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To the editor:

Bendamustine, etoposide, cytarabine, melphalan, and autologous stem cell rescue produce a 72% 3-year PFS in resistant lymphoma

Based on the results of the PARMA and CORAL studies, high-dose chemotherapy (HDT) followed by autologous stem cell rescue has become the standard of care for patients with relapsed, chemosensitive aggressive non-Hodgkin lymphoma (NHL).[1,2] Moreover, HDT/autologous stem cell rescue is considered the therapy of choice for Hodgkin lymphoma (HL) patients in chemosensitive relapse.[3] However, HDT/autologous stem cell rescue is hampered by several major pitfalls, namely the toxicity related to the procedure and the lack of efficacy in chemoresistant patients.

We previously demonstrated the safety of a new HDT regimen with bendamustine, etoposide, cytarabine, and melphalan (BeEAM) prior to autologous stem cell rescue in 43 patients with resistant/relapsed lymphoma.[4] The characteristics of patients are reported in the previously published paper.[4] The study was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice (ICH-GCP), and the current national guidelines for conducting clinical studies. The protocol was approved by the Institutional Ethics Committee. All subjects gave written informed consent. The study was registered at the European Medicines Agency with the European Clinical Trials Database number 2008-002736-15. Transplant-related mortality was 0%. The cumulative incidence of infectious complications was approximately 60%, without any serious adverse events (grades 3-4). Furthermore, this regimen showed significant antilymphoma activity, with 80% of patients being in complete remission after transplant.[5] Disease type (NHL vs HL) and disease status at transplant (chemosensitive vs chemoresistant) were the only statistically significant variables influencing progression-free survival (PFS), whereas disease status at transplant was the only variable affecting overall survival (OS) at the time of writing.[4] However, the primary objective of the study was to determine the 36-month PFS rate, according to Fleming's method ($[p0] = 40\%$, $[p1] = 60\%$, $\alpha = 0.05$, and $1\text{-}\beta = 0.80$). At the time of publication, the median follow-up for surviving patients was short (18 months), and therefore, it was not possible either to establish if we had met the primary end point of the study or to draw final conclusions on the efficacy of this regimen.

With this in mind, we updated the follow-up at 41 months after transplant in order to evaluate the midtime efficacy of the BeEAM regimen in terms of PFS and OS. Responses were again evaluated according to the criteria reported elsewhere by Cheson et al.[5] At present, 31/43 patients are still in complete remission (72%), as documented by both PET and CT scans. Two patients (4.6%) were refractory to

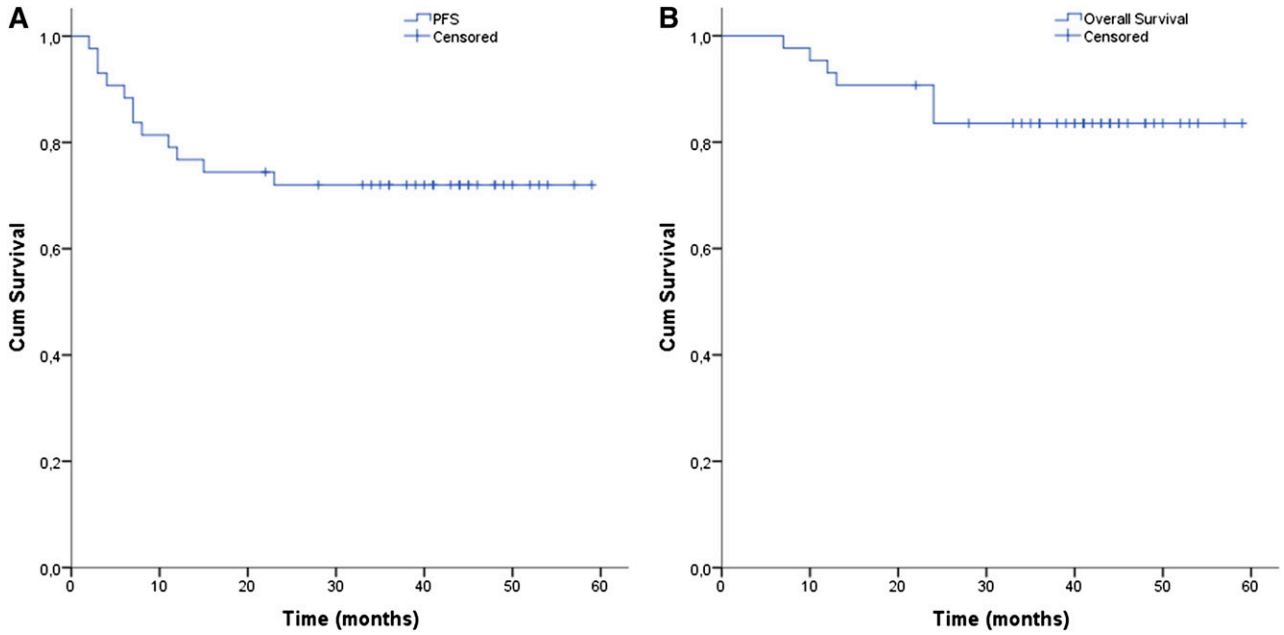

Figure 1. Cumulative survival rates for Hodgkin and non-Hodgkin resistant/relapsed lymphoma patients treated with bendamustine, etoposide, cytarabine and melphalan followed by autologous stem cell rescue. (A) Progression-free and (B) overall survival of all patients.

the HDT regimen and rapidly died after HDT/autologous stem cell rescue, whereas 10/43 patients achieving at least a partial response (23%) relapsed after a median time of 7.5 months (range, 4-23 months) after HDT/autologous stem cell rescue. Five of those 10 patients died (3 NHL, 2 HL), and 5 patients are still alive after relapse.

