4. Laurent C, Delas A, Gaulard P, et al. Breast implant-associated anaplastic large cell lymphoma: two distinct clinicopathological variants with different outcomes. *Ann Oncol*. 2016;27(2):306-314.

5. Clemens MW, Medeiros LJ, Butler CE, et al. Complete surgical excision is essential for the management of patients with breast implant-associated anaplastic large-cell lymphoma. *J Clin Oncol*. 2016;34(2):160-168.

6. Ferrufino-Schmidt MC, Medeiros LJ, Liu H, et al. Clinicopathologic features and prognostic impact of lymph node involvement in patients with breast implant-associated anaplastic large cell lymphoma. *Am J Surg Pathol*. 2018;42(3):293-305.

7. Feldman AL, Dogan A, Smith DI, et al. Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing. *Blood*. 2011;117(3):915-919.

8. Vasmatzis G, Johnson SH, Knudson RA, et al. Genome-wide analysis reveals recurrent structural abnormalities of TP63 and other p53-related genes in peripheral T-cell lymphomas. *Blood*. 2012;120(11):2280-2289.

9. Parrilla Castellar ER, Jaffe ES, Said JW, et al. ALK-negative anaplastic large cell lymphoma is a genetically heterogeneous disease with widely disparate clinical outcomes. *Blood*. 2014;124(9):1473-1480.

10. Vose J, Armitage J, Weisenburger D; International T-Cell Lymphoma Project. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. *J Clin Oncol*. 2008;26(25):4124-4130.

11. Pedersen MB, Hamilton-Dutoit SJ, Bendix K, et al. *DUSP22* and *TP63* rearrangements predict outcome of ALK-negative anaplastic large cell lymphoma: a Danish cohort study. *Blood*. 2017;130(4):554-557.

12. Roden AC, Macon WR, Keeney GL, Myers JL, Feldman AL, Dogan A. Seroma-associated primary anaplastic large-cell lymphoma adjacent to breast implants: an indolent T-cell lymphoproliferative disorder. *Mod Pathol*. 2008;21(4):455-463.

13. Wada DA, Law ME, Hsi ED, et al. Specificity of IRF4 translocations for primary cutaneous anaplastic large cell lymphoma: a multicenter study of 204 skin biopsies. *Mod Pathol*. 2011;24(4):596-605.

14. Lechner MG, Lade S, Liebertz DJ, et al. Breast implant-associated, ALK-negative, T-cell, anaplastic, large-cell lymphoma: establishment and characterization of a model cell line (TLBR-1) for this newly emerging clinical entity. *Cancer*. 2011;117(7):1478-1489.

15. Lechner MG, Megiel C, Church CH, et al. Survival signals and targets for therapy in breast implant-associated ALK–anaplastic large cell lymphoma. *Clin Cancer Res*. 2012;18(17):4549-4559.

16. King RL, Dao LN, McPhail ED, et al. Morphologic Features of ALK-negative Anaplastic Large Cell Lymphomas With DUSP22 Rearrangements. *Am J Surg Pathol*. 2016;40(1):36-43.

17. Wang X, Boddicker RL, Dasari S, et al. Expression of p63 protein in anaplastic large cell lymphoma: implications for genetic subtyping. *Hum Pathol*. 2017;64:19-27.

18. Chiarle R, Simmons WJ, Cai H, et al. Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. *Nat Med*. 2005;11(6):623-629.

19. Khoury JD, Medeiros LJ, Rassidakis GZ, et al. Differential expression and clinical significance of tyrosine-phosphorylated STAT3 in ALK+ and ALK- anaplastic large cell lymphoma. *Clin Cancer Res*. 2003;9(10 Pt 1):3692-3699.

20. Crescenzo R, Abate F, Lasorsa E, et al; European T-Cell Lymphoma Study Group, T-Cell Project: Prospective Collection of Data in Patients with Peripheral T-Cell Lymphoma and the AIRC 5xMille Consortium "Genetics-Driven Targeted Management of Lymphoid Malignancies". Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell*. 2015;27(4):516-532.

21. Song JY, Song L, Herrera AF, et al. Cyclin D1 expression in peripheral T-cell lymphomas. *Mod Pathol*. 2016;29(11):1306-1312.

22. Hu G, Dasari S, Asmann YW, et al. Targetable fusions of the FRK tyrosine kinase in ALK-negative anaplastic large cell lymphoma. *Leukemia*. 2018;32(2):565-569.

