

nonanticoagulant heparin fraction had similar binding properties to histones. Binding of this nonanticoagulant heparin to histones strongly inhibited cytotoxic activity in vitro and translated to impaired inflammation and improved survival in animal models of systemic infection and inflammation.

This effect of heparin on histones fits well into the current ideas on the role of histones in the pathogenesis of sepsis and severe inflammation. Neutrophil extracellular traps (NETs) have recently been identified as important contributors to vascular thrombosis and inflammation.⁶ These NETs consist of extracellular DNA fibers that are present in inflammatory conditions as a result of programmed cell death of inflammatory cells and endothelial cells. NETs seem to be produced to allow inflammatory cells to trap and deactivate microorganisms in the extracellular environment by forming scaffolds of intact chromatin fibers with antimicrobial proteins. However, NETs may also induce endothelial cell death and detrimental inflammatory activity, an effect likely mediated by NET-associated proteases or cationic proteins, including histones.^{6,7} Importantly, it has been demonstrated that activated protein C is an significant inhibitor of histone-mediated detrimental effects in sepsis. Because there is ample interaction between heparin and activated protein C, it is likely that part of the heparin effect on histones is due to modulation of this activated protein C effect, which is in line with very recent observations in mice.⁸

The observation that this novel anti-inflammatory effect of heparin is independent of its anticoagulant activity may be relevant because the risk of hemorrhage is a major limitation of relatively high doses of heparin in patients with severe infection or sepsis who are already vulnerable to this complication due to low levels of platelets and coagulation factors. With the relatively simple technique as demonstrated in the paper by Wildhagen et al, it should be possible to produce heparin mixtures that are only partly anticoagulant but will maintain their full anti-inflammatory properties, providing an optimal therapeutic agent targeted at both coagulation and inflammation in severe sepsis and other types of systemic inflammation.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Wildhagen KCAA, García de Frutos P, Reutelingsperger CP, et al. Nonanticoagulant heparin prevents histone-mediated cytotoxicity in vitro and improves survival in sepsis. *Blood*. 2014;123(7):1098-1101.
2. Levi M, van der Poll T. Inflammation and coagulation. *Crit Care Med*. 2010;38(2 Suppl):S26-S34.
3. Levi M, van der Poll T, Schultz M. New insights into pathways that determine the link between infection and thrombosis. *Neth J Med*. 2012;70(3):114-120.
4. Cornet AD, Smit EG, Beishuizen A, et al. The role of heparin and allied compounds in the treatment of sepsis. *Thromb Haemost*. 2007;98(3):579-586.
5. Xu J, Zhang X, Pelayo R, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med*. 2009;15(11):1318-1321.
6. Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (NET) impact on deep vein thrombosis. *Arterioscler Thromb Vasc Biol*. 2012;32(8):1777-1783.
7. Saffarzadeh M, Juenemann C, Queisser MA, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS ONE*. 2012;7(2):e32366.
8. Kowalska MA, Zhao G, Zhai L, et al. Modulation of protein C activation by histones, platelet factor 4, and heparinoids: New insights into activated protein C formation [published online ahead of print October 31, 2013]. *Arterioscler Thromb Vasc Biol*.

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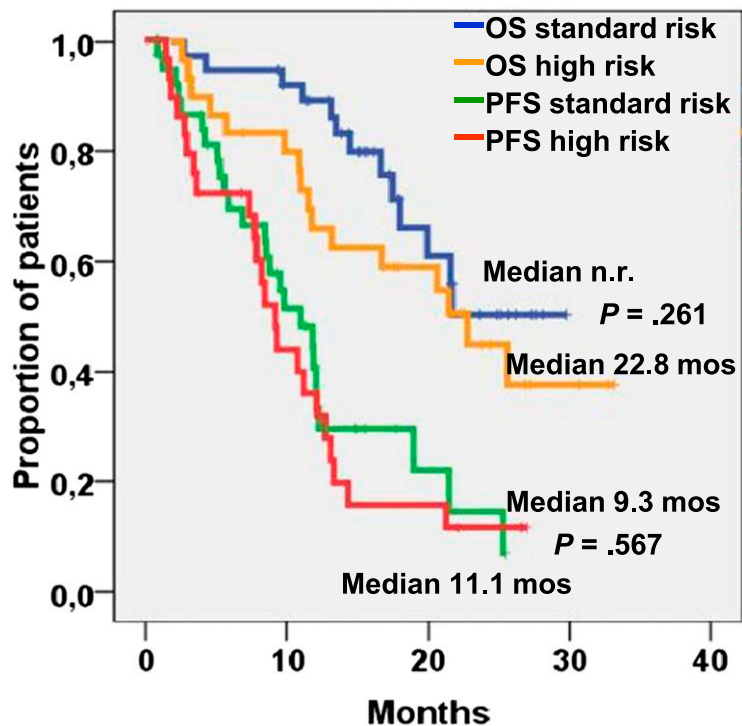
● ● ● CLINICAL TRIALS & OBSERVATIONS

