy with bendamustine plus rituximab in 63 patients with mantle cell lymphoma and low-grade NHL.[8] In these patients, who were in their first to third relapse or who were refractory to previous treatment, bendamustine plus rituximab was associated with an ORR of 90% and a CR rate of 60%.

Subsequently, my coworkers and I conducted a multicenter, phase II study to validate these findings.[9] The study enrolled 67 adults with relapsed indolent B-cell NHL and mantle cell lymphoma without resistance to prior rituximab. In this setting, bendamustine plus rituximab was associated with an ORR of 92%, including 41% CR and 14% unconfirmed CR. These outcomes were observed irrespective of whether patients were considered resistant to prior alkylating agents. Median PFS was 23 months, which again did not vary based on resistance to alkylators.

As patients with indolent lymphoma generally receive an alkylating agent as a component of their therapy, bendamustine has become a standard drug for patients

who have relapsed or are refractory to these agents. However, recent evidence now suggests that bendamustine should probably replace other alkylating agents as initial therapy as well, at least in follicular lymphoma and other indolent lymphomas.

In 2009, Rummel and colleagues presented results from the randomized, phase III StiL study comparing bendamustine plus rituximab against a current standard of care, rituximab plus CHOP chemotherapy (R-CHOP), as first-line treatment of patients with advanced follicular, indolent, and mantle cell lymphomas.[10] In the study, a total of 549 patients requiring treatment for their disease were randomly assigned to rituximab plus either bendamustine or the standard CHOP regimen. The investigators reported impressive results for bendamustine plus rituximab. After a median follow-up of 32 months, bendamustine plus rituximab was superior to R-CHOP in regard to CR rate (40.1% vs 30.8%; P=.0323), median PFS (54.8 vs 34.8 months; hazard ratio, 0.58; 95% confidence interval [CI], 0.43–0.77; P=.0002), and median event-free survival (54 vs 31 months; hazard ratio, 0.60; 95% CI, 0.45–0.78; P=.0002). Moreover, bendamustine plus rituximab was better tolerated than R-CHOP; it was associated with fewer serious adverse events, fewer episodes of grade 3/4 neutropenia and leukocytopenia, a lower rate of alopecia, a lower number of infectious complications, a lower incidence of peripheral neuropathy, and fewer episodes of stomatitis.

Bendamustine in CLL

The impressive phase I and phase II data with bendamustine from Germany[1,2] led to the design of a randomized, open-label, phase III trial comparing bendamustine versus chlorambucil in patients with previously untreated CLL.[11] A total of 319 patients ages 75 years or older with advanced (Binet stage B or C) CLL were randomly assigned to bendamustine (162 patients) or chlorambucil (157 patients). After a median follow-up of 35 months, bendamustine was significantly more effective than chlorambucil in regard to median PFS (21.6 months vs 8.3 months; P<.0001) and ORR (68% [31% CR] vs 31% [2% CR]; P<.0001). The PFS benefit was observed in patients with Binet stage B disease (21.4 vs 9.0 months) and in those with Binet stage C disease (25.4 vs 6.3 months). The median DOR was 21.8 months with bendamustine and 8.0 months with chlorambucil. In this study, bendamustine was associated with a higher incidence of grade 3/4 adverse events (40% in the bendamustine arm vs 19% in the chlorambucil arm) and a higher rate of grade 3/4 infections (8% vs 3%, respectively). However, this trial clearly demonstrated that bendamustine was superior to chlorambucil. These data helped support the FDA approval of bendamustine for use in CLL, both in the relapsed and upfront settings.

In Germany, Clemens Wendtner, MD, is leading the German CLL study group in conducting the phase III CLL-10 trial, which is comparing rituximab plus bendamustine against a current standard first-line therapy—fludarabine, cyclophosphamide, and rituximab (FCR)—in patients with previously untreated CLL. Should rituximab plus bendamustine do as well against FCR as it did against R-CHOP, then we may again alter our therapeutic paradigms for patients with CLL as we are now doing for patients with NHL.

