Niche displacement of human leukemic stem cells uniquely allows their competitive replacement with healthy HSPCs

Allison L. Boyd,1,2,3 Clinton J.V. Campbell,1,2,3 Claudia I. Hopkins,1 Aline Fiebig-Comyn,1 Jennifer Russell,1 Jelena Ulemek,1 Ronan Foley,4 Brian Leber,4 Anargyros Xenocostas,5 Tony J. Collins,1 and Mickie Bhatia1,2,3

Allogeneic hematopoietic stem cell (HSC) transplantation (HSCT) is currently the leading strategy to manage acute myeloid leukemia (AML). However, treatment-related morbidity limits the patient generalizability of HSCT use, and the survival of leukemic stem cells (LSCs) within protective areas of the bone marrow (BM) continues to lead to high relapse rates. Despite growing appreciation for the significance of the LSC microenvironment, it has remained unresolved whether LSCs preferentially situate within normal HSC niches or whether their niche requirements are more promiscuous. Here, we provide functional evidence that the spatial localization of phenotypically primitive human AML cells is restricted to niche elements shared with their normal counterparts, and that their intrinsic ability to initiate and retain occupancy of these niches can be rivaled by healthy hematopoietic stem and progenitor cells (HSPCs). When challenged in competitive BM repopulation assays, primary human leukemia-initiating cells (L-ICs) can be consistently outperformed by HSPCs for BM niche occupancy in a cell dose-dependent manner that ultimately compromises long-term L-IC renewal and subsequent leukemia-initiating capacity. The effectiveness of this approach could be demonstrated using cytokine-induced mobilization of established leukemia from the BM that facilitated the replacement of BM niches with transplanted HSPCs. These findings identify a functional vulnerability of primitive leukemia cells, and suggest that clinical development of these novel transplantation techniques should focus on the dissociation of L-IC–niche interactions to improve competitive replacement with healthy HSPCs during HSCT toward increased survival of patients.

Acute myeloid leukemia (AML) is a hematological neoplasm with a hierarchical cellular structure that is reminiscent of the normal hematopoietic system (Lapidot et al., 1994; Bonnet and Dick, 1997; Hope et al., 2004). Leukemic stem cells (LSCs), which sit at the top of this hierarchy, are particularly resistant to conventional therapeutic measures, contributing to minimum residual disease and ultimately causing patient relapse (Guzman et al., 2002). More recent insights suggest that the BM microenvironment plays a fundamental role in sheltering LSCs (Konopleva et al., 2002) and specifying their self-renewal properties (Raaijmakers et al., 2010; Schepers et al., 2013; Kode et al., 2014). Therefore, niche-targeted consolidation treatment strategies represent a promising mechanism to effectively compromise LSC self-renewal and eliminate minimum residual disease in AML. To inform novel therapeutic efforts toward this goal, it is necessary to develop a thorough understanding of LSC niche characteristics, in relation to those of hematopoietic stem cells (HSCs).

We have previously characterized geographical and molecular features that functionally define the HSC niche in vivo (Guezguez et al., 2009).
bone areas (TBAs) provides a unique molecular microenvironment that preferentially accommodates self-renewing HSCs. Applying the same analytical techniques, we comparatively interrogated whether phenotypically immature leukemic cells share this nonuniform distribution in BM, using xenografted immunodeficient mice established as a reliable surrogate model. After transplantation with primary cells from AML patients or normal human donors, xenografted femurs were dissected along axial planes that delineate the borders between TBA and LBA regions (Fig. 1 A). Flow cytometric measurement of primitive CD45+CD34+ human hematopoietic cells indicated that, like their normal counterparts, immature leukemic cells were markedly more predominant in the cancellous TBA (Fig. 1, B and C). Longitudinal sectioning of frozen decalcified femurs further allowed more precise comparison of microanatomical distribution patterns of normal and leukemic hematopoiesis in situ. Using a high-resolution fluorescence-based imaging platform (Guezguez et al., 2013), human-specific CD45+CD34+ cells could be sensitively and accurately detected, paralleling our flow

RESULTS AND DISCUSSION
Spatial overlap exists between normal and leukemic stem cell-enriched populations in the BM
We have recently described anatomical boundaries within the BM that discretely define the functional localization of healthy HSCs (Guezguez et al., 2013). Relative to diaphyseal long bone areas (LBA), the cellular composition of trabecular

