display higher serum tryptase level, serum alkaline phosphatase level and bone marrow mast cell burden (Table 1).

Next, we focused on SM-AHN-myeloid (n = 178), which included 11 (6%) patients with SM associated with acute myeloid leukemia (SM-AML), 50 (28%) patients with SM associated with myeloproliferative neoplasms (SM-MPN), 24 (13%) patients with SM associated with myelodysplastic syndromes (SM-MDS), 39 (22%) patients with SM associated with chronic myelomonocytic leukemia (SM-CMML) and 54 (30%) patients with SM associated with MDS/MPN/unclassified (SM-MDS/MPN/Unclassified). Figure 1C confirms the survival superiority of SM-AHN-lymphoid, compared to any other specific category of SM-AHN-myeloid. Prognostic comparison in SM-AHN-myeloid (Figure 1C) is confounded by significant variation in age and risk distribution of the specific myeloid components but appears to favor SM-MPN and SM-MDS over other subcategories, including SM-CMML and SM-MDS/MPN/Unclassified (Figure 1C).

We conclude that patients with SM-AHN-lymphoid are phenotypically and prognostically different than those with SM-AHN-myeloid and should be cited as such in both clinical practice and future survival studies in SM. Similarly, prognosis in SM-AHN-myeloid appears to be dictated by the specific AHN component, which underscores the need to be more specific in diagnostic assignment and treatment directions. This is particularly important since sensitivity to drug therapy or to other treatment modalities in SM might be different for neoplastic mast cells vs the associated myeloid neoplasm clone.[5]

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Rituximab, bendamustine and cytarabine (R-BAC) in patients with relapsed-refractory aggressive B-cell lymphoma

To the Editor:

Management of diffuse large B-cell lymphoma (DLBCL) after failure of front-line immunochemotherapy is a treatment challenge, without a clear optimal salvage strategy, especially for patients not candidate to autologous stem cell transplantation (ASCT).[1] Few patients achieve long-term disease control and no standard therapy exists. The British Columbia Cancer Agency reported a median progression-free survival (PFS) and overall survival (OS) of 2.1 and 3.9 months, respectively, in 326 patients who were not candidate for ASCT.[2] Among most widely used salvage regimens, the combination of rituximab and bendamustine (BR) was associated with overall response (OR) ranging from 32 to 63%, and PFS of 3-8 months.[3,4] When combined with cytarabine, bendamustine has demonstrated significant synergistic activity in preclinical studies on DLBCL.[5] Thus, we conducted a pilot trial to explore the efficacy and tolerability of R-BAC (rituximab, bendamustine, and cytarabine) in a cohort of patients with B cell relapsed/refractory (R/R) aggressive lymphoma.

This study enrolled previously treated, histologically confirmed DLBCL, either de-novo or transformed B-cell lymphoma (t-DLBCL), treated with R-BAC between November 2012 and September 2017 in six Centers from Northern Italy. To be included patients had to be: (1) treated with at least one previous line if not eligible for ASCT; (2) treated with two or more previous lines of therapy including ASCT or not; (3) aged 18 years or more, with no upper age limit; (4) not treated with prior bendamustine. The study was approved by the ethics review board of the participating institutions and patients signed informed consent before being enrolled. All histological diagnosis were centrally reviewed by two expert hematopathologists (B.F. and

TABLE 1 Patients characteristics and treatment

Characteristics	N = 39	(%)
Histology		
De novo DLBCL	28/39	*72*
t-DLBCL	11/39	*28*
Age		
Median (range)	68 (21-84)	
>65 years	22	*56*
Gender		
Male	23/39	*59*
ECOG PS		
≥2	17/39	*44*
IPI risk group		
Low (0-1)	9/39	*23*
Intermediate (2-3)	17/39	*44*
High (4-5)	13/39	*33*
Cell-of-origin[a]		
GCB	23/32	*72*
Non-GCB	9/32	*28*
N prior treatments		
Median (range)	2 (1-4)	
Prior therapies		
Upfront R-CHOP or CHOP-like	39/39	*100*
ASCT	6/39	*15*
Cytarabine	14/39	*36*
Lenalidomide	6/39	*15*
Disease status		
Relapsed	18/39	*46*
Refractory	21/39	*54*
R-CHOP-refractory	8/39	*21*
R-DHAP-refractory	4/39	*10*
Double refractory[b]	9/39	*23*
Number of R-BAC cycles		
Median (range)	4 (1-6)	
≥4 cycles	19	*49*
Reason for less than 4 cycles		
PD	10/19	*53*
Toxicity[c]	2/19	*10*
Other[d]	7/19	*37*

