compared overall survival according to molecular classification by producing random pairs of age-matched patients from the publicly available data published by Lenz *et al.*[12] For each patient randomly selected in the GCB subtype, an age-matched patient was randomly selected from the ABC subtype, in order to form 80 GCB-ABC pairs of patients. In the 50 tested random paired combinations, the ABC subtype remains constantly correlated to an unfavorable outcome indicating that the unfavorable prognostic value of the molecular signature is not related to the skewed ABC distribution during aging (Figure 2).

In conclusion, our results indicate that in addition to constitutive factors related to advanced age, the prognosis of DLBCL is also conditioned by intrinsic biological features of the tumor cells. Despite promising results obtained using conventional immuno-chemotherapy, such as R-miniCHOP, new therapeutic strategies in geriatric populations should include molecules able to target oncogenic pathways related to the ABC phenotype, such as the NFKB pathway.

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Bortezomib and high-dose melphalan conditioning for stem cell transplantation for AL amyloidosis: a pilot study

Treatment with high-dose intravenous melphalan followed by autologous stem cell transplantation (HDM/SCT) can induce hematologic responses, organ responses and lead to improvement in survival in selected patients with AL (immunoglobulin light chain) amyloidosis.[1] The depth of hematologic response, in particular the achievement of complete hematologic response (CR), has been shown to be predictive of clinical response, quality of life and improvement in survival.[1-3] The median survival of patients achieving hematologic CR after HDM/SCT in a landmark analysis of patients alive at one year following treatment exceeds ten years compared to 50 months for those not achieving a hematologic CR.[4]

The proteasome inhibitor bortezomib has been approved for treatment of myeloma. Recent studies demonstrate high response rates when bortezomib is used in combination with oral melphalan and prednisone.[5] While the mechanism of action is still not completely understood, *in vitro*, bortezomib senstitizes myeloma cells to DNA-damaging agents such as melphalan, and overcomes chemoresistance.[6] It also acts upon the bone marrow microenvironment, inhibiting nuclear factor-κB activation in bone marrow stromal cells. This leads to a reduction in interleukin-6 production and enhanced apoptosis of myeloma cells.[7] Recently, bortezomib has been incorporated into HDM conditioning for SCT in myeloma.[8] Pre-clinical and phase I/II data have suggested that the optimal timing of administration of a single dose of bortezomib is 24 h after melphalan.[6,9]

Because hematologic CR is a critical determinant of treatment outcome following HDM/SCT, we hypothesized that the addition of bortezomib to HDM/SCT could increase hematologic CR rates in patients with AL amyloidosis. This hypothesis led us to conduct a prospective feasibility pilot study of bortezomib-HDM/SCT for the treatment of AL amyloidosis (*ClinicalTrials.gov: NCT00790647*). The objective of this

Table 1. Patients' characteristics.

Feature N (%)	10 (100)
Age, median (range)	65 (46-68)
Patients ≥65 years, number (%)	2 (20)
Female gender, n (%)	4 (40)
Performance status, median (range)	1 (0-1)
N. organs involved, median (range)	2 (1-4)
1 organ, n (%)	4 (40)
2 organs, n (%)	3 (30)
≥3 organs, n (%)	3 (30)
Types of organs involved	
Renal	9 (90%)
Cardiac	2 (20%)
Liver/GI	3 (30%)
Neuropathy	4 (40%)
% BM plasma cells, median (range)	5 (5-25)
Light chain isotype	
Kappa, n (%)	2 (20)
Lambda, n (%)	8 (80)
Median time from diagnosis to SCT (months) (range)	4.8 (2.4-7.3)
Dose of melphalan, n (%)	
200 mg/m^2	8 (80)
140 mg/m^2	1 (10)
Follow up (months), median (range)	23 (18-31)

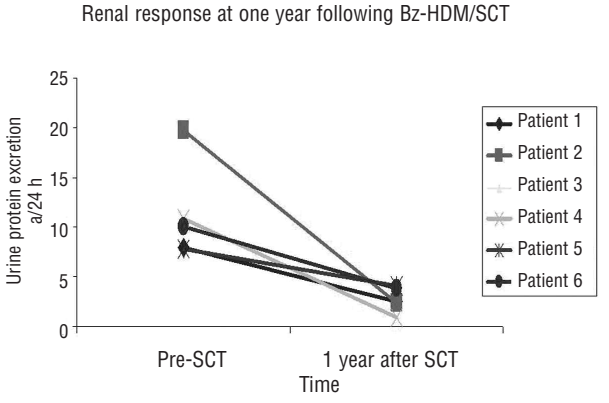

Figure 1. Renal response with improvement in urine protein excretion at one year after Bz-HDM/SCT.

trial was to determine whether the addition of bortezomib to HDM/SCT has the potential to improve hematologic CR rates in patients with AL amyloidosis undergoing HDM/SCT. Additional objectives were to evaluate the tolerability of the combination and its impact upon clinical responses.