Adverse Events Associated With Bendamustine

Long-term toxicity is a significant concern with alkylating agents. Bendamustine does not appear to be associated with a substantial increase in secondary malignancies, as has been observed with other alkylating agents. My colleagues and I conducted a pooled analysis of 161 patients with rituximab-refractory indolent NHL receiving single-agent bendamustine.[7] We observed that secondary malignancies developed in 9 patients. However, not all of the malignancies could be directly attributed to bendamustine. In some cases, patients had received extensive therapy with alkylating agents or radioimmunotherapy, and in other cases, the proximity of the cancer to the bendamustine treatment made the association unlikely.

The other toxicities observed with bendamustine primarily have been infections, cytopenias, skin rash, and fatigue, plus some nausea and vomiting, as would be expected with an alkylating agent. However, bendamustine appears to be tolerated as well as, if not better than, other standard drugs that we use for NHL and CLL.

Future Directions

The implications of these clinical trial results, and the subsequent FDA approvals, are numerous. First, we now have another effective treatment option for patients with follicular and low-grade B-cell NHL, mantle cell lymphoma, and CLL. In fact, given the high response rates observed with bendamustine, any new agent today will likely need to be compared against bendamustine or will be partnered with bendamustine. Second, we now have a foundation upon which to design more effective therapies. Although outcomes for these patients are improving, these malignancies remain largely incurable with current therapies. However, bendamustine, both as monotherapy and in combination with rituximab, has become the basis of many new regimens.

In 2011, Fowler and colleagues published results from the phase II VERTICAL (A Phase II Study of Velcade in Combination With Bendamustine and Rituximab in Subjects With Relapsed or Refractory Follicular Lymphoma) study, which evaluated a 3-drug regimen of bendamustine, rituximab, and the proteasome inhibitor bortezomib in 63 patients with relapsed/refractory NHL.[12] The regimen showed significant activity, with an ORR of 88% and a CR rate of 53%.

Also in 2011, Friedberg and colleagues published results from a multicenter, phase II study evaluating the same combination—bendamustine, bortezomib, and rituximab—in patients with relapsed/refractory indolent and mantle cell NHL.[13] A total of 30 patients, including 7 patients with mantle cell lymphoma, were treated. The combination showed promising efficacy: among the 29 evaluable patients, the ORR was 83%, including 52% CR, and the 2-year PFS rate was 47% after a median follow-up of 2 years. Serious adverse events developed in 8 patients (27%), including 1 patient who died from sepsis.

The regimen of bendamustine, rituximab, and bortezomib is now being evaluated as the initial treatment in a number of NHL histologies, including mantle cell lymphoma. Bendamustine is also being studied in combination with other novel agents, such as the small-molecule proapoptotic drug ABT-263 and the anti-CD20 monoclonal antibody GA101. A randomized, phase II trial sponsored by the Cancer and Leukemia Group B (CALGB) is evaluating combination therapy with bendamustine and the anti-CD20 monoclonal antibody ofatumumab with or without bortezomib.[14] The Eastern Cooperative Oncology Group (ECOG) is planning a similar trial evaluating bendamustine and rituximab with or without bortezomib. Interestingly, one challenge with the rapid rise of bendamustine has been a limited ability to find patients who have not yet received the agent, which makes it difficult to develop new agents in the relapsed/refractory setting.

Bendamustine is also being explored in other diseases that are currently treated with conventional alkylating agents, such as multiple myeloma and Hodgkin lymphoma. In a phase II trial, Moskowitz and colleagues reported a response rate of more than 75% in the setting of relapsed/refractory Hodgkin lymphoma, enabling patients who were not candidates for stem cell transplant to become suitable candidates for that potentially life-saving procedure.[15]

Another unmet need is in the treatment of diffuse large B-cell lymphoma (DLBCL), an aggressive NHL. Currently, R-CHOP remains the standard treatment for these patients. However, the median age of a DLBCL patient is in the mid-60s, and many patients are not suitable candidates for R-CHOP, perhaps due to cardiac or renal impairment. Bendamustine can be used relatively safely in those situations. Several recent studies have evaluated rituximab plus bendamustine in patients with DLBCL, particularly older patients. In a phase II trial of older patients with relapsed or refractory DLBCL (mean age, 74 years), bendamustine plus rituximab was active, demonstrating an ORR of 52% (15% CR).[16]