Figure 1. Healthy and leukemic human CD34+ cells spatially overlap within the BM cavity. (A) Experimental design to study the location of healthy or leukemic human hematopoietic cells within the TBA and LBA of xenografted mouse femurs. (B) Representative flow cytometry plots showing human CD45+CD34+ AML cells within TBA and LBA regions. (C) Scatter plots showing the TBA- and LBA-specific frequencies of human CD45+CD34+ cells derived from AML patients or normal human donors. Each data point represents an individual mouse, and each symbol represents an independent primary human sample. *, $P < 0.05$, paired Student’s t test. (D) Representative whole-bone section images of AML-engrafted mouse femurs immunostained with human-specific CD45 and CD34. Images have been montaged from 82 individual fields. Bar, 1 mm. (E) Representative high magnification images of AML or healthy donor-engrafted mouse femurs immunostained with human-specific CD45 and CD34. Dotted lines indicate the endosteal boundary. Bar, 50 µm. (F) Proximity distribution of healthy or leukemic human CD45+CD34+ cells relative to the nearest bone nucleus. Black line indicates the distribution of CB CD45+CD34+ cells (averaged across 4 replicates), and each colored line represents an independent AML-engrafted femur. Each color represents a different primary human AML sample. $P > 0.20$ when comparing AML CD45+CD34+ versus CB CD45+CD34+ distributions (Chi square).
Healthy HSPCs can outcompete L-ICs to populate common functional BM sites

Although spatial profiling of phenotypically primitive cells provides compelling evidence that HSPCs and L-ICs share common BM niches, ultimately self-renewing cells are stringently defined based on their in vivo performance, requiring rigorous competitive transplantation assays to comprehensively address their predicted functional co-localization. To this end, human leukocyte antigen A2 (HLA-A2) served as a faithful antigen that allowed us to combine and track primary cells from genetically distinct human individuals within mixed chimeric xenografts. Using this clinically relevant system, we then cotransplanted HSPC competitive properties would offer the most durable fashion. This would be an unlikely finding if L-ICs do not appear to have a superior affinity for niche repopulation was striking considering that primitive AML blasts have been reported to express elevated levels of antigens involved in adhesion and chemotraction (De Waele et al., 1999; Jin et al., 2006).

When human cells from healthy/leukemic chimeras were serially transplanted into secondary recipients, we found that leukemic repopulation was equal to or less than that in primary recipient mice, whereas the self-renewal of AML-alone controls was uncompromised or elevated (Fig. 2, K and L). Therefore, the competitive pressure provided by co-injected HSPCs was apparently able to jeopardize L-IC fitness in a durable fashion. This would be an unlikely finding if L-ICs had the capacity to resist HSPC competition by relocation to alternative BM niches. When our model was instead adjusted to simulate the clinical context of preestablished leukemic disease followed by irradiation-conditioned CB Lin− transplantation, leukemic engraftment was able to recur in secondary recipients, indicating less effective replacement of L-ICs (Fig. 2 M). This provides a model of leukemic relapse and complements our CB cotransplant studies, suggesting that equal competition for BM niches is compromised if leukemic engraftment is preestablished.

Mobilizing agents can displace leukemic cells from BM niches

We next reasoned that better capitalization of such unanticipated HSPC competitive properties would offer the most promising direction to improve therapeutic targeting of residual AML-LSCs during HSTC. Murine studies have suggested that small molecule or cytokine-induced mobilization of indigenous HSCs can effect dramatic niche exchange and HSC redistribution throughout BM (Verovskaya et al., 2014) and can also act as a preparative treatment to allow allogeneic HSC engraftment (Chen et al., 2006). We therefore sought to apply the same approach in a xenograft setting, to evaluate

cytometry analysis (Fig. S1, A and B). Enrichment of CD34+ leukemic cells was evident along the surface area of the endosteum (Fig. 1, D and E), a geographical arrangement that has been previously described for both human and murine HSCs (Guezguez et al., 2013; Nombela-Arrieta et al., 2013). A customized quantitative localization analysis based on endosteal proximity (Fig. S1 C) showed that the spatial frequency distribution of CD34+ AML cells is indistinguishable from that of normal HSPC donors (Fig. 1, E and F). This assessment extends observations of general associations of primitive AML cells with paratrabecular features (Ishikawa et al., 2007; Ninomiya et al., 2007; Fig. 1, B and C), and more specifically predicts that the regional distribution of normal and leukemic self-renewal niches are physically superimposed.