Abbreviations: ASCT: autologous stem cell transplantation; CHOP: cyclophosphamide, hydroxydaunorubicin, oncovin and prednisone; DHAP: dexamethasone, cytarabine, cisplatin +/− rituximab; DLBCL: diffuse large B cell lymphoma; ECOG PS: Eastern Cooperative Oncology Group Performance Status; GCB: germinal center B-cell like DLBCL; IPI: International Prognostic Index; N: number; Non-GCB: nongerminal center DLBCL; PD: progressive disease; t-DLBCL: transformed-DLBCL.

[a] Double refractory: R-CHOP and R-DHAP refractory.

[b] Cell of Origin was determined according to the Visco-Young algorithm.

[c] Two patients had infectious complications after the second cycle while they were responding and were withdrawn from study treatment (see text for details).

[d] Other: one patient refused to receive further therapy after he was in PR after 3 cycles; two patients were consolidated with allogeneic stem-cell transplant; four patients proceeded to autologous stem cell transplantation after 2-3 R-BAC cycles.

E.SG.D.). The primary end-point was the complete remission (CR) rate. Secondary end-points included OR, PFS, OS, duration of response (DOR), and safety. The response rate was based on local radiologists evaluation, and was evaluated according to Cheson criteria, 2007. A boundary of 30% CR rate was considered indicative of a positive trial, based on results obtained with BR in similar patient series.[3,4] The R-BAC regimen[6] consisted of rituximab (375 mg/m2, Day 1 of the cycle), bendamustine (70 mg/m^2, Days 1-2, given as a 1-h infusion) and cytarabine (500 mg/m^2, Days 1-3, given as a 2-h infusion starting 2 h after bendamustine), administered every 21 days. Patients were meant to receive at least four cycles, but in case of good tolerance and/or partial response after four cycles patients continued treatment to a maximum of six cycles. Patients older than 70 years or with poor performance status (ECOG >2) not due to lymphoma progression had cytarabine dose limited to Days 1 and 2. The same dose reduction was applied to patients experiencing an excess of toxicity, defined as hematological or non-hematological toxicity of Grade 4 lasting for more than 5 days during any of the treatment cycle. Primary prophylaxis with granulocyte colony-stimulating factor was routinely used starting from Day 5 after chemotherapy completion, and lasting for 3-6 days or until neutrophil count recovery.

Overall, 39 patients were included. Median age was 68 years (range 21-84), and 23 (59%) were males. The majority of patients (29, 74%) were recruited and treated in Vicenza, since other participating centers joined the study later in 2015. All patients had received rituximab-CHOP (R-CHOP) or R-CHOP-like induction treatment, median number of prior treatments was 2 (range 1-4), and six patients (15%) had prior ASCT. The majority of patients (*n* = 21, 54%) had refractory disease, with 23% of patients (*n* = 9) being double refractory (both to R-CHOP and R-DHAP). Median time from initial lymphoma diagnosis to enrollment was 21 months (range 4-120). Median follow-up of the entire population from lymphoma diagnosis was 30 months (12-172). Patients characteristics are listed in Table 1.

Patients received a median of 4 cycles (range 1-6). Early discontinuations (before cycle 4) were due to infections in 2 (1 had febrile neutropenia in two consecutive cycles despite dose reductions, 1 had colitis complicated by salmonellosis), and tumor progression in 10; 2 patients were consolidated with allogeneic stem-cell transplantation after achieving CR; 4 patients were consolidated with ASCT; 20 patients (51%) had to delay or reduce treatment dose at least once along cycles, however, delays lasted <14 days.

