Age-related defects in B lymphopoiesis underlie the myeloid dominance of adult leukemia

Robert A.J. Signer¹, Encarnacion Montecino-Rodriguez¹, Owen N. Witte²³⁴, Jami McLaughlin², Kenneth Dorshkind¹

¹ Department of Pathology and Laboratory Medicine and the Hematopoietic Malignancies Program, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA.

² Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA.

³ Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA.

⁴ Howard Hughes Medical Institute, University of California, Los Angeles, CA 90095, USA.

Corresponding author: Kenneth Dorshkind
Department of Pathology and Laboratory Medicine and the Hematopoietic Malignancies Program
Jonsson Comprehensive Cancer Center
David Geffen School of Medicine
University of California, Los Angeles
10833 Le Conte Avenue
Los Angeles, CA 90095
USA
Tel.: 1-310-206-9535
Fax: 1-310-206-9391
E-mail: kdorshki@mednet.ucla.edu

Scientific Category: Hematopoiesis
Total Word Count: 4879
Abstract Word Count: 147
Running Title: Effects of aging on leukemogenesis
Key Words: aging, BCR-ABL, CML, hematopoiesis, hematopoietic stem cells, leukemia, leukemia stem cells, lymphopoiesis, myelopoiesis, senescence.
Abstract

Reduced lymphopoiesis during aging contributes to declines in immunity, but little consideration has been given to its effect on the development of hematological disease. This report demonstrates that age-related defects in lymphopoiesis underlie the myeloid dominance of adult leukemia. Using a murine model of chronic myeloid leukemia (CML), an adult-onset malignancy arising from transformation of hematopoietic stem cells (HSC) by the BCR-ABL\textsuperscript{P210} oncogene, we demonstrate that young bone marrow (BM) cells transformed with BCR-ABL\textsuperscript{P210} initiated both a myeloproliferative disorder (MPD) and B lymphoid leukemia while BCR-ABL\textsuperscript{P210} transformed old BM cells recapitulated the human disease by inducing a MPD with rare lymphoid involvement. Further, the lesser severity of MPDs initiated from old BCR-ABL\textsuperscript{P210} transduced BM cells revealed unappreciated defects in aged myeloid progenitors. These data demonstrate that aging affects patterns of leukemogenesis and indicate that the effects of senescence on hematopoiesis are more extensive than previously appreciated.
Introduction

Hematopoietic stem cells (HSC) are the precursors from which all mature blood cells are generated\textsuperscript{1,2}. HSC normally maintain the hematopoietic system through balanced differentiation into common myeloid (CMP) and lymphoid specified progenitors. CMP, the earliest myeloid specified progenitors to be defined, subsequently differentiate into granulocyte-macrophage (GMP) and megakaryocyte-erythroid progenitors (MEP) from which most myeloid and erythroid cells respectively arise\textsuperscript{3}. While there is uncertainty regarding the characteristics of the most HSC proximal lymphoid specified progenitors\textsuperscript{4}, there is general agreement that common lymphoid progenitors (CLP)\textsuperscript{5} are a canonical intermediate through which B cell development progresses. The progeny of CLP include pre-pro-B and pro-B cells, and upon successful rearrangement of immunoglobulin (Ig) heavy chain genes, pre-B cells that express Ig \(\mu\) heavy chain in their cytoplasm are produced. Surface IgM\textsuperscript{+} B cells are generated from pre-B cells following rearrangement and expression of Ig light chain genes\textsuperscript{6,7}.

Recent analyses have shown that the balance of hematopoietic cell production becomes severely perturbed during aging. B lymphocyte development begins to decline early in adult life, and the production of B cells is dramatically diminished in aged individuals\textsuperscript{8,9}. This decline is manifest at all stages of B cell development, as both the frequency and number of CLP and their downstream pre-pro-B, pro-B, and pre-B cell progeny are significantly reduced with age\textsuperscript{10-14}. Since HSC accumulate multiple functional defects with increasing age\textsuperscript{15-18}, it has been proposed that the decline in B lymphopoiesis is the result of age-related deficiencies in the potential of HSC to generate lymphoid progeny\textsuperscript{19,20} as well as to proliferative and differentiative defects intrinsic to lymphoid intermediates\textsuperscript{9,21}. Despite increasing evidence that aged HSC do not proliferate and differentiate as efficiently as their young counterparts\textsuperscript{22}, myelopoiesis has been reported to be unaffected by aging\textsuperscript{11,19}. This conclusion is based on the finding that the frequency of CMP and their downstream progeny remains normal or is increased in old mice.

The consequences of HSC aging and reduced lymphocyte production have, for the most part, been considered in the context of their impact on the quality of the adaptive immune response, which is diminished in the elderly\textsuperscript{23}. Less attention has been given to
how these age-related alterations influence disease within the hematopoietic system, and hematopoietic malignancies in particular. For instance, the majority of leukemias that present in children involve lymphoid cells, and these occur at a time when lymphoid progenitor number and proliferation are highest. Conversely, myeloid leukemias tend to predominate in the elderly when lymphopoiesis is waning.