Comment on Ludwig et al, page 985

Bendamustine: the remedy that came in from the cold

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In this issue of *Blood*, Ludwig and colleagues present the data of a phase 2 clinical trial showing that bendamustine, bortezomib, and dexamethasone (BBD) is a very active and well-tolerated regimen in patients with relapsed or refractory multiple myeloma.¹ More importantly, BBD overcomes the adverse prognosis in patients with high-risk cytogenetics.



Progression-free and overall survival in patients with standard and high-risk cytogenetics. See Figure 1B in the article by Ludwig et al that begins on page 985.

Bendamustine, originally termed IMET 3393 and also known as Treakisym, Ribomustin, Levact, Treanda, or SDX-105, is a nitrogen mustard that was developed as a bifunctional molecule with alkylator and antimetabolite properties. Compared with other frequently used alkylating agents, bendamustine induces higher rates of DNA double-strand breaks and is not cross-resistant with melphalan and several other cytotoxic drugs.²

Bendamustine was originally developed in 1963 in East Germany (the former German Democratic Republic) by Ozegowski and Krebs.² Over a 20-year period, it became highly used in the Eastern Bloc before the fall of the Iron Curtain in 1989. More than 18 000 patients using this compound were studied in the mid 1990s, mostly in Germany. In 2008, bendamustine was approved by the US Food and Drug Administration for the treatment of chronic lymphocytic leukemia and indolent B-cell non-Hodgkin lymphoma.

Over the past decade, there was increasing evidence that bendamustine is also very active in multiple myeloma, and it is currently being tested in a clinical trial for amyloid light-chain amyloidosis (<http://clinicaltrials.gov/ct2/show/NCT01222260>). A dose-escalation study of single-agent bendamustine up to 100 mg/m² showed an overall response rate (ORR) of 55%, including minor responses (MRs).³ Pönisch et al performed a phase I clinical trial testing the combination of bendamustine, prednisolone, and thalidomide for relapsed or refractory multiple myeloma.⁴ Fixed doses of bendamustine (60 mg/m²) and prednisolone (100 mg) with escalating doses of thalidomide (50, 100, and 200 mg) achieved a high response rate of 86%, including 14% complete responses. A direct comparison with current data is difficult due to the use of different response criteria without free light-chain assessment. In another phase 1/2 trial, our group established the maximum tolerated dose (MTD) of the combination of bendamustine (75 mg/m²), lenalidomide (10 mg), and dexamethasone (40 mg) with an ORR of 50%. Most of the patients were pretreated with lenalidomide and bortezomib and had a median of 3 prior treatment lines.⁵ In a different phase 1/2 trial with less heavily pretreated patients, the MTD was not reached using up to 75 mg/m² of bendamustine and 25 mg lenalidomide with an ORR of 75%.⁶

The most frequently observed adverse events when combining bendamustine with lenalidomide are neutropenia and prolonged thrombocytopenia. Therefore, a combination of bendamustine with a less hematotoxic agent such as bortezomib provides a very promising approach that will allow the administration of adequate therapeutic doses. Indeed, Pönisch et al conducted a clinical trial using bendamustine, bortezomib, and prednisone and observed a response rate of 69%.⁷ The Intergroupe Francophone du Myélome presented data obtained with bendamustine, bortezomib, and dexamethasone in elderly patients after 1 line of therapy with responses rates up to 67.1%.⁸ Berenson et al conducted a phase 1/2 trial of bendamustine (90 mg/m²) in combination with a fixed low dose of bortezomib (1 mg/m²) without corticosteroids in heavily pretreated patients resulting in an ORR of 33% and 48%, including MRs.⁹

The current study by Ludwig et al¹ published in this issue of *Blood* is the largest prospective phase 2 trial using BBD for relapsed or refractory multiple myeloma. BBD resulted in a very high response rate of 60.9% or 75.9% if MRs were included. Including only patients who received ≥ 2 cycles of therapy (71 patients), the ORR increased to 67.7% or 84.6% (with MRs). As expected, the response rates were lower in patients pretreated with bortezomib, lenalidomide, or both, in patients exposed to ≥ 2 prior lines of lenalidomide-based therapy, and in those with relapsed and/or refractory disease to the last treatment regimen. Nevertheless, 5 of the 8 patients who had been pre-exposed to 2 or 3 lines of bortezomib achieved at least a partial response or better, suggesting that the BBD regimen induces meaningful responses even after multiple treatments in relapsed/refractory patients.