23. Blombery P, Thompson ER, Jones K, et al. Whole exome sequencing reveals activating JAK1 and STAT3 mutations in breast implant-associated anaplastic large cell lymphoma anaplastic large cell lymphoma. *Haematologica*. 2016;101(9):e387-e390.

24. Di Napoli A, Jain P, Duranti E, et al. Targeted next generation sequencing of breast implant-associated anaplastic large cell lymphoma reveals mutations in JAK/STAT signalling pathway genes, TP53 and DNMT3A. *Br J Haematol*. 2018;180(5):741-744.

25. Chen J, Zhang Y, Petrus MN, et al. Cytokine receptor signaling is required for the survival of ALK- anaplastic large cell lymphoma, even in the presence of JAK1/STAT3 mutations. *Proc Natl Acad Sci USA*. 2017;114(15):3975-3980.

26. Koskela HL, Eldfors S, Ellonen P, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med*. 2012;366(20):1905-1913.

27. Boddicker RL, Razidlo GL, Dasari S, et al. Integrated mate-pair and RNA sequencing identifies novel, targetable gene fusions in peripheral T-cell lymphoma. *Blood*. 2016;128(9):1234-1245.

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TO THE EDITOR:

Short course of bortezomib in anemic patients with relapsed cold agglutinin disease: a phase 2 prospective GIMEMA study

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Cold agglutinin disease (CAD) is a chronic hemolytic disorder caused by anti–red blood cell immunoglobulin M (IgM) autoantibodies most often monoclonal with k light-chain restriction.[1,2] The autoantibody reacts at temperatures lower than the body temperature, causing autoagglutination and complement-dependent red blood cell destruction. CAD is a rare disease,

Table 1. Characteristics of patients enrolled

Patients enrolled	21	
Median age, y	70.4	Range 53 to 85
M/F ratio	1/2	
Associated B-cell disorder	9/21 (42.9%)	B-CLL: 3 B-cell NHL: 6
Previous treatments		
Corticosteroids	9	
Rituximab	12	
Cyclophosphamide	3	
Azathioprine	1	
Splenectomy	1	
Hgb concentration (median)	87 g/L	Range 78-102
Transfusion dependence	10/21 (47.6%)	
Monoclonal gammopathy at serum electrophoresis	13/20* (65%)	Ig isotype: Mk 11, Gk 1, Mk+Gl 1 Paraprotein level <10% of total serum proteins in all 13 cases
Clonal B-cell disorder		
Detectable clonal B cells in PB	7/19* (36.8%)	Number of clonal B cells: 15-339/μL
B-lymphoid marrow infiltrate	9/21 (42.9%)	Lymphoid infiltrate: 5%-15% of total cellularity
No evidence of monoclonal gammopathy nor of clonal B-cell disorder	6/20* (30%)	
Comorbidities		
Cardiovascular	6	Atrial fibrillation (1), chronic ischemic disorder (3), arterial hypertension (2), ictus cerebri (1)
Metabolic	5	Diabetes (3), hypothyroidism (1), osteoporosis (1)
Hepatic	3	Prior HBV infection (1), chronic HCV hepatitis (2)
Neoplastic	3	Breast cancer (2), thyroid papillary carcinoma (1)

B-CLL, B-cell chronic lymphocytic leukemia; F, female; HBV, hepatitis B virus; HCV, hepatitis C virus; M, male; NHL, non-Hodgkin lymphoma; PB, peripheral blood.
*Number of evaluable patients.

accounting for ~15% of autoimmune hemolytic anemias.[3,4] Although clinical manifestations (acrocyanosis, Raynaud phenomenon, and anemia) can be mitigated by avoiding cold exposure, the median hemoglobin level in unselected CAD patients is 89 g/L.[5] Moreover, CAD is a relapsing disease, requiring transfusions and multiple therapies to obtain disease control.[6]

There are few therapies for symptomatic CAD patients. Both corticosteroids and immunosuppressants are less effective in CAD than in warm autoimmune hemolytic anemias.[7,8] Rituximab is presently considered the standard treatment; however, the response rate is ~50%. Responses are almost exclusively partial, and their median duration is only 12 months.[9-11] Its efficacy can be significantly increased and prolonged by the association with fludarabine or bendamustine, which, however, also adds the common side effects of these chemotherapeutic agents in patients without a clinically overt neoplastic disorder.[12-14]

Bortezomib is a proteasome inhibitor that has been successfully used in the treatment of immunoglobulin-producing diseases, including multiple myeloma, light-chain amyloidosis, and Waldenstrom macroglobulinemia.[15] The presence of an underlying B-cell clonal disorder in most cases of CAD[3,16] together with the favorable effect of few infusions of bortezomib reported in 2 patients with refractory CAD[17] prompted us to investigate the potential benefit of a short course of bortezomib in anemic CAD patients refractory or relapsed after previous treatments.