This clinical trial was approved by the Institutional Review Board of the Boston University Medical Campus. Eligibility criteria for participation in this clinical trial were as described in previous HDM/SCT protocols.[1] Patients with grade 3 peripheral sensory neuropathy from AL amyloidosis were excluded. Peripheral blood stem cells were mobilized with G-CSF, and a minimum of 2.5×10^6 CD 34$^+$ cells/kg were required for transplantation. Bortezomib was administered at 1 mg/m^2 on Days -6, -3, +1, and +4 and HDM was administered at 140 or 200 mg/m^2 in two divided doses on Days -2 and -1, depending upon age and co-morbidities. The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3, was used to grade adverse events. Treatment-related mortality was defined as death occurring from the time of stem cell mobilization through Day +100 following HDM/SCT. Hematologic and clinical responses were assessed six and 12 months after HDM/SCT. The response criteria for hematologic and clinical/organ response used were standards defined by the consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis.[10]

Ten subjects with AL amyloidosis were enrolled in this clinical trial from October 2008 to November 2009. The median age was 65 years (range 46-68) and 60% were men. The median number of organs involved was 2 (range 1-4). Two patients (20%) had cardiac involvement; both had elevated BNP (B-type natriuretic peptide) and troponin I levels (Mayo clinic cardiac stage III).[11] The median BNP level for all patients was 84 pg/mL (range 8-325). Two patients (20%) had received prior treatment with 2 cycles of bortezomib and dexamethasone. Other patients' characteristics are shown in Table 1.

Of the 10 subjects enrolled, one was removed from the study prior to treatment because of cardiac arrhythmias during stem cell collection that precluded HDM/SCT. This patient subsequently underwent orthotopic heart transplantation followed by HDM/SCT. Of the 9 remaining subjects on the trial, 8 received 200 mg/m^2 of HDM and one received 140 mg/m^2, being over 65 years of age. There was no treatment-related mortality. The median times to neutrophil and platelet engraftment were Days +10 and +14 after SCT, respectively. One of the 9 subjects developed grade 4 mucositis, one developed grade 3 renal failure not requiring dialysis, and 3 developed grade 3 infectious complications (enterococcus urinary tract infection, clostridium difficile colitis, and influenza A pneumonia).

Hematologic responses were achieved in 89% of treated subjects (8 of 9), of which 6 (67%) were hematologic CRs. Thus, according to intention-to-treat, 80% (8 of 10) had a hematologic response to treatment. Only one treated subject presented with worsening of hematologic parameters, necessitating additional treatment. There have been no hematologic relapses at a median follow up of 23 months (range 18-31). Seventy-eight percent of treated patients (7 of 9) had an organ response at one year following bortezomib-HDM/SCT; 6 with renal and one with hepatic response (Figure 1). All subjects are alive and well after a median follow up of 29 months from the time of diagnosis and 23 months from study enrollment.

In conclusion, this pilot study demonstrates that the addition of bortezomib to the conditioning regimen for HDM/SCT is feasible and well tolerated by patients with AL amyloidosis. The combination resulted in no increase in adverse events over those typically seen with HDM alone. Furthermore, this combination produced a high rate of hematologic and organ responses. Although this pilot study only included a small number of highly selected patients, the CR rate of 67% of treated patients compares favorably with that of 40% seen in previous series using melphalan alone for conditioning.[5] This suggests that there may be additive or synergistic activity of bortezomib and melphalan, due to the activity of bortezomib as a chemosensitizer[6] and its effect on the bone

marrow microenvironment.[7] We plan to carry out a second clinical trial using bortezomib for initial induction therapy as well as incorporating it into the conditioning regimen. Based upon the results of these two studies, the regimen with superior phase II results will be compared to a standard melphalan-based SCT in a randomized phase III study, with the goal of determining whether the addition of bortezomib leads to a higher rate of hematologic and clinical responses, and better progression-free and overall survival.

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Managing individuals with propensity to myeloid malignancies due to germline *RUNX1* deficiency

With great interest we read the recent review on familial myelodysplastic syndromes (MDS) published in this journal by Liew and Owen.[1] Besides telomere disorders and familial monosomy 7, the review focused on familial platelet disorder with propensity to myeloid malignancies (FPDMM) and also surveyed syndromic cases of heterozygous loss of chromosome 21q22. To further highlight the clinical diversity of FPDMM and to discuss the challenges posed by the clinical management of patients with germline *RUNX1* deficiency, we report here on a patient with a constitutional loss of *RUNX1* due to a *de novo* deletion of 21q22.

Due to chronic, idiopathic thrombocytopenia, retrospectively found to have been already present in childhood, mild anemia and neutropenia, cytogenetic investigations were performed in a 19-year old patient and displayed a loss of one *RUNX1* allele in bone marrow cells. There was no evidence of MDS or acute myeloid leukemia (AML). Following genetic counseling, karyotyping and fluorescence *in situ* hybridization of phytohemagglutinin-stimulated peripheral blood cells confirmed a heterozygous deletion in 21q22 (Figure 1A, *Online Supplementary Appendix*). High-resolution array comparative genomic hybridization (aCGH) displayed a 1.6 Mb deletion in the long arm of a chromosome 21 involving among others *RUNX1* (Figure 1B). Breakpoint spanning long distance PCR reconfirmed the deletion in DNA isolated from peripheral blood and a buccal swab (Figure 1C). Mutations of the remaining *RUNX1* allele were excluded by DNA sequencing.

In contrast to the reviewed syndromic cases with deletions in 21q22 that, with the exception of one case,[2] displayed a complex phenotype,[1] our patient did not show any growth or developmental delay, dysmorphic features or other abnormalities. Most of the previously reported patients had been described in early childhood when a complex phenotype probably prompted cytogenetic analyses. However, as demonstrated by our patient, deletions of 21q22 including *RUNX1* do not necessarily lead to a complex phenotype, highlighting again the clinical variability of FPDMM.[1]

In view of the early onset of leukemias in 3 out of 12 patients, Liew and Owen hypothesized that the age of leukemic transformation seems to be earlier in patients