Bendamustine is also being used as the basis of a new generation of cooperative group trials evaluating different approaches for the first-line treatment of mantle cell lymphoma. In one study, patients older than 65 years will receive bendamustine plus rituximab or bendamustine, rituximab, or bortezomib, followed by maintenance rituximab or maintenance rituximab plus lenalidomide. A trial in younger patients (<60 years) is comparing a standard regimen of rituximab plus cyclophosphamide, vincristine, doxorubicin, and dexamethasone, plus methotrexate and cytarabine (hyper-CVAD) followed by stem cell transplantation versus rituximab plus bendamustine followed by stem cell transplantation. Each of these trials has the potential to alter our treatment paradigms for these diseases.

We are also fortunate to have a large number of new and active agents available for CLL and lymphoma. These include almost a dozen anti-CD20 monoclonal antibodies as well as monoclonal antibodies directed at other targets. In addition, there are novel signaling pathway-inhibiting drugs, including the PI3-kinase inhibitor CAL-101 and the Bruton's tyrosine kinase inhibitor PCI32765. Both of these oral agents are active and well tolerated, with minimal side effects in patients with CLL/small lymphocytic lymphoma and various histologies of NHL.[17,18]

The plan now will be to develop regimens that maximize the activity of these agents; the leading contender to combine them with is bendamustine. Numerous trials investigating these approaches are currently in development in cancer centers and cooperative groups. In our center, we have several bendamustine-based studies: a phase I trial evaluating bendamustine, lenalidomide, and rituximab in patients with lymphomas, and a trial evaluating bendamustine plus ofatumumab in patients with relapsed/refractory CLL. We are also conducting a trial of bendamustine with or without the anti-CD20 monoclonal antibody GA-101 in patients with rituximab-refractory follicular lymphoma.

One could envision development of regimens in which bendamustine substitutes for drugs like cyclophosphamide. It is possible that a B-HOP regimen might be more effective than CHOP-like therapy. Overall, although there is much work yet to be done to determine the best use of bendamustine in different clinical settings, it is clear that bendamustine is more than just another

alkylating agent—rather, it has substantially altered treatment paradigms, resulting in improved outcomes for many patients with lymphoid malignancies.

We must continue to make progress in the treatment of our patients. To do so, it is critical that we accrue patients to high-quality clinical trials testing novel agents and novel combinations. It is also essential that scientific correlates be incorporated into these trials, so that we can gain a better understanding of the mechanisms of these drugs and why they may be effective in certain patient populations and ineffective in others. The eventual goal will be to individualize our therapies, enhancing efficacy and minimizing toxicity, and thereby improving the outcome of patients with CLL and NHL.

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Bendamustine: History

- Initially synthesized in the 1960s in the former East German Democratic Republic
- Intended to be a less expensive form of other effective drugs available at the time
- Designed to have both alkylating and antimetabolite properties and an acceptable toxicity profile

Bendamustine: Structural Elements

- 2-chloroethylamine alkylating group
- Benzimidazole ring
- Butyric acid side chain

Bendamustine: Mechanisms of Action

- Activates the DNA-damage stress response
- Induces apoptosis
- Inhibits mitotic checkpoints
- Induces mitotic catastrophe

Alkylating Agents: DNA Damage

- Alkylating agents exert cytotoxic effects primarily through their effects on DNA:
  - Through alkylation, a process in which an alkyl group is added to a DNA residue
  - By cross-linking strands of DNA, creating links both between strands (interstrand cross-linking) and within strands (intrastrand cross-linking). Both alkylation and DNA cross-linking can cause DNA breaks, which also activate DNA repair mechanisms
- In addition to their direct effects on DNA, alkylating agents also exert indirect effects on cell division, as cells that have undergone DNA damage exhibit inhibited DNA replication and transcription

Bendamustine: Differences From Other Alkylating Agents

- The benzimidazole ring system, which is unique to bendamustine, may provide enhanced antitumor activity
- The heterocyclic ring structure could allow bendamustine to better penetrate and localize within DNA and remain there for a longer period of time
- Bendamustine induces more DNA double-strand breaks than other alkylating agents
- The DNA double-strand breaks induced by bendamustine persist longer than those induced by other alkylating agents

