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# Bendamustine plus rituximab for chronic cold agglutinin disease: results of a Nordic prospective multicenter trial

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## Key Points

- Bendamustine-rituximab therapy results in high overall and CR rates with sustained remissions in CAD.
- Bendamustine plus rituximab may be considered in first line for most patients with CAD requiring therapy.

**Primary chronic cold agglutinin disease (CAD) is a well-defined clinicopathologic entity in which a bone marrow clonal B-cell lymphoproliferation results in autoimmune hemolytic anemia and cold-induced circulatory symptoms. Rituximab monotherapy and fludarabine-rituximab in combination are documented treatment options. In a prospective, nonrandomized multicenter trial, 45 eligible patients received rituximab 375 mg/m$^2$ day 1 and bendamustine 90 mg/m$^2$ days 1 and 2 for 4 cycles at a 28-day interval. Thirty-two patients (71%) responded; 18 (40%) achieved complete response (CR) and 14 (31%) partial response (PR). Among 14 patients previously treated with rituximab or fludarabine-rituximab, 7 (50%) responded to bendamustine-rituximab (3 CR and 4 PR). Hemoglobin levels increased by a median of 4.4 g/dL in the complete responders, 3.9 g/dL in those achieving PR, and 3.7 g/dL in the whole cohort. The 10th percentile of response duration was not reached after 32 months. Grade 3-4 neutropenia occurred in 15 patients (33%), but only 5 (11%) experienced infection with or without neutropenia. Thirteen patients (29%) had their dose of bendamustine reduced. In conclusion, bendamustine-rituximab combination therapy is highly efficient, sufficiently safe, and may be considered in first line for patients with CAD requiring therapy. The trial was registered at www.clinicaltrials.gov as #NCT02689986. (*Blood*. 2017;130(4):537-541)**

## Introduction

Primary chronic cold agglutinin disease (CAD) is an autoimmune hemolytic anemia mediated by cold agglutinins (CAs), without any obvious underlying disease such as aggressive lymphoma, other overt malignancies, or specific infections.[1,2] It accounts for approximately 15% of autoimmune hemolytic anemia.[3,4] In typical primary CAD, a well-defined clonal lymphoproliferative disorder (LPD) of the bone marrow, termed "CA-associated LPD" and shown to be distinct from lymphoplasmacytic lymphoma, causes production of monoclonal CA.[5-7] The CAs are almost invariably of the immunoglobulin (Ig) Mκ class and have specificity for the I carbohydrate antigen on the erythrocyte surface.[8-11] Temperatures below normal central body temperature facilitate binding to the antigen, causing agglutination and complement-dependent hemolysis.[2,9,12] The predominant mechanism of erythrocyte breakdown, known as extravascular hemolysis, is phagocytosis of cells opsonized with complement protein C3b, generated by the classical pathway.[2,13-15] Essential clinical manifestations are anemia and, in up to 90% of the patients, cold-induced acrocyanosis and/or Raynaud phenomena.[10]

Although most literature emphasizes the importance of avoiding cold exposure, descriptive studies have shown that 70% to 80% of unselected patients have received pharmacologic treatment.[10,16] Hemoglobin (Hb) levels <8.0 g/dL in one-third of the patients also highlight the need for effective medical therapy.[10] Corticosteroids are generally ineffective in treating CAD.[4,10,16,17] Documented effective therapies are either directed at the pathogenic B-cell clone or designed to target the complement-mediated hemolysis.[1,14,18-22] B-cell–directed approaches have the advantage of not requiring infinite treatment. However, rituximab monotherapy resulted in a modest response rate of 50%, almost exclusively partial responses (PRs), and a median response duration of only 12 months.[18,19] Rituximab-fludarabine in combination demonstrated an overall response rate of 70% with 20% complete

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responses (CRs) and a median response duration of more than 66 months, but with some toxicity.[20]

Bendamustine is an antineoplastic drug that combines the 2-chlorethylamine group of the nitrogen mustard–derived alkylating agents with the benzimidazole ring structures of the purine analogs.[23] Combined treatment with bendamustine and rituximab has shown high efficacy and acceptable toxicity in a variety of B-cell LPD.[24-26] Favorable effect in CAD has been reported in a single patient.[27,28] In the present study, we have investigated the potential of bendamustine plus rituximab for patients with CAD requiring therapy.