A prospective multicenter, phase 2, open-label study was conducted within the GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) and registered at ClinicalTrials.gov (NCT01696474). It was approved by the Ethical Committees of participating centers. Data were collected and managed using REDCap electronic data capture.[18]

The primary objective of the study was the achievement of transfusion independence or at least a significant rise (>20 g/L) in hemoglobin. Secondary objectives were safety, duration of hematologic response, and effect on underlying B-cell disorders, if present. Diagnosis of CAD required the presence of chronic hemolysis and the detection of cryoagglutinins at 4°C (direct antiglobulin test strongly positive for complement C3 and negative/weakly positive for IgG). Other inclusion criteria were patients refractory or relapsed after at least 1 previous treatment, and hemoglobin concentration <100 g/L determined at least

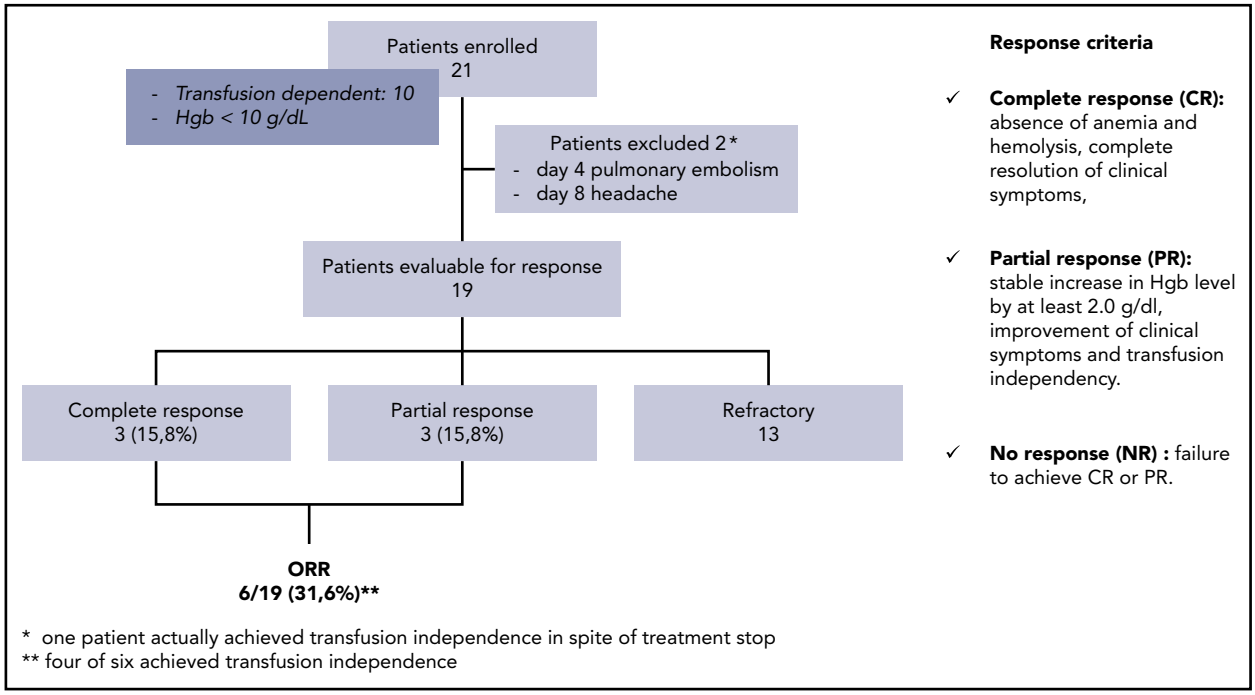

Figure 1. Flowchart of patients enrolled. Response was evaluated at 3 months after treatment start. Hgb, hemoglobin; ORR, overall response rate.

monthly during the 2 months before entering the trial. Exclusion criteria were concomitant clonal B-cell disorder requiring specific treatment, preexisting peripheral neuropathy, documented hypersensitivity to bortezomib, pregnancy, or psychiatric disorders. Twenty-one patients have been enrolled by 6 centers between April 2012 and March 2016. Eligible patients received a single course of bortezomib (1.3 mg/sqm IV on days 1, 4, 8, 11). Prophylaxis of herpes zoster reactivation was given with oral acyclovir (400 mg twice daily) until 1 month after the end of bortezomib. The response criteria were the same previously published.[19,12]

A summary of baseline characteristics of patients is provided in Table 1, and results achieved after bortezomib administration are summarized in Figure 1. Among the 19 evaluable patients, 3 achieved a complete response (15.8%) and 3 achieved a partial response (15.8%), for an overall response rate of 31.6%, including transfusion independence in 4 patients. Response was not correlated to previous treatments, including rituximab, nor to the presence of B-cell clonal disorder.