CML, the most common MPD in humans, typifies this pattern. CML presents as a myeloid hyperplasia that occurs almost exclusively in adults and its incidence increases with age. Over 90% of CML patients possess the Philadelphia chromosome, a reciprocal translocation t(9q34;22q11) that fuses the breakpoint cluster region (BCR) and Abelson tyrosine kinase (ABL) genes and encodes a 210 kDa chimeric protein with constitutive tyrosine kinase activity. The occurrence of the BCR-ABL P210 translocation in HSC has led to CML being classified as a disease of stem cell origin. Since HSC have multilineage differentiation potential, their transformation would be expected to result in disease with representation of both the myeloid and lymphoid lineages. However, this is not the case, since BCR-ABL P210 induced leukemia predominantly presents as CML and rarely causes disease with lymphoid involvement.

This clinical presentation of CML, combined with the fact that it is a disease of middle and old age, led us to hypothesize that the age-related decline in lymphopoiesis is a factor that contributes to the myeloid predominance of this and other adult-onset leukemias. In order to test this premise, we examined the pattern of disease induced by BCR-ABL P210 transformation of BM cells from young and old mice. The results of these experiments support the validity of our hypothesis and further reveal that, in contrast to what has been generally accepted, myelopoiesis is also compromised by aging. Taken together, this study demonstrates that immune senescence contributes to the myeloid dominance of adult leukemia and indicates that the effects of aging on blood cell development are more extensive than is currently appreciated.
Materials and Methods

Mice

Four to 7 week old C57BL/6J (B6) and B6.129S7-Rag1<sup>1<sup>tm1Mom/J</sup></sup> (Rag1<sup>-/-</sup>) mice were purchased from The Jackson Laboratory, and B6.SJL mice were obtained from Taconic Farms. Ten to 24 month old B6 mice were purchased from the National Institute on Aging colony. Animals were housed in the vivarium of the University of California at Los Angeles Division of Laboratory Animal Medicine. Experiments were conducted according to the guidelines of the UCLA Institutional Animal Care and Use Committee.

B6, B6.SJL, and Rag1<sup>-/-</sup> recipients were preconditioned with 500 R 12-30 hours before intravenous injection of transformed cells from a <sup>137</sup>Cs irradiator (120 R/min; Mark I-68A; JL Shepperd and Associates).

Generation of retroviral stocks

The retroviral vector pMSCV<sup>40</sup> containing a 5' LTR-driven BCR-ABL internal ribosome entry site (IRES) enhanced GFP (EGFP) or a 5' LTR-driven IRES EGFP was used to generate high-titer helper-free retrovirus following transient cotransfection of 293T cells. 293T cells were grown on Poly-L-Lysine (Sigma) coated 10 cm tissue culture treated plates (Becton Dickinson) in Iscove’s modified Dulbecco’s minimum essential medium (IMDM, Mediatech) supplemented with 10% fetal calf serum (FCS, Hyclone), 1mM L-glutamine, 100 U/ml streptomycin, and 100 µg/ml penicillin (complete IMDM; all from Gibco). Transfections were done by co-precipitating 15 µg of retroviral vector with 15 µg of an ecotropic packaging vector<sup>41</sup> with the CalPhos Mammalian Transfection Kit (BD Biosciences). Medium was replaced every 12 hours for 3 days with complete IMDM. Viral stocks were prepared by pooling supernatants collected at 36, 48, and 60 hours post-transfection. Viral titers were determined following infection of 3T3 cells with serial dilutions of the pooled virus supernatant, and found to range between 2 x 10<sup>6</sup> and 7 x 10<sup>6</sup> virus particles/ml.

Retroviral transduction and bone marrow transplantation

Young mice were administered a single intravenous dose of 5-Fluorouracil (5-FU, 150 mg/kg body weight; Sigma). Due to their higher susceptibility to 5-FU, middle-age and
old mice were administered a dose of 115 mg/kg body weight. On the eighth day after 5-FU administration, BM cell suspensions were prepared as described\textsuperscript{42}. Cells were distributed in 5 ml polystyrene tubes (Becton Dickinson) and incubated with 1 ml of retrovirus supplemented with 10% horse serum (Hyclone), 1mM L-glutamine, 100 U/ml streptomycin, and 100 µg/ml penicillin, 8 µg/ml polybrene (Sigma), 50 µM β-mercaptoethanol (Sigma), 25 mM HEPES (Gibco), 100 ng/ml SCF (Biosource), 100 ng/ml Flt-3L (R&D), and 10 ng/ml IL-11 (R&D) for 2 hours at 37 °C in 5% CO\textsubscript{2} and air and constant humidity. Cells were centrifuged for 5 minutes at 400 g, and viral supernatant was replaced with 1 ml of virus stock supplemented as described above. This procedure was repeated twice. After 6 hours, cells were washed, counted, and resuspended in PBS. Purified pro/pre-B cells were infected in a modified progenitor cell culture system\textsuperscript{42}. One million cells were seeded in 6 well plates in RPMI 1640 (Gibco) supplemented with 10% FCS, 1mM L-glutamine, 100 U/ml streptomycin, 100 µg/ml penicillin, 8 µg/ml polybrene, 50 µM β-mercaptoethanol, 50 µg/ml gentamycin (Sigma), 20 ng/ml SCF, 20 ng/ml IL-3, 20 ng/ml IL-6 (Biosource), 10 ng/ml Flt-3L, and 10 ng/ml IL-7. 0.4-µm transwell inserts in which confluent S17 stromal layers had been pre-established were inserted into each well. Retrovirus was added to the bottom well 4 times over a 24 hour period, at the end of which cells were washed, counted, and resuspended in PBS. Five to 7 week old irradiated B6, B6.SJL, or Rag1\textsuperscript{-/-} mice received an intravenous injection of 2 x 10\textsuperscript{5} transduced BM cells or 1 x 10\textsuperscript{5} pro/pre B cells per mouse. For secondary transplants 5 x 10\textsuperscript{6} splenocytes from primary diseased mice were injected intravenously into irradiated 5-7 week old B6 recipients.