Healthy HSPCs can outcompete L-ICs to populate common functional BM sites
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Using this clinically relevant system, we then cotransplanted constant cell numbers of AML with escalating doses of CB Lin− cells to determine whether normal and leukemic repopulating cells can compete with each other to seed common BM niches (Fig. 2 D). All AML samples chosen for competitive repopulation experiments generated exclusively myeloid leukemic grafts (Fig. S3 A), and cotransplanted CB-HSPCs consistently gave rise to multilineage hematopoiesis (Fig. S3 B). Leukemic cell doses were chosen that would generate considerable levels of disease burden, to ensure that our model would accurately represent clinical situations of poor prognosis that would require HSCT therapy. Similar to our observations in paired CB donor–transplantation experiments, the proportions of AML patient-derived cells within the human grafts were consistently reduced by CB competition in a cell dose-dependent manner (Fig. 2, E and F). Remarkably, this translated into suppression of the absolute leukemic burden at higher CB Lin− cell doses, unless the overall human reconstitution was low (AML Patient #1; Fig. 2, G and H). In this particular case of low human reconstitution, the presence of any competing CB cells was sufficient to compromise leukemic engraftment regardless of CB cell dose used (Fig. 2 I), and this observation was not unlike absolute engraftment profiles observed in double CB cotransplant experiments (Fig. 2 J). Overall, this demonstrates that normal and leukemic repopulating cells can compete dynamically to populate vacant BM niches and that L-ICs can be outcompeted by CB-HSPCs in a cell dose-dependent manner as long as niche availability is limiting. Our finding that L-ICs do not appear to have a superior affinity for niche repopulation was striking considering that primitive AML blasts have been reported to express elevated levels of antigens involved in adhesion and chemotraction (De Waele et al., 1999; Jin et al., 2006).

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Figure 2. Cotransplantation of healthy HSPCs with AML MNCs can competitively reduce leukemic progression. (A) Experimental design to study competitive niche repopulation between HSPCs enriched from pairs of healthy human CB donors. (B) Gating strategy and representative flow cytometry plots showing relative donor cell proportions within human populations before and after co-injection into xenograft recipients. Top left plot shows that CD2+ T cells were absent from all injected Lin− populations, precluding any immune reactions within the recipient mice. (C) Scatter plot of flow cytometry data showing that in mouse BM grafts, the relative donor CD45+ cell frequencies closely recapitulated their original proportions within injected CD34+ populations, consistently across four independent experiments using different CB donor pairs. Dotted lines represent 95% confidence intervals. Each data point represents an individual transplanted mouse. ***, P < 0.0001, linear regression. (D) Experimental design to study the effects of BM niche competition between healthy and leukemic human repopulating cells. AML cell numbers were kept constant with increasing dose titrations of CB Lin− cells. (E) Example gating strategy to assess the relative reconstitution of primary AML samples in the presence of competing CB-HSPCs, expressed as a proportion of the total human graft. (F) Flow cytometry data showing that HSPC competition reduces the leukemic contribution to human grafts in a cell dose-dependent manner for all AML samples tested (three independent experiments). Cotransplantation of CB Pair #1 is also shown for comparison.
its therapeutic value to promote competition of transplanted HSPCs versus niche-occupant L-ICs. Initially, we assessed whether engrafted HSPCs and L-ICs would respond to mobilization treatment in similar ways based on their mutual dependence on CXCR4–CXCL12 for anchorage and retention in the BM (Peled et al., 1999; Petit et al., 2002; Tavor et al., 2004). Starting with CB-engrafted mice, we applied a mobilization regimen using two CXCR4–CXCL12 antagonists (granulocyte colony-stimulating factor; G-CSF and AMD3100), adapted from Petit et al. (2002) and Broxmeyer et al. (2005; Fig. 3 A). Consistent with previous studies (Nervi et al., 2009), we found that CXCR4 antagonism effectively mobilized xenografted human cells from the BM into the periphery within 1 h of treatment (Fig. 3 B). Although human hematopoietic cells were present in splenic isolates from both mobilized and control-treated mice, transplantation of these cells into secondary recipients revealed functional repopulating activity only among human cells that had been recovered from the spleens of mobilized mice (Fig. 3 C). This rigorous assessment of primitive functional characteristics is evidence that mobilization treatment successfully caused physical displacement of human long-term repopulating cells out of the BM, causing their redistribution into peripheral hematopoietic sites. We next applied the same treatment strategy to xenografts of primary human AML to establish whether leukemic cells could equally be displaced from the mouse BM (Fig. 3 D). Consistent with the niche similarities shared by normal and leukemic cells, mobilization treatment was also able to effect movement of human leukemic cells from the BM into the peripheral blood (PB) and spleens of diseased chimeric mice (Fig. 3 E). Recovered AML cells expressed appropriate tissue-specific and treatment-specific levels of CXCR4 (with the highest expression observed within the BM of mobilized mice; Petit et al., 2002; Fig. S4). Together, this suggests that the functional integrity of the CXCR4–SDF1 axis is intact and targetable in primitive AML cells.