## Methods

### Study outline

We conducted a prospective, uncontrolled multicenter trial with 16 participating hospitals in Norway, Finland, and Denmark. The study was registered at www.clinicaltrials.gov as "The CAD5 Study." Our primary objective was to assess the efficacy of bendamustine plus rituximab in CAD. Secondary objectives were safety in the whole cohort and efficacy in patients who had previously received rituximab or rituximab-fludarabine therapy. The Regional Committee for Medical and Health Research Ethics of Southeast Norway and the Norwegian Medicines Agency approved the protocol, which was subsequently approved by the corresponding regulatory authorities in Finland and Denmark. Written informed consent was obtained from each patient in accordance with the Declaration of Helsinki. Patients were enrolled between October 1, 2012, and December 31, 2015. We have included in this report 3 consecutive patients who fully met the inclusion and exclusion criteria, treated according to the same protocol immediately before and after the enrollment period.

### Inclusion and exclusion criteria

To be eligible for the trial, each patient had to be diagnosed with CAD and require therapy because of anemia, substantial cold-induced circulatory symptoms, or both. The criteria for CAD were chronic hemolysis combined with CA titer 64 or higher at 4°C and a typical pattern for the direct antiglobulin test (DAT).[29] The typical DAT pattern was defined as a strongly positive monospecific DAT for complement protein C3d and a negative (or only weakly positive) test for IgG.[10,29] Serum and bone marrow examinations had to confirm a clonal B-cell lymphoproliferation, as described by the presence of a monoclonal Ig band in serum and/or a clonal expansion in bone marrow of lymphocytes with a phenotype consistent with CAD, demonstrated by immune histochemistry, flow cytometry, or both. Central analysis of the bone marrow samples at an expert laboratory (Department of Pathology, Oslo University Hospital; U.R.) was encouraged.[5]

Patients who had a secondary CA syndrome (eg, associated with an apparent or aggressive lymphoma)[2,29] were ineligible for the study. Further exclusion criteria were nonlymphatic malignant disease other than basal cell carcinoma of the skin, known HIV infection, hepatitis B or C, liver failure, parenchymal liver disease with serum bilirubin >51 μmol/L (3.0 mg/dL), pregnancy, breastfeeding, unwillingness to use safe contraception if relevant, any contraindication to therapy with the study drugs, age <18 years, and inability to cooperate. Previous therapy for CAD or high serum bilirubin levels considered due to hemolysis were not exclusion criteria.

### Therapy

Eligible patients received 4 cycles at 28-day intervals of rituximab intravenously 375 mg/m$^2$ day 1 and bendamustine 90 mg/m$^2$ days 1 and 2. Dose reduction of bendamustine was allowed according to strict protocol guidelines if the patients had received previous cytotoxic therapy or experienced drug-related adverse events (AEs). Growth factor or antimicrobial prophylaxis was not used.

**Table 1. Baseline data**

| | Median | Range |
|---|---|---|
| Age, y | 74 | 48-86 |
| Hemoglobin level, g/dL | 9.5 | 4.5-14.8 |
| Reticulocyte count, $\times 10^9$/L | 158 | 72-300 |
| Bilirubin, μmol/L | 45 | 15-153 |
| Lactate dehydrogenase, U/L | 468 | 181-952 |
| Haptoglobin, g/L | <0.1 | <0.1-2.8 |
| Total IgM concentration, g/L | 4.1 | 1.0-27.2 |
| Cold agglutinin titer, 4°C | 2048 | 64-64000 |
| Complement C4 level, g/L | <0.06 | <0.06-0.24 |
| Ratio between κ- and λ-positive B cells in bone marrow aspirate | 7.1 | 0.2-92 |

Patients were assessed before each cycle of therapy and thereafter monthly for 6 months. Blood samples were collected at each visit, and cold-induced circulatory symptoms were assessed using a 0 to 2 scale (0, no improvement; 2, complete resolution). Any AEs were recorded. Bone marrow histology and flow cytometry were performed at 4 months after completion of therapy. After the initial 6-month follow-up, we obtained clinical, hematologic, and biochemical data at approximately 3-month intervals for the remaining time of the trial.