**Immunophenotypic analysis of leukemic cells and cell sorting**

BM and spleen cell suspensions were prepared as previously described\textsuperscript{42}. Cell suspensions were incubated with anti-CD16/32 (FcγRII-III; clone 2.4G2; eBiosciences) or total mouse IgG (for CMP and GMP stains only; Axell) to reduce nonspecific labeling. Cells were incubated with combinations of antibodies to the following cell surface determinants, conjugated to fluoro-isothiocyanate, phycoerythrin, tricolor, indodicarbocyanine, biotin, or allophycocyanin: CD3ε (clone KT31.1), CD4 (clone GK1.5), CD8α (clone 53-6.7), CD11b (clone M1/70), CD16/32 (FcγRII/III; clone 2.4G2),
CD19 (clone 1D3), CD45.1 (clone A20), CD45.2 (clone 104), CD45R (B220, clone RA3-6B2), CD117 (c-Kit, clone 2B8), CD127 (IL-7Rα, clone A7R34), Ter119 (clone Ter-119), TCRβ (clone H57-597), TCRγδ (clone UC7-13D5), NK1.1 (clone PK136), Ly-6C (clone AL-21), AA4.1 (clone C1qRp), Sca-1 (clone E13-161.7), IgM (clone II/41), and Gr-1 (clone RB6-8C5). Biotinylated cells were visualized by incubation with tricolor-, allophycocyanin-, or allophycocyanin-Alexa Fluor 750-conjugated streptavidin. All reagents were obtained from Becton Dickinson or eBiosciences except for goat anti-mouse IgM (Southern Biotech), tricolor-conjugated CD11b and allophycocyanin-Alexa Fluor 750-conjugated streptavidin (Caltag Laboratories). All incubations were for approximately 30 minutes at 4°C. After the last wash, live cells were acquired with Cell Quest Software (Becton Dickinson) on a FACScan, FACSCalibur or Cytek modified FACSscan (all BD Biosciences) located at the Flow Cytometry Core of Jonsson Comprehensive Cancer Center at the University of California at Los Angeles.

Disease phenotype was established by determining the frequencies of BM or spleen cells co-expressing GFP (BCR-ABL210) and lineage specific cell surface antigens (Myeloid: CD11b+Gr-1+, B lymphoid: CD45R+CD19+, Erythroid: Ter119+, T-Lymphoid: CD4+ and/or CD8+). Disease was considered present when at least 7.5% of the total GFP+ cells in either the BM or spleen co-expressed the specific cell surface antigens.

Populations enriched for CMP and GMP3 were defined as Lin− (Lin = CD3ε, CD8α, CD45R, Gr-1, Ter-119, TCRβ, TCRγδ, and NK1.1) Sca-1−CD127− CD16/32+CD117hi and Lin−Sca-1−CD127−CD16/32hiCD117hi, respectively. Populations enriched for HSC38 were defined as Lin− (Lin = CD3ε, CD8α, CD11b, CD45R, Gr-1, Ter-119, TCRβ, TCRγδ, and NK1.1) Sca-1hiCD117hi. Pre-pro B cells and pro/pre-B cells43 were defined as Lin− (Lin = CD3ε, CD4, CD8α, CD11b, Gr-1, Ter-119, Ly-6C, IgM, TCRβ, TCRγδ, and NK1.1) CD45R+CD19−AA4.1+ and Lin− CD45R+CD19+AA4.1+, respectively.

Cells to be purified were resuspended in minimum essential medium-α (αMEM; Gibco) supplemented with 2% FCS, 25 mM Hepes, 1mM L-glutamine, 100 U/ml streptomycin, 100 µg/ml penicillin, and 50 µg/ml gentamycin before being sorted on a FACSaria (Becton Dickinson). Sorted fractions were examined by reanalysis, and were routinely 95% pure.
**B cell progenitor cultures**

BM cells from day 8 5-FU treated mice or purified pro/pre-B cells were retrovirally transduced as described above. 3 x 10^5 BM cells or 3.8 x 10^5 GFP^+ pro/pre-B cells (which had been expanded for one week in the modified progenitor assay described above) were seeded onto S17 stromal cells in T12.5 flasks (Becton Dickinson) in RPMI 1640 supplemented with 5% FCS, 50 \( \mu \)M \( \beta \)-mercaptoethanol, 1 mM L-glutamine, 100 U/ml streptomycin, and 100 \( \mu \)g/ml penicillin^45. Cultures were incubated at 37 °C in 5% CO\(_2\) and air and constant humidity, and fed twice weekly for up to three weeks.