This evidence that BM-resident leukemic cells can successfully be physically dislodged from their inhabited niches offers hope that leukemic mobilization could represent a mechanism to promote better competitive repopulation by HSPCs during therapeutic transplantation. Due to the inability of serial transplantation to discriminate between LSCs localized within specialized niches versus those transiting through nonniche BM space during mobilization, we explored the effects of mobilization on niche accessibility by challenging injected CB-HSPCs to home to the marrow after leukemic graft displacement. In our initial leukemia mobilization experiments, we found that within 1 d after treatment, the spatial configuration of the leukemic grafts had begun returning to a normal nonmobilized state, and that AML cells had completely resumed their original tissue distribution by 1 wk after mobilization (unpublished data). We therefore reasoned that rapid infusion of competitive CB-HSPCs would be necessary after leukemic mobilization, 1 h after mobilization treatment of AML–engrafted mice, lipophilic dye-labeled CB Lin<sup>−</sup> cells were injected i.v. and the dissemination of these fluorescently labeled cells was assessed 1 d later (Fig. 3 F). Although a significant proportion of dye-labeled cells were localized to the spleen under both conditions at this time point, there was a modest reduction of these cells in premobilized mice (unpublished data). This was reflected by a twofold increase in CB Lin<sup>−</sup> cells homed to the BM (Fig. 3 G), suggesting that leukemic displacement led to increased niche availability. The observation of enhanced HSPC homing ability as a consequence of leukemic niche displacement supports our earlier interpretation that primitive normal and leukemic cells do share and compete for common microanatomical niches, and reinforces the prediction that leukemic mobilization can enhance competitive L-IC replacement by transplanted HSPCs.

**Leukemic mobilization facilitates competitive reconstitution and leukemia elimination by transplanted HSPCs**

Next, we evaluated the long-term effectiveness of preHSCT mobilization in the context of established leukemic disease by injecting large numbers of healthy CB-HSPCs after leukemic mobilization, to saturate the newly vacant BM niches (Fig. 4 A). Importantly, the injected CB Lin<sup>−</sup> cell doses were chosen such that the numbers of infused CD34<sup>+</sup> cells/kg body weight were representative of realistic cellular doses achievable in clinical human transplantation from adult HSPC sources (Körbling et al., 1995; Scheid et al., 1999; Table 1). Across three different patients,
mobilization preconditioning consistently ameliorated the ability of injected HSPCs to competitively reconstitute the leukemic xenograft microenvironment relative to nonmobilized controls (Fig. 4 B). In each case, this was accompanied by a statistically significant reduction in overall leukemic burden, as measured by overall leukemic cell frequencies in BM (Fig. 4 C) or total leukemia cell numbers per mouse (not depicted). Established healthy human grafts could be similarly suppressed by mobilization followed by transplantation with either normal or leukemic human cells (Fig. 4 D–G). This supports the notion that the temporal sequence of events is a fundamental determinant of competitive repopulation, and suggests that this is independent of the normal or leukemic nature of competing stem or progenitor cells. 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preconditioning persisted after each individual chimeric graft was serially transplanted into a single secondary recipient mouse (Fig. 4, H and I; and Table 2). In several cases, mobilization pretreatment involved complete elimination of L-IC repopulative capacity, with robust CB engraftment serving as a powerful positive internal control. This suggests that leukemic BM mobilization represents a promising HSCT preconditioning strategy, which can improve long-term management of leukemic disease.

