To the editor:

Role of primacy of birth in HLA-identical sibling transplantation

HLA-identical siblings are prime donors for allogenic hematopoietic stem cell transplantation (HSCT). Despite matching for MHC antigens, the risk for graft-versus-host disease (GVHD) remains high.[1] Fetomaternal microchimerism has been associated with better survival of maternal compared with paternal grafts[2,3] in non–T-cell–depleted, haploidentical HSCT. Fetomaternal[4-6] and transmaternal sibling microchimerism[7] have been associated with autoimmune diseases. In analogy, we hypothesized that pretransplantation encounters of recipient cells with their later donors through fetomaternal trafficking should have an impact on outcome in HLA-identical sibling HSCT.

This retrospective single-center cohort study analyzed overall survival (OS), transplantation-related mortality (TRM), relapse mortality (RM), and incidence and severity of acute and chronic GVHD after HLA-identical sibling HSCT. We defined 3 groups based on birth sequence: firstborn donor (FD), firstborn recipient (FR), and others (FO). A total of 311 consecutive patients with complete information on sibling sequence that received transplants from 1980 to 2004 were included. Recipient and donor age and sex, diagnosis, stage of the disease, transplantation date and conditioning regimen, stem-cell source (bone marrow versus peripheral blood stem cells), graft manipulation (T-cell depletion), acute and chronic GVHD and dates of relapse and last clinical visit or death were recorded. A total of 97 patients were FRs, and 107 recipients received a graft from an FD. A total of 107 patients were neither firstborn nor received a graft from a firstborn sibling (FO), and served as a control group. FR patients were by definition older than FD patients, and FO patients had more siblings. No other significant differences for pretransplantation variables were observed (data not shown). A total of 187 (60.1%) patients were alive with a median follow-up of 8.9 years. OS at 10 years was better in FR than FD patients (49.6% vs 63.7%, $P = .014$; Figure 1A), and cumulative incidence of death from relapse was lower in FR (Figure 1B). Cumulative incidence of grade 2 or higher acute GVHD was 41.2% in FR versus 57.0% in FD ($P = .035$; Figure 1C). FO patients showed intermediate results. TRM and chronic GVHD were not different among groups (Figure 1D and data not shown). Multivariate analyses strongly confirmed the findings of the univariate tests and excluded recipient/donor age or sibling number as reasons for the observed results (Table 1).

Table 1. Multivariate analysis for OS and RH

	Relative risk	95% confidence interval	*P*
OS			
FD	1.00	—	—
FR	0.58	0.38-0.92	.02
FO	0.79	0.53-1.21	.07
Early disease	1.00	—	—
Advanced disease	1.75	1.19-2.58	.005
Year of transplantation (risk reduction per year)	0.97	0.94-0.99	.048
RM			
FD	1.00	—	—
FR	0.19	0.08-0.46	< .001
FO	0.65	0.34-1.23	.19
Early disease	1.00	—	—
Advanced disease	2.72	1.57-4.70	< .001
Year of transplantation (risk reduction per year)	0.97	0.94-0.99	.048

Multivariate analysis for survival and relapse mortality by Cox proportional hazard models. Variables included in the final models were tested by using a time-dependent covariate method to determine whether the proportional hazards assumption was met. Only significant ($P < .05$) results are shown.

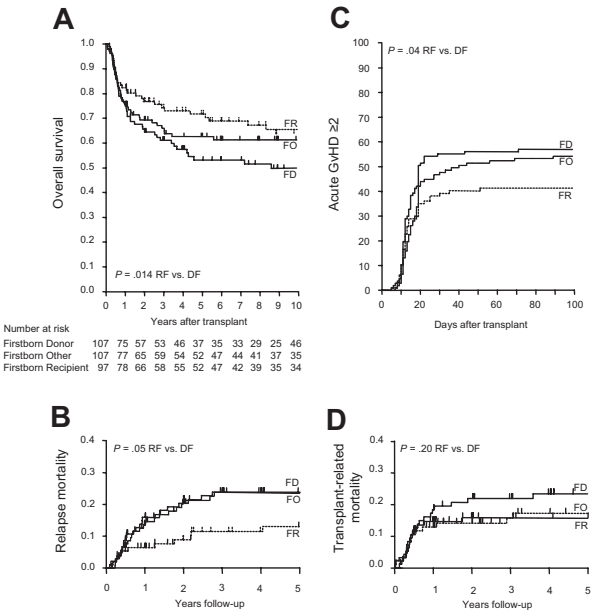

Figure 1. Transplant outcomes depending on sibling sequence. Kaplan-Meier estimate and log-rank comparison of OS (A). Cumulative incidence of RM (B), incidence of acute GVHD (C), and TRM (D). Comparisons were done using the Gray test.

We describe a significant impact of sibling primacy on the incidence of acute GVHD, relapse incidence, and overall survival in HLA-identical HSCT: patients who were born as a first child in a family had the best survival, with a significant reduction in acute GVHD and relapse mortality. Possible mechanisms include fetomaternal and transmaternal sibling cell trafficking and tolerization of the donor. If confirmed, birth order could be integrated into the donor-selection algorithm in HLA-identical sibling HSCT, or matched unrelated donors might be preferred over a high-risk HLA-identical sibling in some cases.