**Myeloid cultures**

Myeloid colony assays were performed by resuspending target cells in 1 ml of methylcellulose supplemented with 30% FCS, 40% aMEM, 50 \( \mu \)M \( \beta \)-mercaptoethanol, 1 mM L-glutamine, 100 U/ml streptomycin, 100 \( \mu \)g/ml penicillin, 50 \( \mu \)g/ml gentamycin, 20 ng/ml SCF, 10 ng/ml GM-CSF (Biosource), 30 ng/ml IL-3, and 10 ng/ml IL-11 in 3.5 cm petri dishes (Becton Dickinson) in triplicate^3. Colonies were counted on day 8. Individual colonies were picked under a dissecting microscope, resuspended in 300 \( \mu \)l of PBS, and analyzed for GFP expression on a FACScan for 30 seconds at a fixed flow rate of 60 \( \mu \)l/min. Live cells were gated, and total cell numbers were calculated as [total live events/(flow rate x acquisition time)] x sample volume.

Liquid suspension cultures were initiated by seeding 1.5 x 10^5 whole BM cells or 2 x 10^3 sorted CMP in complete IMDM supplemented with 50 \( \mu \)M \( \beta \)-mercaptoethanol, 50 \( \mu \)g/ml gentamycin, 20 ng/ml SCF, 10 ng/ml GM-CSF, 30 ng/ml IL-3, 30 ng/ml IL-6, and 10 ng/ml IL-11 at 37 °C in 5% CO\(_2\) and air and constant humidity. Cultures were fed on day 4, and on day 6 cells were harvested and assessed by immunostaining with Gr-1 and CD11b as described above.

**Statistical analysis**

Unless indicated otherwise, data are expressed as a mean ± SEM. Differences between groups were tested by a two-tailed, unpaired t-test, with an \( \alpha \) of 0.05.
Results

Hematopoietic cell age alters BCR-ABL<sup>P210</sup> induced leukemia phenotype

To assess the impact of aging on BCR-ABL<sup>P210</sup> induced leukemogenesis, BM cells harvested from day 8 5-FU treated young (5-7 weeks) or old (90-104 weeks) mice were infected with a retrovirus carrying a bicistronic IRES expression vector encoding BCR-ABL<sup>P210</sup> and an EGFP reporter gene and subsequently injected into sublethally irradiated syngeneic young mice (BM<sup>BCR-ABL</sup> recipients; Figure 1A). Control animals were similarly transplanted with BM cells infected with a retrovirus containing EGFP alone (BM<sup>EGFP</sup> recipients). The use of congenic CD45.1<sup>+</sup> recipients in one experiment confirmed that GFP<sup>+</sup> (BCR-ABL<sup>+</sup>) leukemic cells co-expressed the donor derived CD45.2 cell surface antigen (data not shown).

Recipients of both young and old BM<sup>BCR-ABL</sup> cells exhibited weight loss, cachexia, and poor grooming and were sacrificed between 2 to 7 weeks post-transplantation. These animals had decreased BM and increased splenic cellularity compared to BM<sup>EGFP</sup> recipients (Figure 1B and 1C). Hematopoietic infiltration of the liver and lungs with occasional lymphadenopathy was evident at necropsy (data not shown). In contrast, all BM<sup>EGFP</sup> recipients were observed for up to four months post-transplantation and remained healthy.

Disease patterns in BM<sup>BCR-ABL</sup> recipients were assessed by phenotypic and morphologic characterization of hematopoietic cells in their BM and spleen (Figure 1D and Table 1). Ninety percent (26/29) of young and 100% (24/24) of old BM<sup>BCR-ABL</sup> recipients developed MPDs characterized by the expansion of GFP<sup>+</sup>Gr-1<sup>+</sup>CD11b<sup>+</sup> granulocytes in the BM and spleen that was frequently accompanied by the presence of GFP<sup>+</sup>Ter119<sup>+</sup> erythroid cells (Figure 1D and 1E). Similar to the situation in humans, 96% (23/24) of old BM<sup>BCR-ABL</sup> recipients lacked significant B lineage involvement in their disease, as determined by the minimal frequency of GFP<sup>+</sup>CD45R<sup>+</sup>CD19<sup>+</sup> B lineage cells and absence of lymphoid blasts in their BM and spleen. In stark contrast, 35% (9/26) of young BM<sup>BCR-ABL</sup> recipients that developed MPDs concurrently developed B lymphoid leukemia. In addition, 10% (3/29) of young BM<sup>BCR-ABL</sup> recipients presented with B lymphoid leukemia without significant myeloid involvement, a disease profile that was never observed in any old BM<sup>BCR-ABL</sup> recipients.
Overall, 41% (12/29) of young BM^{BCR-ABL} recipients developed B lymphoid leukemia compared with 4% (1/24) of old BM^{BCR-ABL} recipients (Figure 1E). These differences were not the result of decreased engraftment of old cells\textsuperscript{18}, because engraftment of GFP\textsuperscript{+} cells was comparable in the BM of recipients of young and old BM^{EGFP} (Table 1), all recipients of old BM^{BCR-ABL} developed myeloid disease, and increasing the number of transplanted old BM^{BCR-ABL} cells did not increase the incidence of lymphoid disease in the recipients (data not shown). Therefore, these data strongly indicate that BCR-ABL\textsuperscript{P210} transduced old hematopoietic cells lack significant potential to initiate B lymphoid leukemia.