Overall, OR was 69% (CI 58-76), and CR was 41% (CI 32-48). Among different histologies, OR was 78% in *de novo* DLBCL (CR 48%) and 55% in t-DLBCL (CR 27%). Refractory patients had an OR of 48% (CR 24%). The nine double refractory patients had an OR of 56% (2 CR, 3 PR, 2 stable disease, 2 progressive disease). Interestingly, tumor shrinkage was observed in the majority of patients (79%), irrespective of tumor histology (*P* = .23 by Mann-Whitney test), as shown in Figure 1A. Median follow-up for survivors from study entry was 14 months (range 2-55). Median PFS was 13.9 months, and median OS was 17.3 months (Figure 1B,C). Median duration of response was 16 months, significantly different between CR and PR (nr vs. 9.9 months, *P* = .05, Figure 1D). The two patients receiving allogeneic transplant are still in remission after 52 and 25 months, respectively. Of the four patients that were consolidated with ASCT, two experienced early relapse (2 and 5 months later) and two are still in complete remission after 4 and 7 months, respectively. At univariate analysis, the variables associated with a significantly impaired PFS were refractory as opposed to relapsed disease (*P* = .03),

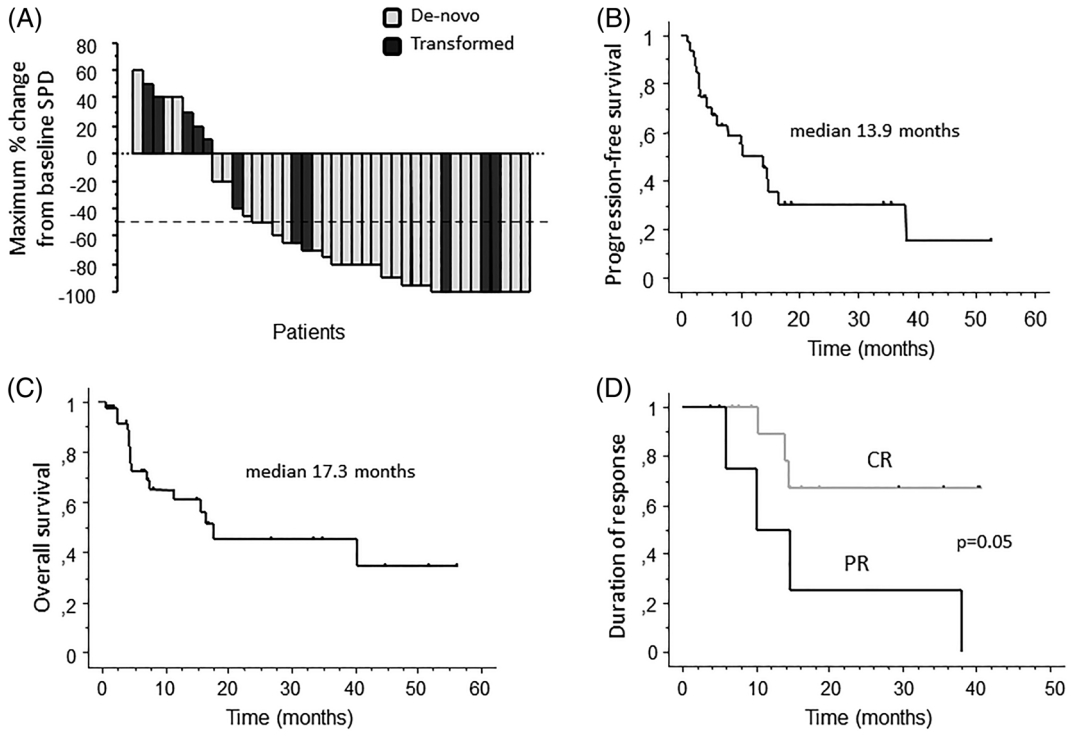

FIGURE 1 A, Waterfall plot indicating maximum % of change from baseline sum of products of greatest diameters (SPD). Tumor shrinkage was seen in 79% (31/39). Response was quantified by local radiologists of each participating center. B,C, Median PFS (Figure 1A) and OS (Figure 1B) in 39 patients with relapsed-refractory aggressive B-cell lymphomas treated with R-BAC. D, Duration of response (nr vs. 9.9 months, P = .05)

shorter time since prior anti-lymphoma treatment (<6 vs. ≥6 months, P = .007), and nongerminal center (GCB) cell-of-origin (non-GCB vs. GCB, P = .01). Interestingly, international prognostic index (P = .15), t-DLBCL as opposed to *de novo* DLBCL (P = .87), and number of prior lines of therapy (1 vs. ≥2) were not associated with PFS (P = .57). Cox proportional hazard model identified refractoriness to prior treatment as the strongest independent prognostic variable in terms of PFS (P = .01, hazard ratio, 3.88; 95% CI 1.37-11.71).