### Outcomes

We used previously published response criteria.[10,18,20] CR was defined as normal Hb level with absence of hemolysis, full resolution of any circulatory symptoms, undetectable monoclonal serum protein, and no signs of clonal lymphoproliferation as assessed by bone marrow histology, immunohistochemistry, and flow cytometry. The criteria for PR were a stable increase in Hb level by at least 2.0 g/dL or to the normal range, a decline in serum IgM level by at least 50% or to the normal range, transfusion independence, and improvement of clinical symptoms. No response (NR) was defined as failure to achieve CR or PR.

We defined time to response (TTR) as the time from start of therapy to the achievement of any degree of response. For responders who were anemic at baseline, we also calculated time to best response (TTBR), defined as the time from start of therapy to observation of the highest Hb level achieved ±0.5 g/dL. Response duration was the time from response to relapse or death. The criteria for relapse were a decrease in Hb level by at least 2.0 g/dL from the highest level observed or to <10.0 g/dL and/or recurrence of clinical symptoms. AE were graded according to the Common Terminology Criteria, version 4.0 (2009).[30]

### Statistics

Median values were determined to express the central tendency of variables with skewed distribution. We used the Wilcoxon test to calculate the statistical significance of differences between paired continuous data, the Fisher exact test to determine the significance of differences between frequencies in cross tables of nominal variables, and Spearman ρ to examine correlations between numeric variables when at least 1 variable was ordinal.

## Results

### Baseline characteristics

Forty-five patients (20 men and 25 women) were included, with a median age of 74 years (range, 48-86) and median disease duration of 4 years (range, 0-18). Thirty-three patients (73%) were reported to have cold-induced circulatory symptoms, whereas 24 patients (53%) had experienced exacerbation during febrile disease. Median Hb level was 9.5 g/dL (range, 4.5-14.8; 7 patients with compensated hemolysis were included because of cold-induced circulatory symptoms requiring therapy). Additional baseline data are shown in Table 1.

DAT was positive for C3d only in 36 patients (80%), whereas 9 patients (20%) had a DAT strongly positive for C3d and weakly

**Table 2. Response rates**

| Response level | n | % |
|---|---|---|
| CR | 18 | 40 |
| PR | 14 | 31 |
| NR | 13 | 29 |
| All patients | 45 | 100 |

positive for IgG. The serum monoclonal Ig class was IgMκ in 39 patients (87%); IgGκ and IgAλ in 1 patient each, and not detectable or not evaluable in 4 patients (9%).

Histologic signs of LPD were present in 35 (85%) of 41 evaluable bone marrow biopsies obtained before therapy. In the whole cohort of 45 patients, we were able to confirm clonal bone marrow B lymphocytes in 40 (89%), as demonstrated by histology and flow cytometry in 34 (76%), by histology only in 1 (2%), and by flow cytometry only in 5 (11%). In the remaining 5 patients (11%), the only evidence of a clonal disease was monoclonal serum Ig. Among 27 pretreatment bone marrow biopsies submitted for central analysis, CAD-associated LPD was found in 25 (93%), whereas 2 (7%) were consistent with possible or probable CAD-associated LPD.

Twenty-seven patients (60%) were previously untreated, whereas 10 patients (22%) had previously received rituximab monotherapy, 4 (9%) rituximab-fludarabine combination therapy, and 4 (9%) other therapies.