This conclusion was confirmed by transplanting BCR-ABL\textsuperscript{P210} transduced young and old BM cells into \textit{Rag1}\textsuperscript{-/-} recipient mice, whose lack of lymphocytes creates a more favorable environment for normal and dysplastic lymphoid development\textsuperscript{47-49}. All BM^{BCR-ABL} \textit{Rag1}\textsuperscript{-/-} recipients developed MPDs, and recipients of young BM^{BCR-ABL} also developed B lymphoid leukemia with an incidence markedly higher (63%; 5/8) than observed in wild type mice. In contrast, none (0/7) of the old BM^{BCR-ABL} \textit{Rag1}\textsuperscript{-/-} recipients showed any significant B lineage component to their disease (Figure 1F).

The incidence of B lymphoid leukemia correlates with age-related declines in B lymphopoiesis

The decline in B lymphopoiesis does not abruptly initiate in old age, but instead begins in relatively young animals and progresses gradually thereafter\textsuperscript{8}. For example, middle-age mice (42-44 weeks) have approximately half the number of B lineage cells as young mice (Figure 2A and 2B). Similarly, the frequency of pre-pro-B, pro-B, and pre-B cells is progressively reduced in the BM of 5-FU treated mice of increasing age, demonstrating a reduced capacity to generate B lineage cells \textit{de novo} in old mice\textsuperscript{11}.

If the age-related decline in B cell production underlies the reduced capacity of BM^{BCR-ABL} cells to initiate B lymphoid leukemia, then the incidence of BCR-ABL\textsuperscript{P210} induced lymphoid disease should decline gradually with increasing age. This hypothesis was tested by transplanting BCR-ABL\textsuperscript{P210} transduced BM cells from young, middle-age, and old mice into \textit{Rag1}\textsuperscript{-/-} recipients. As shown in Figure 2C, the incidence of B lymphoid leukemia is highest amongst young BM^{BCR-ABL} recipients (63%; 5/8), intermediate in
middle-age BM$^{\text{BCR-ABL}}$ recipients (25%; 2/8), and nil in recipients of old BM$^{\text{BCR-ABL}}$ (0%; 0/7).

**Cell intrinsic defects diminish the leukemogenic potential of aged B lineage cells**

In addition to its emergence from HSC$^{36}$, B lymphoid leukemia can also initiate from direct transformation of committed lymphoid progenitors$^{50,52}$. This raised the possibility that the reduced incidence of B lymphoid leukemia from aged BM cells observed in the above study resulted from the lower number of lymphoid progenitors available for transformation (Figure 2B). However, this was not the case. When an equivalent number of BCR-ABL$^{P210}$ transduced pro/pre-B cells from young and old mice were injected into sublethally irradiated $\text{Rag}^{-/-}$ recipients, 25% of recipients of young BCR-ABL$^{P210}$ transduced B lineage cells developed B lymphoid leukemia 8 weeks later, while none of the recipients of old BCR-ABL$^{P210}$ transduced pro/pre-B cells developed disease (Figure 3A).

Further *in vitro* analysis showed that young and old BCR-ABL$^{P210}$ transduced 5-FU BM differed significantly in the potential to establish long-term B lineage cultures. Following 3 weeks in B lymphoid permissive conditions, recovery of GFP$^+$ B lineage cells in cultures initiated with young BM$^{\text{BCR-ABL}}$ was 100 times higher than in cultures established with young BM$^{\text{EGFP}}$ cells (Figure 3B). In contrast, BCR-ABL$^{P210}$ transformed old BM cells expanded only 6 fold when compared to old BM$^{\text{EGFP}}$ cells (Figure 3C). Morphologic and phenotypic analyses confirmed these observations (Figure 3D). As in the *in vivo* experiments, this was not due to differences in numbers of B lineage progenitors present in young and old BM. When an equivalent number of young and old BCR-ABL$^{P210}$ transduced pro/pre-B cells were cultured in B lymphoid permissive conditions the young BCR-ABL$^{P210}$ transduced pro/pre-B cells had expanded approximately 10 fold after 5 days, while no increase in cell number was observed in the cultures established with old B lineage cells (Figure 3E).

Taken together, these observations indicate that BCR-ABL$^{P210}$ expression cannot overcome the proliferative and differentiative defects that have accumulated in aged B lymphoid precursors, and these defects underlie their reduced leukemogenic potential.
Decreased severity of MPDs derived from old BM$^{\text{BCR-ABL}}$

Compared to recipients of old BM$^{\text{BCR-ABL}}$ cells, young BM$^{\text{BCR-ABL}}$ recipients consistently presented with increased wasting and a more pronounced invasion of organs such as liver and lung with leukemic cells (data not shown). Although young and old BM$^{\text{BCR-ABL}}$ recipients had comparable numbers of cells in their BM and spleen (Figure 1B and 1C), the proportion of cells that were GFP$^+$ was higher in young BM$^{\text{BCR-ABL}}$ recipients. For example, the proportion of GFP$^+$ cells in the spleen of young and old BM$^{\text{BCR-ABL}}$ recipients was 62% and 52%, respectively and 47% and 29% in the BM (P<0.002), respectively. Since this increased tumor burden in young BM$^{\text{BCR-ABL}}$ recipients could have reflected the addition of B lymphoid leukemia to their MPDs, we compared young and old BM$^{\text{BCR-ABL}}$ recipients that developed MPDs without B lineage involvement. Surprisingly, the difference in tumor burden was accentuated in these animals due to a higher frequency of GFP$^+$Gr-1$^+$CD11b$^+$ cells present in their BM and spleen (Figure 4A and 4B). This also resulted in tissue disruption as demonstrated by an enhanced displacement of endogenous B lineage cells in the spleen (Figure 4C).