Finally, to put our findings into a broader context for clinical application, we considered the predictive value of pretreatment disease levels toward therapeutic outcomes of mobilization HSCT. The robust therapeutic responses described for AML Patients #4 and #5 were associated with marginal frequencies of peripherally circulating leukemic blasts in primary mice at the time of treatment (Fig. 5 A), which provides an accurate surrogate representation of BM infiltration (Fig. 5 B). In contrast, mice treated under conditions of abundant...
Table 1. CD34+ cell doses injected in mobilization-conditioned transplantation experiments, expressed per kg of recipient body weight

<table>
<thead>
<tr>
<th>Sample</th>
<th>CD34+ cells × 10^6/kg body weight</th>
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<tbody>
<tr>
<td>AML Patient 3</td>
<td>19.4 ± 0.4</td>
</tr>
<tr>
<td>AML Patient 4</td>
<td>10.9 ± 0.2</td>
</tr>
<tr>
<td>AML Patient 5</td>
<td>15 ± 1</td>
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All doses are below reported clinically used values of 25.9 × 10^6 CD34+ cells/kg from adult HSPC sources (Scheid et al., 1999) and 40.1 × 10^6 CD34+ cells/kg (Körbling et al., 1995).

Table 2. Self-renewal analysis of human AML-CB cotransplants with or without mobilization treatment

<table>
<thead>
<tr>
<th>Engraftment outcomes</th>
<th>AML Patient 3</th>
<th>AML Patient 4</th>
<th>AML Patient 5</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Mobilized</td>
<td>Control</td>
</tr>
<tr>
<td>AML only</td>
<td>0/4</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>AML + CB</td>
<td>4/4</td>
<td>3/3</td>
<td>2/3</td>
</tr>
<tr>
<td>CB only</td>
<td>0/4</td>
<td>0/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Total mice</td>
<td>7</td>
<td>8</td>
<td>6</td>
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For each of three AML samples, it is indicated how many secondary transplant recipients were observed to have AML-LIC self-renewal, HSPC self-renewal, or both.

LSC versus HSPC self-renewal. We instead suggest that novel approaches to cell-based therapy offer a more promising consolidation strategy to challenge LSC survival within the BM. To our knowledge, this represents the first demonstration that similar functional properties shared by LSCs and HSCs can be used as a therapeutic advantage in a preclinical transplantation model. Our study identifies a currently unexploited ability of HSPCs to rival L-ICs for BM niche territory, achievable by mobilization of resident L-ICs before healthy HSPC transplantation. This repositions transplanted HSPCs as powerful therapeutic effectors that can actively participate in LSC elimination, in contrast to their traditional reconstitutive role that is secondary to aggressive antileukemic therapy. The value in this novel mechanistic strategy is that LSC elimination can be accomplished without cytotoxic myeloablative procedures and augments the safety and therapeutic index of HSCT through the repurposing of agents already known to be well-tolerated for patient administration (Bradley et al., 2012).

This potential for improved reduced-intensity conditioning could allow larger patient populations to be considered as HSCT candidates, and complements other toxicity-limiting strategies such as techniques that selectively deliver radiation targeted specifically to the BM, sparing other organs (Wong et al., 2006). The clinical application of leukemia mobilization for the purpose of HSCT is currently unprecedented, unlike the strategy of G-CSF–mediated chemotoxic sensitization (Saïto et al., 2010), which has been subject to numerous patient trials over the past decade that have led to somewhat conflicting interpretations regarding efficacy (Esteve et al., 1999; Löwenberg et al., 2003; Büchner et al., 2004). Based on the anticipated safety and the promising preclinical observations reported here, the testing and optimization of mobilization preconditioning as a new HSCT approach for AML patients is currently planned, including the evaluation of adult BM as a compatible HSPC source due to the greater CD34+ cell numbers that can be procured (Körbling et al., 1995; Scheid et al., 1999; Wagner et al., 2002). Additionally, although a single round of mobilization-primed transplantation provided an effective technique of disease suppression under conditions of considerable leukemic burden (AML Patients #3 and #5), further refinement of this procedure could involve repeated cycles of mobilization and HSPC infusion (Colvin et al., 2004; Bhattacharya et al., 2009) to capitalize on multiple windows of opportunity to competitively eliminate L-ICs. Ultimately, the use of mobilization pretreatment either alone or in conjunction...
with low-intensity HSCT regimens offers a novel strategy toward the development of more tolerable AML transplantation therapy with superior eradication of residual disease.