Median PFS and OS were still not reached (Figure 1A-B). Conversely, 3-year PFS was 72%, allowing our study to meet its primary end point (p[1]=60%). Interestingly, disease type (HL vs NHL) at transplant is no longer influencing PFS, and still does not influence OS. On the other hand, disease status at transplant (chemosensitive vs chemoresistant) is confirmed as a strong predictor of both PFS and OS ($P = .002$ and $P = .009$, respectively). However, the study was not designed to have the statistical power to compare disease entities, and therefore, the possible effect of HDT/autologous stem cell rescue regimen on disease type should be interpreted with caution, and needs additional confirmation in further trials. At present, 1 patient developed myelodysplasia after transplant. No other late effects were observed up to now.

Following the publication of the randomized CORAL study, whose results were less favorable than those reported in non-randomized studies,[6] it has become critical to evaluate novel and effective HDT schedules in order to increase the number of clinical responses among relapsed/refractory lymphoma patients.[7] In this regard, by meeting the primary end point of our BeEAM study, we showed that well-designed clinical trials incorporating new drugs in well-established HDT regimens may increase the number of lymphoma patients who become long-term survivors. This study provides the proof of principle for testing the BeEAM regimen in randomized phase 2-3 studies.

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Contribution: G.V. and A.I. equally proposed, designed, and supervised the study and wrote the manuscript; L.M., P.M.S., S.C., P.G., F.G., G.S., G.M., F.G., R.G., M.G., A.S., and F.F. treated the patients, collected the data, and commented on the manuscript; M.R., F.L., and A.I. were responsible for statistical analysis; and E.M.O. and M.D.C. commented on the manuscript.

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To the editor:

Blockade of endothelial cell protein C receptor augments factor VIIa hemostatic effect in hemophilia treatment

Endothelial cell protein C receptor (EPCR) plays a critical role in downregulating blood coagulation by promoting the activation of anticoagulant protein C by the thrombin:thrombomodulin (TM) complex.[1] Recent studies from our laboratory and others showed that prohemostatic clotting factor, factor VII (and factor VIIa [FVIIa]) also binds EPCR with the same affinity as that of protein C and activated protein C (APC).[2-4] At present, the pathophysiological significance of the EPCR interaction with FVIIa in hemostasis is unclear. We postulated earlier that FVIIa binding to EPCR may augment the hemostatic effect of FVIIa in therapeutic conditions where high concentrations of FVIIa were used to restore hemostasis.[2,5,6] In these conditions, FVIIa concentration in plasma may reach as high as that of plasma protein C, and therefore, effectively competes with protein C for limited EPCR on the endothelium. This would result in the downregulation of protein C/APC-mediated anticoagulant pathway, allowing FVIIa-induced thrombin generation without impediment. If this hypothesis is correct, then blockade of EPCR-mediated anticoagulant pathway by other means should also augment FVIIa's hemostatic effect in therapeutic conditions. Recently, Pavani et al[7] showed that modified mouse FVIIa that binds EPCR exhibited superior hemostatic activity compared with wild-type mouse FVIIa and suggested that FVIIa tethering to EPCR on the endothelium may provide an extended locale of procoagulant reactions that is responsible for the procoagulant effect of FVIIa. It is conceivable that both the downregulation of APC generation and EPCR-dependent FXa generation contribute to the hemostatic effect of FVIIa in therapeutic conditions.

To determine the potential mechanism of EPCR-dependent FVIIa's hemostatic effect in hemophilia, we investigated here the effect of mouse EPCR blocking (monoclonal antibody [mAb] 1560) and nonblocking antibodies (mAb 1567) on FVIIa-induced hemostasis in the hemophilia A mouse using the saphenous vein bleeding model.[8] Both the blocking and nonblocking antibody bind to EPCR; the blocking antibody inhibits thrombin:TM-mediated protein C activation, whereas the nonblocking antibody does not.[9] Neither antibody binds to mouse APC.[9] The blocking antibody inhibits human FVIIa binding to mouse EPCR, whereas the nonblocking antibody does not.[10] Saline or 3 different concentrations of human recombinant FVIIa were administered to hemophilia A mice via the tail vein 5 minutes before inducing saphenous vein injury. Following the injury, the time to hemostasis for each bleeding episode was recorded over a 30-minute time period, and the average time to achieve hemostasis (ATH) was calculated. Volume of blood loss was determined by measuring hemoglobin levels (see Figure 1 for details). Hemophilia A mice failed to achieve hemostasis within the 30-minute experimental time frame, whereas wild-type mice had an ATH of 64 seconds (Figure 1A). Administration of 1 mg/kg of FVIIa to hemophilia A mice had no