The increase in leukemic myeloid cells in young BM$^{\text{BCR-ABL}}$ recipients was accompanied by an increased frequency and number of GFP$^+$ HSC, CMP and GMP in the BM and spleen (Figure 4D and 4E), and splenocytes from young BM$^{\text{BCR-ABL}}$ recipients formed more GFP$^+$ colonies than splenocytes from old BM$^{\text{BCR-ABL}}$ recipients when tested in myeloid colony assays (data not shown).

This discrepancy in tumor burden did not result from an increased transduction efficiency of young BM cells. Following transduction, young and old BM cells were used to establish myeloid colonies in two independent experiments. Eight days later, 12 colonies derived from young and old BM$^{\text{EGFP}}$ were examined for GFP expression by flow cytometry. The number of young and old derived colonies that contained GFP expressing cells (8/12) was the same.

Identification of myelopoietic defects in old mice

The above data were surprising because the frequency and absolute number of HSC and myeloid progenitors is increased in the BM of old mice (Figure 5A)$^{11,19}$. Therefore, we considered the possibility that aging affected the quality of old myeloid progenitors.
Further analysis revealed that while old BM cells formed 1.3 fold more colonies than young BM cells, this value was less than would be predicted from the 2.3 fold increase in myeloid progenitor frequency observed by flow cytometry. This could be due in part to a 2-3 fold increase in the number of apoptotic Annexin V+ CMP and GMP in old as compared to young BM (Figure 5B). In addition, individual colonies derived from old BM cells contained approximately 50% fewer cells than those derived from young BM cells (Figure 5C), and old CMP similarly generated smaller colonies than their counterparts from young mice (Figure 5D).

This age-related reduction in myelopoietic potential was also observed when whole BM cells or sorted CMP isolated from young and old mice were grown in liquid culture supplemented with myelopoietic cytokines. Consistent with results from the colony assays, BM cells (Figure 5E) and CMP (Figure 5F) from old mice produced approximately 45% less Gr-1+CD11b+ myeloid cells than those isolated from young animals. Taken together, these results demonstrate that aged myeloid progenitors harbor intrinsic proliferative and/or differentiative defects.

**Age-related hematopoietic defects alter the potential of leukemia stem cells (LSC)**

Hematopoietic stem and progenitor cells play a critical role in the pathogenesis of CML. HSC have been implicated as the leukemic cell of origin, while committed myeloid and lymphoid progenitors have been deemed the LSC in more advanced stages of disease. Consequently, we assessed whether age-related differences in the pathogenesis of BCR-ABL induced leukemia was a reflection of changes intrinsic to LSC. Since LSC are defined by their potential to transplant disease, splenocytes isolated from diseased mice grafted with young or old BM^BCR-ABL^ were transplanted into sublethally irradiated syngeneic mice. Disease patterns and progression were then analyzed in these secondary recipients.

Secondary recipients developed disease symptoms within 3 weeks post-transplantation and were sacrificed. All these mice (27/27) presented with MPDs. However, 60% (9/15) of mice grafted with splenocytes from young BM^BCR-ABL^ recipients also presented with B lymphoid leukemia (Figure 6A). In contrast, none (0/12) of the mice grafted with splenocytes from old BM^BCR-ABL^ recipients developed lymphoid
disease (Figure 6B). These data suggest that either no B lymphoid LSC were produced following BCR-ABL$^{P210}$ transduction of old hematopoietic cells, or that if they were produced, they have intrinsic defects that disrupt their leukemia initiating potential.

In addition, we compared secondary recipients that developed MPDs without B lineage involvement for disease severity. Leukemic burden was 3 fold greater in secondary recipients transplanted with young BM$^{BCR-ABL}$ derived tumors when compared with secondary recipients of old BM$^{BCR-ABL}$ derived tumors (Figure 6C and 6D). These data demonstrate that myeloid LSC derived from young BM$^{BCR-ABL}$ have a greater expansive potential through either enhanced self-renewal and/or increased production of mature leukemic myeloid progeny. Taken together, these data indicate that age-related intrinsic hematopoietic defects ultimately alter the leukemogenic potential of LSC generated following transformation of hematopoietic cells by BCR-ABL$^{P210}$.

**Discussion**
The present report demonstrates that age-related intrinsic defects that accumulate in B lineage cells limit lymphoid involvement in CML. This finding provides a biological explanation for the myeloid predominance of adult-onset leukemia. Although this study focused on B cell development, T cell production also declines with increasing age$^{21}$, which may explain the extreme rarity with which T cell leukemia presents in older humans.

In the murine CML model used in this study, transformation of young BM cells with BCR-ABL$^{P210}$ consistently resulted in myeloid and lymphoid disease while transformation of old BM cells primarily resulted in a MPD with no lymphoid involvement. Since the 5-FU BM used in these experiments contains HSC as well as B lineage specified progenitors, B lymphoid leukemia could have developed from transformation of either population. However, the origin of the leukemia does not influence the interpretation of our results, which demonstrate that age-related intrinsic defects that accumulate in B lineage cells limit their involvement in CML. This conclusion is supported by the observation that BCR-ABL$^{P210}$ transduced old B lineage precursors lack lymphoid leukemia initiating potential. Furthermore, expression of a powerful oncogene such as BCR-ABL$^{P210}$, which greatly enhanced the growth of young
B lineage cells, was unable to significantly augment the growth of old B cell progenitors \textit{in vitro}. These data, combined with intrinsic age-related defects in the ability of HSC to generate lymphoid progeny\textsuperscript{19}, can explain the clinical presentation of CML. Inefficient production of early lymphoid progenitors from older HSC, in combination with intrinsic proliferative and developmental defects in the progenitors that are generated, would contribute to a low incidence of lymphoid leukemia and a predominance of myeloid disease in the aged. In contrast, young HSC efficiently generate lymphoid progeny, which in turn are highly susceptible to the effects of BCR-ABL\textsuperscript{32}, thereby resulting in MPD and B lymphoid leukemia\textsuperscript{56}.