Four of 6 responding patients (66.7%; 95% confidence interval 37.9-100) maintained the response after a median follow-up of 16 months (range 10-31). One patient relapsed after >1 year and obtained a second remission after a second course of bortezomib. All patients were alive at last follow-up except 1 who died of septic shock during off treatment follow-up, 10 months after study entry. Median hemoglobin levels (g/L) before and after treatment were 87 and 96 in the whole cohort, 85 and 114 in responding patients, and 88 and 92 in nonresponding patients, respectively. The level of monoclonal immunoglobulin remained stable after treatment in 10/13 cases and decreased to <50% of pretreatment levels in 3 patients. The marrow B-cell lymphoid infiltrate persisted in 3 of 5 patients, decreased by >50% in 1 case, and became undetectable in 1. In 4 patients, bone marrow biopsy was not repeated. No correlation was found between the response to bortezomib and any of the pre- and the posttreatment characteristics of patients.

Treatment-related toxicity, including neurotoxicity, was rarely detected. A total of 19 adverse events of any grade were reported. Among 13 grade 1 to 2 adverse events, 1 was considered related to study drug (anemia grade 2). Six grade 3 to 4 adverse events were detected (headache, diarrhea, increased bilirubin levels, anemia, pulmonary embolism), among which only 1 (upper respiratory tract infection) was considered related to study drug.

There is an active search for novel therapies in symptomatic patients with CAD, to overcome the relatively low efficacy of rituximab and the potential toxicity of cytostatic agents like fludarabine and bendamustine, which currently represent state-of-the-art treatments. Therapies designed to target complement-mediated hemolysis of CAD are being actively investigated. Eculizumab has been reported effective in reducing transfusion requirement in preliminary reports,[20,21] although in steady-state CAD most of hemolysis is not intravascular and C5 mediated. Moreover, in paroxysmal nocturnal hemoglobinuria eculizumab has been reported to increase C3b-mediated extravascular hemolysis,[22] which is assumed to predominate in CAD. Thus, its efficacy in CAD needs to be confirmed in larger studies. More recently, the humanized antibody BIVV009 specific for the complement subunit C1s was proven effective in blocking the classical complement pathway activation and the resulting extravascular hemolysis both in vitro and in a phase 1 study.[23,24] The antibody has received US Food and Drug Administration Breakthrough Therapy designation, and pivotal trials are currently ongoing (ClinicalTrial.gov: #NCT03347396; #NCT03347422).

Bortezomib has proven very effective in B-cell clonal lymphoproliferative disorders, characterized by the presence of a

monoclonal IgM (eg, Waldenstrom macroglobulinemia), like in most cases of primary CAD. In the present study, a brief course of bortezomib obtained an overall response and a CR rate of 32% and of 16%, respectively, in patients with CAD. These results represent a proof of concept of the activity of bortezomib in a proportion of patients with CAD, which had been suggested to date only by case reports of single patients.[17] Treatment was well tolerated, and toxicity was limited. All patients had received prior treatments, including rituximab and cytostatic agents, further supporting the efficacy of bortezomib. Moreover, despite the short duration of treatment, two-thirds of responding patients enjoyed a long-lasting remission without the need of further treatment.

No significant effect of 4 infusions of bortezomib on the underlying B-cell clone was expected. However, some activity was shown by the >50% decrease of the monoclonal immunoglobulin in 23% of evaluable cases and by the reduction or disappearance of the lymphoid infiltrate in bone marrow in 2/5 patients, suggesting that a more prolonged administration may be beneficial also on the underlying B-cell disorder.

In conclusion, this study shows that a short course of bortezomib may be effective in one-third of patients with symptomatic CAD failing previous treatments, with acceptable toxicity and long-lasting benefit in the majority of responding patients. These data provide a rationale for further investigating the potential benefits of bortezomib either by using a more prolonged treatment schedule or in combination with other agents, particularly rituximab.