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Contribution: C.B. collected and analyzed data and wrote the paper; M.S. analyzed data and wrote the paper; A.G. designed the study and wrote the paper; J.P. analyzed data; A.B., A.T., S.M.-M., M.P., J.H., D.H., D.T., and A.D. collected data and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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To the editor:

Proteasome inhibitor bortezomib-induced apoptosis in natural killer (NK)–cell leukemia and lymphoma: an in vitro and in vivo preclinical evaluation

According to the World Health Organization classification, the natural killer (NK)–cell neoplasms consist of 2 separate entities: aggressive NK–cell leukemia (ANKL) and extranodal NK-cell lymphoma, nasal type (ENKL).[1] Both of them are aggressive diseases with poor survival.[2] We have previously reported constitutively active nuclear factor-κB signaling in ENKL.[3] Here, we report that this earlier finding has given us a promising lead for NK-cell neoplasm treatment. Bortezomib, the proteasome inhibitor, targets nuclear factor-κB activation and can be used to treat multiple myeloma and mantle-cell lymphoma.[4] We show in this study that bortezomib also has anticancer activity against ANKL and ENKL. Our results from both in vitro cytotoxicity assay and in vivo animal model give substantial support for planning a high priority clinical trial.

We used the cell viability assay to examine bortezomib's cytotoxicity on NK leukemia (KHYG-1, YT, and NK-92) and NK lymphoma (NK-YS) cell lines and short-term primary cultures from tumor biopsies of 2 patients with ENKL (Figure 1A).[5] From the cytotoxicity results, we estimated that the median inhibitory concentrations (IC_{50}) of bortezomib were 2.4 to 5 ng/mL in these neoplastic NK-cell lines and primary ENKL patient samples,

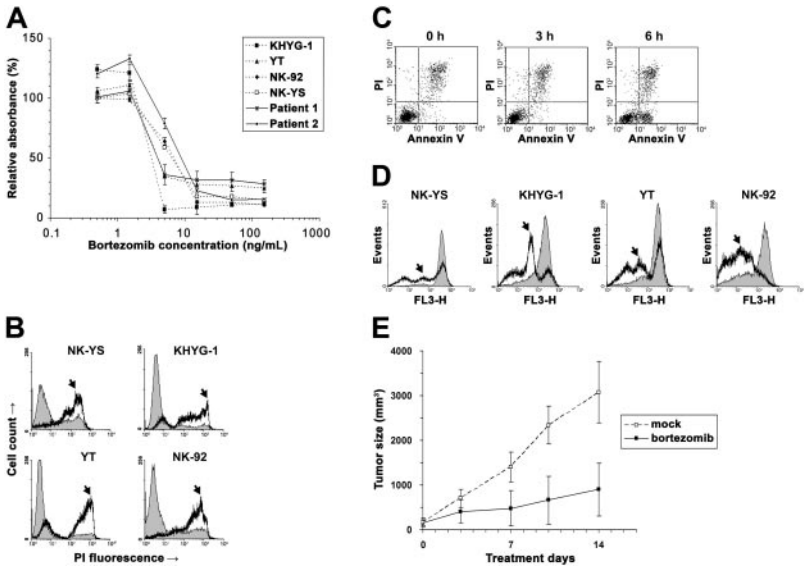

Figure 1. Bortezomib induced apoptosis in the neoplastic NK cells. (A) The NK lymphoma (NK-YS) and NK leukemia (KHYG-1, YT and NK-92) cell lines and short-term primary cultures from tumor biopsies of 2 patients with ENKL were treated with 0.5 to 150 ng/mL bortezomib for 24 hours. Viable cells were measured in triplicate with the MTS assay (Promega, Madison, WI), and results are presented as relative absorbance equal to the percentage of the average reading in untreated cells (±1 standard deviation). (B) After NK-cell lines were treated with 15 ng/mL bortezomib for 24 hours, the cells were collected and stained immediately with 5 μg/mL PI (Sigma, St Louis, MO) to distinguish viable cells from nonviable cells. The fluorescence was measured using the flow cytometer FACSCalibur (BD Biosciences, San Jose, CA). Data were analyzed by the WinMDI v2.8 software (Joseph Trotter, http://facs.scripps.edu/). Histograms of PI fluorescence were overlaid to show the altered distribution between before (thin line) and after (thick line) incubation with bortezomib. Because the dead cells are permeable to PI and thus stainable by PI, they correspond to the population with high fluorescence signals in the chart (arrows). (C) After treatment with 5 ng/mL bortezomib, KHYG-1 cells were stained with Annexin V/PI (BD Biosciences) and examined cytometrically. A population of apoptotic cells appeared in 6 hours with the Annexin V^+/PI^- phenotype. (D) After treatment with 15 ng/mL bortezomib, neoplastic NK cells were collected at different times and stained with Mitotracker Red (Molecular Probes, Eugene, OR) for $\Delta m\psi$ detection with flow cytometry. Histograms were overlaid to show the altered distribution (arrows) between the time before (thin line) and after 9-hour incubations with bortezomib (thick line). (E) Bortezomib inhibited YT cells in vivo. Four-week-old nude mice (n = 20) were injected subcutaneously in a single flank with 4×10^6 YT cells. Implanted tumors successfully engrafted in 10 mice. The tumor-bearing animals were injected intraperitoneally with bortezomib (n = 5) at 1 mg/kg twice a week or with vehicle only (n = 5). The subcutaneous tumor was measured with a caliper, and tumor size was calculated by the following formula: volume = $0.166 \times \pi \times$ length $\times$ width.^{2} The experiment lasted for 2 weeks until the animals were killed due to big tumor size ($<$ 20 mm) in mock-treated mice. Means (±1 standard deviation) are shown. We compared the data by *t* test and found the result statistically significant ($P = .001$).