Our finding that B lymphoid leukemia initiated more frequently with young than old BM\textsuperscript{BCR-ABL} is consistent with the clinical observation that the incidence of B lineage leukemia is highest in children. That children are predisposed to lymphoid leukemia is consistent with results from murine studies demonstrating that B lineage progenitors from neonates and young adults cycle at levels above their aged counterparts\textsuperscript{11}. While this increased proliferation may be necessary to fill peripheral lymphoid compartments, it could increase the chance that an aberrant genetic event could occur, particularly since developing B lineage cells possess active gene rearrangement machinery\textsuperscript{57}. This possibility, combined with the presence of chromosomal translocations in neonatal B lineage progenitors that cause a predisposition to leukemia development\textsuperscript{58}, may increase the likelihood of transformation during early B lymphopoiesis.

The observation that MPDs initiated from young BM\textsuperscript{BCR-ABL} cells were more aggressive than those initiated from old BM\textsuperscript{BCR-ABL} cells was quite unexpected. This pattern led to the discovery that defects do in fact accumulate in aged myeloid progenitors. Thus, it is reasonable to propose that the milder MPDs are the result of age-related defects in growth and survival of myeloid progenitors and/or their progeny. It is surprising that age-related changes in myeloid progenitors were not previously reported. However, previous studies primarily examined myeloid progenitors as a population rather than on a per-cell basis, and as a result, the cell intrinsic defects defined herein had been overlooked. The age-related defects that accumulate in myeloid progenitors are similar to the those exhibited by aged HSC, including increased frequency and cycling, decreased survival, and diminished per-cell repopulating potential\textsuperscript{22}. These observations suggest...
that age-related defects intrinsic to myeloid progenitors may be the result of intrinsic changes in old HSC. However, whether old HSC and CMP share common molecular changes\(^5^9\) remains to be determined.

While the detrimental effects of advancing age and decreased lymphocyte production on the adaptive immune response have been well documented\(^2^3\), the consequences of aging on the innate immune system remain largely unexplored\(^6^0\). The innate immune system is a critical first responder to infection. Despite defects in old myeloid progenitors, their increased number may compensate for the fact that they do not produce progeny as efficiently as their young counterparts, which results in a relatively normal number of mature myeloid cells. However, reports of diminished function of aged neutrophils\(^6^1\), macrophages\(^6^2\), and dendritic cells\(^6^3\) indicates that the innate immune response may become significantly compromised with age. Consequently, the unexpected observation that myeloid progenitors from old mice are intrinsically defective suggests that age-related defects may contribute the overall reduction in myeloid cell function in the elderly.

While this study used CML as a model with which to investigate the larger question of how aging affects leukemia development, the data nevertheless provide new insights into this disease. First, the demonstration that the degree of lymphoid disease mediated by BCR-ABL\(^\text{p210}\) is related to overall levels of B lymphopoiesis provides an explanation for the paradoxical observation that while CML is considered a stem cell disease, it presents as a MPD with relatively rare lymphoid involvement. Second, age-related defects in myeloid progenitors may explain in part why human CML presents with a chronic rather than an acute course\(^6^4\). Finally, we demonstrate that patterns of lymphoid and myeloid disease exhibited in primary recipients of young and old BM\(^\text{BCR-ABL}\) are conserved following transplantation of leukemic cells into secondary recipients. This finding suggests an intrinsic role for senescence in governing the behavior of LSC and demonstrates the impact of aging on disease development.

**Acknowledgements**

This work was supported by grants from the National Institutes of Health (AG-21459) and the U.S. Department of Defense (W81XWHO410795). O.N.W is an investigator of
the Howard Hughes Medical Institute. R.A.J.S. is supported by a fellowship from the California Institute for Regenerative Medicine (TI-00005). The UCLA Flow Cytometry Core Facility is supported by grants from the National Institutes of Health (CA-16042, AI-28697).

Author Contributions
The study was designed by R.A.J.S., E.M.-R., O.N.W. and K.D.; experiments were performed and data was analyzed by R.A.J.S. with assistance from E.M.-R.; critical reagents were provided by J.M.; the manuscript was written by R.A.J.S., E.M.-R., and K.D.
References

Figure 1. Age alters the phenotype of BCR-ABL\textsuperscript{P210} induced leukemia.