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Authorship

Contribution: G.R. and W.B. designed and performed research and wrote the paper; F.Z. and D.G. performed research and wrote the paper; B.F., F.B., M.D., M.F., E.L., F.R.M., F.S., and M.M. included and followed patients; F.P. and P.F. monitored and analyzed data; and all authors reviewed and approved the final version of the manuscript.

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REFERENCES

1. Petz LD, Garratty G. Immune Hemolytic Anemias. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2004.

2. Berentsen S. Cold agglutinin disease. *Hematology Am Soc Hematol Educ Program*. 2016;2016:226-231.

3. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. *Blood*. 2013;122(7):1114-1121.

4. Barcellini W, Fattizzo B, Zaninoni A, et al. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients. *Blood*. 2014;124(19):2930-2936.

5. Berentsen S, Ulvestad E, Langholm R, et al. Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. *Haematologica*. 2006;91(4):460-466.

6. Mullins M, Jiang X, Bylsma LC, et al. Cold agglutinin disease burden: a longitudinal analysis of anemia, medications, transfusions, and health care utilization. *Blood Adv*. 2017;1(13):839-848.

7. Berentsen S. How I manage cold agglutinin disease. *Br J Haematol*. 2011; 153(3):309-317.

8. Zanella A, Barcellini W. Treatment of autoimmune hemolytic anemias. *Haematologica*. 2014;99(10):1547-1554.

9. Barcellini W. Current treatment strategies in autoimmune hemolytic disorders. *Expert Rev Hematol*. 2015;8(5):681-691.

10. Schöllkopf C, Kjeldsen L, Bjerrum OW, et al. Rituximab in chronic cold agglutinin disease: a prospective study of 20 patients. *Leuk Lymphoma*. 2006;47(2):253-260.

11. Berentsen S, Ulvestad E, Gjertsen BT, et al. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. *Blood*. 2004;103(8):2925-2928.

12. Berentsen S, Randen U, Vågan AM, et al. High response rate and durable remissions following fludarabine and rituximab combination therapy for chronic cold agglutinin disease. *Blood*. 2010;116(17):3180-3184.

13. Berentsen S, Randen U, Oksman M, et al. Bendamustine plus rituximab for chronic cold agglutinin disease: results of a Nordic prospective multicenter trial. *Blood*. 2017;130(4):537-541.

14. Berentsen S. How I manage patients with cold agglutinin disease. *Br J Haematol*. 2018;181(3):320-330.

15. Treon SP. How I treat Waldenström macroglobulinemia. *Blood*. 2015; 126(6):721-732.

16. Małecka A, Trøen G, Tierens A, et al. Frequent somatic mutations of KMT2D (MLL2) and CARD11 genes in primary cold agglutinin disease [published online ahead of print 19 December 2017]. *Br J Haematol*. doi:10.1111/bjh.15063.

17. Carson KR, Beckwith LG, Mehta J. Successful treatment of IgM-mediated autoimmune hemolytic anemia with bortezomib. *Blood*. 2010;115(4):915.

18. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381.

19. Barcellini W, Zaja F, Zaninoni A, et al. Low-dose rituximab in adult patients with idiopathic autoimmune hemolytic anemia: clinical efficacy and biologic studies. *Blood*. 2012;119(16):3691-3697.

20. Röth A, Hüttmann A, Rother RP, Dührsen U, Philipp T. Long-term efficacy of the complement inhibitor eculizumab in cold agglutinin disease. *Blood*. 2009;113(16):3885-3886.

21. Roth A, Bommer M, Huttmann A, et al. Complement inhibition with Eculizumab in patients with cold agglutinin disease (CAD): results from a prospective Phase II Trial (DECADE trial) [abstract]. *Blood*. 2015;126(23). Abstract 274.

22. Risitano AM, Marotta S. Toward complement inhibition 2.0: Next generation anticomplement agents for paroxysmal nocturnal hemoglobinuria. *Am J Hematol*. 2018;93(4):564-577.

23. Shi J, Rose EL, Singh A, et al. TNT003, an inhibitor of the serine protease C1s, prevents complement activation induced by cold agglutinins. *Blood*. 2014;123(26):4015-4022.

24. Jaeger U, D'Sa S, Schoergenhofer C, et al. Long term efficacy, safety and PK/PD profile of the anti-C1s antibody (BIVV009) in primary cold agglutinin disease patients [abstract]. *Blood*. 2017;130(suppl 1). Abstract 703.

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