### Response data

Table 2 shows the response rates. Thirty-two patients (71%) responded to therapy. Eighteen patients (40%) achieved CR and 14 (31%) PR, whereas 13 (29%) were nonresponders. In previously treated patients, we observed an overall response rate of 9/17 (53%); 3 (18%) CR, 6 (35%) PR, and 8 (47%) NR. Among 10 patients who had previously received rituximab monotherapy, 4 responded to bendamustine-rituximab, with 2 CR and 2 PR. In 3 of these responders (2 CR, 1 PR), rituximab monotherapy had failed. Among 4 patients with a history of fludarabine-rituximab therapy, 1 achieved CR and 2 PR after the bendamustine-rituximab combination. Two of these (1 CR, 1 PR) had not responded to fludarabine-rituximab. Median TTR was 1.9 months (range, 0.25-12) in the whole group of responders, 1.5 months (range, 0.25-10) in those who developed CR, and 2.25 (range, 0.25-12) with PR (correlation not statistically significant). In most of the 28 responders with anemia at baseline, Hb levels continued to improve after achievement of response, showing a median TTBR of 7.0 months (range, 1.5-30; no significant correlation with response level).

We observed a median increase in Hb levels by 4.0 g/dL in the responders; 4.4 g/dL (range, 0.2-11.8) in those who achieved CR and 3.9 g/dL (0.0-7.6) in those who had a PR. There was no significant change in Hb in the nonresponders. Further laboratory response data are provided in Table 3. In the responders, median bilirubin levels declined to 11 μmol/L (range, 4-37) and median lactate dehydrogenase levels to 196 U/L (range, 121-331). Acrocyanosis and Raynaud symptoms resolved completely in 17 patients and improved in additional 11 (50% and 32%, respectively, of those with such symptoms before treatment).

By definition, all patients who achieved CR had complete resolution of any demonstrable bone marrow LPD by follow-up histology. In the PR group, complete histologic resolution was found in 4 patients (29%) and significant but incomplete regression in 1 patient. In the remaining 9 partial responders, rebiopsy was either not relevant (no demonstrable LPD by histology at baseline), not done because of patient or local investigator decision, or technically unsuccessful. Partial histologic regression was also observed in 3 (23%) of the nonresponders. All responders taken together, histologic regression was complete in 17 patients (53%), partial in 1 (3%), and not relevant or not evaluable in 14 (44%).

Response levels did not significantly correlate with age, pretreatment Hb level, IgM level, CA titer, serum C4 concentration, or the ratio between κ- and λ-positive B cells in bone marrow aspirate. We also categorized patients into 2 groups, those with circulatory symptoms only (fully compensated hemolysis) and those with manifest anemia, but we found no significant association between group and response level. Furthermore, there was no significant difference in overall response rate between patients who had their dose of bendamustine reduced and those who had not.

Median follow-up was 36 months (range, 3.0-65) from inclusion for the whole cohort and 32 months (2.0-59) from achievement of response in the combined CR and PR group. During this period, 3 patients (9% of the responders) relapsed; 1 CR at 12 months and 2 PR at 1 and 9 months, respectively. The remaining 29 responders (91%) experienced sustained remission. Median observed response duration (to censoring, relapse, or death) was 32 months (range, 1.0-59). By censoring the data after median 32 months after response follow-up, the 10th percentile of response duration was still not reached.

### Safety and tolerance

Neutropenia accounted for all hematologic toxicity grade >1 (Table 4). Fifteen patients (33%) had 1 to 3 episodes each of neutropenia grade 3-4, including 9 patients (20%) who developed neutropenia grade 4. Three patients (7%) had 1 to 2 episodes each of febrile neutropenia, all of which were grade 3 and readily manageable. Varicella-zoster virus reactivation or *Pneumocystis jirovecii* pneumonia did not occur. Following therapy, we did not observe worsened anemia with reticulocytopenia in any patient (lowest posttherapy reticulocyte count in nonresponders: $51 \times 10^9$/L).