Most interestingly, BBD resulted in fast responses, with a time to response and time to best response of 31 days and 111 days, respectively. This is of high clinical relevance, because rapid tumor control usually corresponds with fast improvement of clinical symptoms and is especially important in patients with high tumor burden and aggressive disease. Similar findings have also been reported in studies using bendamustine with lenalidomide and corticosteroids.^{5,6}

Besides the induction of fast and high responses by this regimen, probably the most

significant finding of this study is that BBD achieved similar response rates but, even more importantly, comparable progression-free survival and overall survival in patients with or without cytogenetically defined high-risk features (see figure). This suggests that the combination of bortezomib and bendamustine is even more effective than bortezomib alone in overcoming the adverse prognosis of multiple myeloma patients with high-risk cytogenetics.

Pharmacologic studies have shown that bendamustine can be given safely in patients with renal failure. A study on a small group of patients with stage IV or V renal impairment showed no unexpected toxicity and a response rate of 55% when a single dose of 120 mg/m² bendamustine was given in combination with daily 100 mg thalidomide and weekly dexamethasone for the first 3 weeks of a 4-week cycle.¹⁰ This suggests that the combination of bendamustine, bortezomib, and dexamethasone provides a very effective and safe option in high-risk patients with renal failure and adverse cytogenetics.

In summary, the study by Ludwig and colleagues shows that BBD

- is a highly effective regimen in heavily pretreated patients;
- overcomes adverse cytogenetics;
- induces very fast remissions;
- can be given in renal failure; and
- is a safe combination with a well-defined toxicity profile.

Based on the excellent response data, especially in patients with adverse cytogenetics, and a favorable toxicity profile, combinations of bendamustine with second-class proteasome inhibitors such as carfilzomib are promising and currently tested in the first- and second-line setting (<http://clinicaltrials.gov/ct2/show/NCT02002598>).

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Ludwig H, Kasparu H, Leitgeb C, et al. Bendamustine-bortezomib-dexamethasone is an active and well tolerated regimen in patients with relapsed or refractory multiple myeloma. *Blood*. 2014;123(7):985-991.
2. Leoni LM, Hartley JA. Mechanism of action: the unique pattern of bendamustine-induced cytotoxicity. *Semin Hematol*. 2011;48(suppl 1):S12-S23.
3. Knop S, Straka C, Haen M, Schwedes R, Hebart H, Einsele H. The efficacy and toxicity of bendamustine in recurrent multiple myeloma after high-dose chemotherapy. *Haematologica*. 2005;90(9):1287-1288.

4. Pönisch W, Rozanski M, Goldschmidt H, et al; East German Study Group of Haematology and Oncology (OSHO). Combined bendamustine, prednisolone and thalidomide for refractory or relapsed multiple myeloma after autologous stem-cell transplantation or conventional chemotherapy: results of a Phase I clinical trial. *Br J Haematol*. 2008;143(2):191-200.
5. Lentzsch S, O'Sullivan A, Kennedy RC, et al. Combination of bendamustine, lenalidomide, and dexamethasone (BLD) in patients with relapsed or refractory multiple myeloma is feasible and highly effective: results of phase 1/2 open-label, dose escalation study. *Blood*. 2012;119(20):4608-4613.
6. Pönisch W, Heyn S, Beck J, et al. Lenalidomide, bendamustine and prednisolone exhibits a favourable safety and efficacy profile in relapsed or refractory multiple myeloma: final results of a phase 1 clinical trial OSHO - #077. *Br J Haematol*. 2013;162(2):202-209.
7. Pönisch W, Bourgeois M, Moll B, et al. Combined bendamustine, prednisone and bortezomib (BPV) in

patients with relapsed or refractory multiple myeloma. *J Cancer Res Clin Oncol*. 2013;139(3):499-508.

8. Rodon P, Hulin C, Pegourie B, et al. Bendamustine, bortezomib and dexamethasone (BVD) in elderly MM progressive after 1st line therapy (IFM-2009-01 trial): predictive factors of favourable outcome [abstract]. *Haematologica*. 2013;98(suppl 1):100. Abstract P231.
9. Berenson JR, Yellin O, Bessudo A, et al. Phase I/II trial assessing bendamustine plus bortezomib combination therapy for the treatment of patients with relapsed or refractory multiple myeloma. *Br J Haematol*. 2013;160(3):321-330.
10. Ramasamy K, Hazel B, Mahmood S, Corderoy S, Schey S. Bendamustine in combination with thalidomide and dexamethasone is an effective therapy for myeloma patients with end stage renal disease. *Br J Haematol*. 2011;155(5):632-634.