The primary toxicity was reversible myelosuppression. Grades 3-4 neutropenia (median duration 3 days, range 0-7) and thrombocytopenia (median duration 4 days, range 0-8) occurred in 45% and 32% of patients, respectively. Febrile neutropenia occurred in two patients (5%). There was no clear trend towards an increase in cytopenia severity or transfusion requirements with subsequent cycles. Most nonhematological adverse events attributed to R-BAC were Grades 1-2, leaded by isolated gamma-glutamyl transferase elevation (16%). Skin rash, sometimes accompanied by localized itchy areas of cutaneous desquamation, was reported in 15%. No secondary malignancy was observed during follow-up. Of the 15 deaths reported during the study, all patients except one were attributed to disease progression. This patient died of unknown cause while he was in remission. No toxic death has been reported. No alopecia was observed.

In conclusion, R-BAC demonstrated promising anti-tumor activity in this series of patients with de-novo or transformed aggressive lymphomas (2 median prior lines, and 54% refractory disease) unsuitable for ASCT, with tumor shrinkage observed in 79% of patients. The response rate compared favorably with previously reported prospective phase two studies in *R/R* patients with DLBCL treated with the combination of BR.[3,4] Indeed, our patients presented with higher risk features compared to patients enrolled in these trials, due to a worse performance status, higher IPI and greater number of previous treatment lines.

Patients treated with R-BAC experienced mainly hematological toxicity, as expected, which was managed with dose reductions. Importantly, R-BAC was administered in outpatient setting in the majority of patients. Based on our observations, R-BAC may warrant further investigation in this setting, both as a bridge to allogeneic stem-cell transplantation, or in combination with targeted therapies.

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CONFLICT OF INTEREST

All authors approved the final version of the paper for publication. The authors have no conflicts of interest to disclose. The results

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Centrifugation-free washing reduces buildup of potassium and free hemoglobin in washed red blood cells after the procedure

To the Editor:

Stored red blood cells (RBCs) are washed in saline before transfusion to remove residual plasma proteins and other contaminants present in the storage medium. However, the levels of extracellular potassium and free hemoglobin (Hb) in RBC units increase precipitously after washing because of the cellular damage induced by centrifugation. Here we tested whether our recently-developed centrifugation-free washing system could reduce the accumulation of potassium and Hb in the supernatant of washed RBCs during 24-h storage. Samples from several RBC units (n = 7, storage duration 39-42 days) were washed in normal saline either using the prototype of the centrifugation-free washing system, or via centrifugation. The indicators of mechanical damage (supernatant potassium, free Hb) and key metrics of RBC *in vitro* quality (ATP, 2,3-DPG, deformability) were measured at 0, 3, 6 and 24 h after washing. RBC samples washed using the centrifugation-free washing system had significantly lower concentration of free Hb (0.6 ± 0.1 vs. 0.8 ± 0.2 mg/mL) and potassium (3.3 ± 0.6 vs. 5.11 ± 0.8 mmol/L) immediately after washing, and their concentrations increased significantly less after the 24-hour storage (Hb: 1.0 ± 0.2 vs. 2.1 ± 0.3 mg/mL; potassium: 4.9 ± 0.6 vs. 10.5 ± 0.9 mmol/L), than those washed by centrifugation. None of the other measured parameters changed significantly during the short-term storage. This study shows that our centrifugation-free washing system could significantly reduce the buildup of potassium and Hb in the supernatant of washed RBCs, potentially reducing the risks that may be associated with their transfusion.

Stored red blood cells (RBCs) are washed before transfusion to reduce the risk of complications associated with the residual plasma proteins, excessive potassium, free hemoglobin (Hb) and other contaminants that may be present in the unit.[1,2] Washing is typically performed by diluting a standard 350 mL RBC unit with 1-2 L of normal saline, and then concentrating the diluted RBCs back to the desired hematocrit—with a centrifuge or a centrifugation-based cell processor—and discarding the supernatant.[2,3] Centrifugation, however, subjects stored RBCs to substantial mechanical damage, inducing hemolysis and increasing fragility of the cells.[3] As a result, the levels of free Hb and potassium in RBC units increase progressively after washing, reducing the clinical value of the procedure and subjecting the recipients to a host of potential adverse outcomes.[4] In this