Nonhematologic AE occurred in 17 patients (38%) and were grade 1-2 in 9 (20%) and grade 3 in 8 (18%). These AE were gastrointestinal discomfort (mostly mild nausea) in 5 patients (11%), rash in 4 (9%), non-neutropenic infection requiring treatment in 2 (4%), atrial fibrillation in 1 (2%), neuropathy in 1 (2%), and other nonhematologic

**Table 3. Laboratory response data**

| | Increase in Hb level | | | Highest Hb level posttherapy | | Decrease in total IgM concentration | | | CA titer after therapy/CA titer before therapy | |
|---|---|---|---|---|---|---|---|---|---|---|
| Response level | Median, g/dL | Range, g/dL | *P* | Median, g/dL | Range, g/dL | Median, % of baseline | Range, % of baseline | *P* | Median ratio | *P* |
| CR | 4.4 | 0.2-11.8 | <.001 | 14.2 | 13.0-16.8 | 76 | 52-95 | <.001 | 0.000 | .01 |
| PR | 3.9 | 0.0-7.6 | <.001 | 12.5 | 10.0-15.0 | 74 | 37-96 | .02 | 0.250 | NS |
| NR | 0.0 | −2.5 to 1.6 | NS | 10.5 | 7.4-14.9 | 55 | −23 to 74 | .01 | 1.000 | NS |
| All patients | 3.7 | −2.5 to 11.8 | <.001 | 13.2 | 7.4-16.8 | 67 | −23 to 96 | .001 | 0.125 | .05 |

Seven patients with hemolysis without anemia were included because of circulatory symptoms requiring therapy, which explains the lower range of Hb level increase among the responders and the higher range of posttherapy Hb levels among the nonresponders. *P* values indicate statistical significance of changes.

NS, not significant.

**Table 4. Essential toxicity data**

| | n | % |
|---|---|---|
| Neutropenia grade 3-4 | 15 | 33 |
| Neutropenia grade 4 | 9 | 20 |
| Infection (with or without neutropenia) | 5 | 11 |
| Varicella-zoster virus reactivation | 0 | 0 |
| Dose reduction | 13 | 29 |
| All patients | 45 | 100 |

AE in 4 (9%). During therapy, 13 patients (29%) were hospitalized for a median of 8 days (range, 1-19; all causes taken together), whereas the remaining 32 (71%) did not require hospitalization. Thirteen patients (29%) had at least 1 dose of bendamustine reduced because of AE, mostly neutropenia. In 7 of these (16% of total), bendamustine therapy was discontinued before planned completion. Frequencies of neutropenia or dose reduction did not correlate with age (younger than median vs median or older).

Three nonresponders died during follow-up. An 84-year-old, frail woman died in a nursing home of pneumonia after 5 months without having experienced any hematologic AE. An 85-year-old man died after 1 year from acute myelogenous leukemia secondary to a myelodysplastic syndrome that was, in retrospect, present before therapy for CAD. An 80-year-old man died 3 weeks after receiving his third therapy cycle. He developed generalized weakness and hypothermia, which started immediately after administration of rituximab and did not improve. We considered his death related to rituximab, but unrelated to bendamustine.

Two non-neutropenic serious AEs were reported among the 42 surviving patients. A 71-year-old female CR suffered from recurrent pneumonia, starting 9 months after completion of therapy. Diagnostic workup showed sustained lymphopenia with hypogammaglobulinemia, which was successfully managed by immunoglobulin substitution. A 77-year-old man developed grade 3 polyneuropathy during his third therapy cycle. Bendamustine was discontinued, his neurologic symptoms resolved completely, and he achieved complete remission of CAD.

## Discussion

The overall response and CR rates show that combined therapy with bendamustine and rituximab is very efficient in primary CAD. The median rise in Hb levels by 4.4 g/dL in the CR and 3.7 g/dL in the whole cohort underscore the high efficacy. Remissions were durable with a 10the percentile response duration not reached at censoring after 32 months, indicating an expected response duration much longer than the observed duration. Because only 3 patients have relapsed so far, it is too early to use a Kaplan-Meier plot to determine the expected response duration. Although half of the responders had achieved remission by 1.9 months, the upper range of TTR was very long (12 months), indicating that 4 cycles of therapy may be appropriate even in patients who have not responded by the end of their fourth cycle. Of interest, responses often continued to improve for a long time after the response criteria had been met, as shown by the long median TTBR of 7 months with a maximum of 30 months.