MATERIALS AND METHODS

Primary human samples. Primary blasts were obtained from PB apheresis or BM aspirates of AML patients (Table S1), and healthy hematopoietic cells were isolated from CB samples. Informed consent was obtained from all sample donors in accordance with Research Ethics Board-approved protocols at McMaster University and the London Health Sciences Centre. Mononuclear cells were recovered by density gradient centrifugation (Ficoll-Paque Premium; GE Healthcare), and remaining red blood cells were lysed using ammonium chloride solution (StemCell Technologies). Lineage depletion of CB samples was performed using a commercially available kit (StemCell Technologies), according to the manufacturer’s instructions.

Xenotransplant assays. NOD/Prkdc<sup>scid</sup> and NOD/SCID/B2M<sup>−/−</sup> mice were used as xenotransplantation recipients. Mice were bred in a barrier facility and all experimental protocols were approved by the Animal Care Council of McMaster University. Immunodeficient mice 6–10 wk of age were sublethally irradiated (350 rads, 137Cs) 24 h before initial transplantation to induce and establish either healthy or leukemic human hematopoiesis. However, upon serial transplantation, HLA-A2<sup>+</sup> CB cells failed to self-renew, suggesting inadequate niche colonization. Cell-intrinsic CB-HSPC deficits are ruled out by HLA-A2<sup>+</sup> engraftment after serial transplantation of CB-alone controls. Plots are representative of 3–5 mice per group.

Quantitative immunofluorescent microscopy. Whole femurs extracted from mice xenografted with healthy or leukemic hematopoietic cells were fixed overnight in 4% paraformaldehyde at 4°C, followed by overnight decalciﬁcation in formic acid (Immunocal; Decal). Femur specimens were subsequently snap frozen in OCT compound (Sakura) and sectioned at a 5-µm thickness with a cryostat microtome, using the CryoLam tape-transfer system (Leica). After blocking with 20% donkey serum (Jackson ImmunoResearch Laboratories) and mouse Fc receptor blocking (BD), slides were incubated with anti–human CD45 rat monoclonal antibody ab30446 and anti–human CD34 rabbit monoclonal antibody ab81289 (both 1:50 dilution; Abcam). Donkey-raised secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 647 fluorophores were used for the detection of immunopositive cells (Life Technologies). Fluorescent montage images of immunostained bone marrow sections were acquired at 20× using an Operetta high content imaging system (Perkin Elmer), and assembled with Columbus analysis software (Perkin Elmer).
In vivo mobilization treatment of xenografts. After the establishment of human grafts, mice were injected subcutaneously with mobilization agents adapted according to published treatment schedules (Petit et al., 2002; Broxmeyer et al., 2005). This consisted of three consecutive days of subcutaneous G-CSF injections at 300 μg/kg, followed by a single SQ injection of AMD3100 (Sigma-Aldrich; 5 mg/kg) on the fourth day. Vehicle control animals were injected with equivalent volumes of saline. 1-2 h after the final injection, mice were either transplanted i.v. with CB Lin− cells (or saline), or were killed for analysis of human cell tissue distribution.

Hematology. PB was collected from the mandibular vein of mice 1 h after treatment with mobilization agents or with vehicle. White blood cell (WBC) counts were used to identify individual cell nuclei, and the fluorescence intensities of the remaining channels were then quantified for each nuclear and perinuclear region. Negative staining controls were used to set positive signal thresholds. Endosteal cells were defined as CD45+CD34+ cells in areas with low surrounding nuclear density and high perinuclear autofluorescence in the DAPI channel. X-Y spatial nuclei coordinates were then used to calculate the distance of each human CD45+CD34+ cell to the nearest endosteal-defined cell to generate spatial distribution histograms.

Statistical analysis. Data are represented as mean ± SEM (SEM). Unpaired two-tailed Student’s t tests, one-way ANOVAs, or linear regressions were used for statistical comparisons, with the exception of the localization-based frequency distributions of normal versus leukemic CD45+CD34+ cells, which were statistically compared using χ² analysis. Statistical analyses were performed using Prism (version 5.0a; GraphPad) or MATLAB (MathWorks) software, and the criterion for statistical significance was P < 0.05.

Online supplemental material. Fig. S1 shows gating strategies to quantify primitive leukemic cells in xenografted mouse femurs. Fig. S2 shows that HLA-A2 mismatching provides a means to track hematopoietic cells from individual human donors transplanted into immunodeficient mice. Fig. S3 shows multilineage gating strategy for cotransplanted human AML and CB samples. Fig. S4 shows gating strategy to evaluate the influence of AML graft mobilization on CXCR4 expression. Table S1 lists clinical details of AML samples used. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20140131/D1C1.

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