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● ● ● IMMUNOBIOLOGY

Comment on Huang et al, page 1012

The polyphony of BACH2

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In this issue of *Blood*, Huang and colleagues examine the role of BACH2, a transcription factor known to be expressed highly in germinal center B cells.¹⁻³

B cells undergo very striking changes as they differentiate from stem cells to become the effector cells of humoral immunity. This process requires that B cells undergo the germinal center reaction where B cells migrate through lymph nodes and interact with other immune cells including dendritic cells and helper T cells. In germinal centers, these B cells proliferate rapidly as they undergo somatic hypermutation and class-switch recombination—2 mechanisms through which B cells develop affinity for antigens. Indeed, these normal B cells have a doubling time of 6 hours, that is, more rapid than most tumors. B cells with affinity for antigen further differentiate into memory B cells and plasma cells, which are the effector cells of humoral immunity.

BCL6 is well known as a major regulator of germinal center differentiation. Mice lacking BCL6 expression are unable to form germinal centers. BCL6 promotes the rapid proliferation of B cells and blocks the DNA damage response that accompanies somatic hypermutation. Not surprisingly, this process is fraught with the potential for oncogenic

transformation. Indeed, most B-cell tumors arise from either germinal center or post-germinal center B-cell stages. Thus, it appears likely that there are other genes beyond BCL6 that aid in closely regulating this important process.

Huang and colleagues generated transgenic mice that expressed BCL6 and BACH2 variably. They demonstrate convincingly that the mice that are deficient in both BCL6 and BACH2 show profoundly decreased formation of germinal center B cells and a predisposition toward increased plasma cells.⁴ They further demonstrate through genome-wide DNA-binding experiments that these transcription factors bind overlapping sites in the genome. They also demonstrate biochemically that BCL6 stabilizes BACH2 protein expression. Taken together, these experiments shed new light on the role of this important transcription factor in this important process.

The role of BACH2 has recently been described in the function of T cells⁵ and alveolar macrophages.⁶ In early B cells, BACH2 regulates the tumor suppressor role of p53, which suggests it has a more extensive role

in cancers. BACH2 mutations occur in about 5% of diffuse large B-cell lymphomas,⁷ as well as other cancers,⁸ although their specific role is unknown. Future work will be needed to define the role of BACH2 in cancer and the potential role of other collaborating transcription factors and genes in B-cell differentiation.

Although much of the study of biology has focused on the role of individual genes, it is becoming increasingly apparent that most important biological processes are governed by multiple genes working in concerted fashion.

The multifaceted actions of BACH2 in different contexts are reminiscent of a polyphonic composition with intertwining melodies carried by different sections of the orchestra. The Baroque era composer J. S. Bach (1685-1750) was one of the foremost composers in this style and his work remains widely performed to this day. Although the full name BTB and CNC homology 1, basic leucine zipper transcription factor 2 is difficult to remember, recalling the polyphonic work of Bach can remind us of the gene BACH2 and its many functions.

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REFERENCES

1. Huang C, Geng H, Boss I, Wang L, Melnick A. Cooperative transcriptional repression by BCL6 and BACH2 in germinal center B-cell differentiation. *Blood*. 2014;123(7):1012-1020.
2. Shaffer AL, Rosenwald A, Hurt EM, et al. Signatures of the immune response. *Immunity*. 2001;15(3):375-385.
3. Zhang J, Jima DD, Jacobs C, et al. Patterns of microRNA expression characterize stages of human B-cell differentiation. *Blood*. 2009;113(19):4586-4594.
4. Kometani K, Nakagawa R, Shinnakasu R, et al. Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. *Immunity*. 2013;39(1):136-147.
5. Roychoudhuri R, Hirahara K, Mousavi K, et al. BACH2 represses effector programs to stabilize T(reg)-mediated immune homeostasis. *Nature*. 2013;498(7455):506-510.
6. Nakamura A, Ebina-Shibuya R, Itoh-Nakadai A, et al. Transcription repressor Bach2 is required for pulmonary surfactant homeostasis and alveolar macrophage function. *J Exp Med*. 2013;210(11):2191-2204.
7. Zhang J, Grubor V, Love CL, et al. Genetic heterogeneity of diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A*. 2013;110(4):1398-1403.
8. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res*. 2011;39(Database issue):D945-D950.

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