Our results confirmed presence of CAD-associated LPD in most patients.[5] Samples were not prospectively assessed for the *MYD88* L265P mutation as part of this trial. In a related study published separately by our group, however, a sensitive polymerase chain reaction technique was used to analyze bone marrow samples from 27 of the same patients, all of which were negative for *MYD88* L265P.[7]

Following B-cell–directed therapies for CAD, one would expect clinical response to be related to histologic resolution of the bone marrow LPD. In the study presented here, as in our fludarabine-rituximab study, the results confirm this notion.[20] The decline in IgM levels and CA titers in the responders as well as the whole cohort is consistent with this. The importance of eradication or substantial reduction of the pathogenic B-cell clone is also illustrated by our small, previous trial of cladribine monotherapy, in which the lack of clinical response seemed associated with poor efficacy in targeting the bone marrow LPD.[31] Even in the present study, however, we did find some patients who achieved a significant reduction in IgM levels and CA titers without enjoying a clinical response.

Most clinical AE were mild and could be attributed to known nonhematologic toxicity of bendamustine.[23,26] The frequency of neutropenia grade 3-4 was considerable. In most patients, however, this did not translate into clinical AE, because only 5 individuals (11%) experienced infection (including 2 cases of non-neutropenic infection). The death rate of 3/45 patients during 36 months is not unexpected in this cohort of mainly old people. Thus, bendamustine-rituximab therapy is considered safe in patients with CAD.

For the clinician, the high efficacy of treatment with bendamustine plus rituximab will raise the question of whether this regimen should be preferred over fludarabine-rituximab.[20] Because of limited statistical power, randomized controlled trials are very difficult to perform in this uncommon disease, even at an international level.[1,20,32] One randomized trial did compare bendamustine-rituximab directly with fludarabine-rituximab in non-Hodgkin lymphoma, showing superior efficacy of the bendamustine combination along with similar rates of AE.[26] Because of the entirely different study population, however, these results are hardly relevant for CAD. Comparison of independent nonrandomized trials should be interpreted with caution. The baseline characteristics presented here, however, matched well with those reported in our fludarabine-rituximab trial (Table 1), and the inclusion and response criteria were identical.[20] To a reasonable extent, this should allow comparison of essential results between the 2 studies.

The overall response rates were similar, but the CR rate was nearly doubled in the present study as compared with the fludarabine-rituximab trial (40% vs 21%; Table 2).[20] The observed response duration was equally long or longer with bendamustine plus rituximab; therefore, we consider this regimen at least as efficient as fludarabine plus rituximab. The frequency of neutropenia grade 3-4 seems lower following therapy with bendamustine-rituximab (33% vs 41%; Table 4), but the significance of this modest difference between independent trials is uncertain. There was a striking difference in the infection rate (11% following the bendamustine-rituximab combination compared with 59% after fludarabine-rituximab). Varicella-zoster virus reactivation, observed in 10% of patients treated with fludarabine-rituximab, did not occur in the present trial. The frequency of dose reduction was also lower with bendamustine plus rituximab (29% vs 45%). It should be emphasized, however, that dose reduction according to the protocol was based on cell counts rather than clinical AE. The study described here did not address the risk of late secondary malignancies, which is a known risk with fludarabine-based therapies.[33,34]

In conclusion, chemoimmunotherapy with bendamustine and rituximab results in a high total response rate, a very high rate of CR, and long response duration. Even patients previously treated with rituximab or fludarabine-rituximab may respond. The safety profile

appears more favorable than with the fludarabine-rituximab combination. Bendamustine plus rituximab may be considered in first line for most patients with CAD requiring therapy.

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## Authorship

Contribution: S.B. and G.E.T. conceived the idea; S.B., U.R., and G.E.T. wrote the protocol; S.B., M.O. and H.B. served as principal investigators in Norway, Finland, and Denmark, respectively; U.R. assessed all bone marrow biopsies submitted for central evaluation; T.H.A.T., J.D., E.G., E.H., R.B., J.H.S., and I.A.N. were local investigators; A.M. performed molecular biologic examinations; S.B. analyzed the data and wrote the manuscript in cooperation with G.E.T.; and all authors revised the paper and approved the submitted version.

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