DNA Repair Mechanisms

- Multiple DNA repair mechanisms play a role in the detoxification of cells that have undergone DNA damage. These include:
  - Base excision repair
  - Homologous recombination
  - The DNA mismatch-repair system
  - Alkyltransferase-based DNA repair

Bendamustine: Mechanisms of Cell Death

- Bendamustine retains activity in cancer cells that are resistant to conventional alkylating agents. In addition to inducing apoptosis, bendamustine also appears to:
  - Induce cell death through an alternative mechanism called mitotic catastrophe
  - Induce a potent, rapid adenosine triphosphate (ATP) depletion
  - Induce reactive oxygen species stress pathways

Single-Agent Bendamustine in Indolent Lymphoma: Phase II Data

Study	Population (N)	Results
Friedberg 2008	Rituximab-refractory, indolent and transformed NHL (76)	ORR, 77%, with 15% CR, 19% CRu, and 43% PR. Median DOR, 6.7 months
Kahl 2010	Rituximab-refractory, indolent B-cell NHL (100)	ORR, 75%, with 14% CR, 3% CRu, and 58% PR. Median DOR, 9.2 months. Median PFS, 9.3 months
Cheson 2010	Pooled analysis of data from Friedberg and Kahl (161)	ORR, 76%, with 23% CR and CRu. The DOR was 10 months. Notably, in patients with alkylator-refractory disease, the ORR was 59%, including 12% CR and CRu

Bendamustine and Rituximab in Indolent Lymphoma: Phase II Data

Study	Population (N)	Results
Rummel 2005	Mantle cell lymphoma and low-grade NHL (63). Patients were in their first to third relapse or were refractory to previous treatment	ORR, 90%, with a CR rate of 60%
Robinson 2008	Relapsed indolent B-cell NHL and mantle cell lymphoma without resistance to prior rituximab (67)	ORR of 92%, with 41% CR and 14% unconfirmed CR. Median PFS, 23 months

The Phase III StiL Trial

- Randomized, phase III study comparing bendamustine plus rituximab against a current standard of care, R-CHOP, as first-line treatment of patients with advanced follicular, indolent, and mantle cell lymphomas
- A total of 549 patients requiring treatment for their disease were randomly assigned to rituximab plus either bendamustine or the standard CHOP regimen
- After a median follow-up of 32 months, bendamustine plus rituximab was superior to R-CHOP in regard to CR rate (40.1% vs 30.8%; P=.0323), median PFS (54.8 vs 34.8 months; HR, 0.58; 95% CI, 0.43–0.77; P=.0002), and median event-free survival (54 vs 31 months; HR, 0.60; 95% CI, 0.45–0.78; P=.0002).

Bendamustine in Chronic Lymphocytic Leukemia: Phase III Data

- Knauf and colleagues conducted a randomized, open-label, parallel-group, phase III trial comparing bendamustine versus chlorambucil in patients with previously untreated CLL (N=319)
- After a median follow-up of 35 months, bendamustine was significantly more effective than chlorambucil in regard to median PFS (21.6 months vs 8.3 months; P<.0001) and ORR (68% [31% CR] vs 31% [2% CR]; P<.0001)
- The median DOR was 21.8 months with bendamustine and 8.0 months with chlorambucil
- Bendamustine was associated with a higher incidence of grade 3/4 adverse events (40% in the bendamustine arm vs 19% in the chlorambucil arm) and a higher rate of grade 3/4 infections (8% vs 3%, respectively)

Bendamustine in Combination Therapy: Phase II Data

Study	Population (N)	Regimen	Results
Fowler 2009	Relapsed/refractory NHL (63)	Bendamustine, rituximab, and bortezomib	ORR, 86%, with 53% CR
Friedberg 2011	Relapsed/refractory indolent and mantle cell NHL (29)	Bendamustine, rituximab, and bortezomib	ORR, 83%, with 52% CR. The 2-year PFS rate was 47% after a median follow-up of 2 years

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