(A) BM from young or old B6 mice was harvested on day 8 following 5-FU treatment, infected with a retrovirus carrying a bicistronic IRES expression vector encoding BCR-ABL\textsuperscript{P210} and a reporter EGFP gene, and transplanted into sublethally irradiated, young syngeneic or congenic recipients. Control recipients received BM transduced with EGFP alone. Leukemias are accompanied by decreased BM (B) and increased splenic (C) cellularity. Cell numbers represent mean values ± SEM obtained from 5 independent experiments with 29 recipients of young BM\textsuperscript{BCR-ABL}, 24 recipients of old BM\textsuperscript{BCR-ABL}, 9 recipients of young BM\textsuperscript{EGFP}, and 5 recipients of old BM\textsuperscript{EGFP}. (D) Leukemic cells in the BM were characterized by flow cytometry for expression of GFP (BCR-ABL\textsuperscript{P210}) in combination with lineage specific cell surface antigens. Examples of recipients with a MPD (upper panels), B lymphoid leukemia (middle panels), and both a MPD and lymphoid leukemia (lower panels) are shown. Recipients that developed a MPD had increased granulocytes and decreased lymphocytes in the spleen (upper right panel), while the ones that developed B lymphoid leukemia had increased lymphoid cells and blasts in the spleen (middle right panel) compared to controls. Cytospin preparations of spleen cells were visualized following wright giemsa staining (400x magnification).

Summary of the incidence of leukemia by phenotype in B6 (E) and \textit{Rag1}\textsuperscript{-/-} (F) recipients of young and old BM\textsuperscript{BCR-ABL} cells. Data in (E) are based on the same recipients as in (B) and (C). Data in (F) are based on 8 recipients of young and 7 recipients of old BM\textsuperscript{BCR-ABL} cells.

Figure 2. B lymphoid leukemogenic potential declines in parallel with age-related declines in B lymphopoiesis.

(A) Immunostaining used to define pre-pro-B cells (Lin\textsuperscript{-}CD19\textsuperscript{-}CD45R\textsuperscript{+}AA4.1\textsuperscript{+}) and pro/pre-B cells (Lin\textsuperscript{-}CD19\textsuperscript{+}CD45R\textsuperscript{+}AA4.1\textsuperscript{+}) in murine BM. (B) The frequency of lymphoid progenitor populations in the BM progressively declines in mice of increasing age. Groups of young (5-7 weeks; n=4), middle-age (42-44 weeks; n=2) and old (90-104 weeks; n=3) mice were analyzed. Steady state frequencies are presented as the mean ± SEM, and 2 pooled middle-age mice. 5-FU frequencies are presented from the pooled BM of 4 young, 7 middle-age, and 4 old mice. Total B lineage cells represents
CD19⁺CD45R⁺ cells. Pre-pro B cell frequencies are 0.103% in young, 0.073% in middle-age, and 0.01% in old 5-FU treated mice, respectively. (C) The incidence of B lymphoid leukemia in \textit{Rag1}⁻/⁻ recipients of BM \textit{BCR-ABL} cells is reduced with increasing BM age. Recipients of middle-age (n=8) BM \textit{BCR-ABL} cells develop B lymphoid leukemia less frequently than recipients of young (n=8) BM \textit{BCR-ABL} cells, but more frequently than recipients of old (n=7) BM \textit{BCR-ABL} cells. The recipients of young and old BM \textit{BCR-ABL} are the same as shown in Figure 1F.

**Figure 3. Age-related intrinsic defects in B lineage progenitors diminish their leukemogenic potential.**

(A) Pro/pre-B cells (Lin⁻CD19⁺CD45R⁺AA4.1⁺) purified from the BM of young and old mice were transduced with BCR-ABL \textit{P210} and the same number of young and old cells was transplanted into \textit{Rag1}⁻/⁻ recipients. 8 weeks later, 25% of recipients of BCR-ABL transduced young pro/pre-B cells (n=8) developed B lymphoid leukemia, while recipients of old pro/pre-B cells (n=8) did not develop any characteristics of disease. 3 x 10⁵ young (B) and old (C) BM cells transduced with EGFP or BCR-ABL \textit{P210} were used to establish hematopoietic cultures in B lineage permissive conditions. Cultures were examined 3 weeks later. Young BM \textit{BCR-ABL} cells expanded 100 fold while only a 6-fold expansion was observed with old cells compared to controls. (D) Phenotypic and morphologic analysis of cultures described in B and C. Cultures derived from young \textit{BCR-ABL} have increased cellularity and a higher frequency of GFP⁺ B lineage cells compared to those initiated from old \textit{BCR-ABL} which produced cells primarily with a myeloid morphology. (E) 3.8 x 10⁵ BCR-ABL \textit{P210} expressing (GFP⁺) young and old pro/pre-B cells were seeded on stromal layers in B lineage permissive conditions. After 5 days, the number of GFP⁺ cells increased 10 fold in the cultures seeded with young pro/pre-B cells, while the old pro/pre-B cells did not show any significant expansion. One of two representative experiments is shown.

**Figure 4. MPDs derived from old BM \textit{BCR-ABL} are characterized by a reduced tumor burden.**
The frequency of total leukemic GFP\(^+\) (BCR-ABL\(^+\)) cells and leukemic myeloid GFP\(^+\)Gr-1\(^+\)CD11b\(^+\) cells in the BM (A) and spleen (B) of recipients of young BM\(^{\text{BCR-ABL}}\) cells is increased compared to recipients of old BM\(^{\text{BCR-ABL}}\) cells. (C) Decreased frequency of B lineage cells in the spleen of young BM\(^{\text{BCR-ABL}}\) recipients compared to old BM\(^{\text{BCR-ABL}}\) recipients. Cell frequency in (A-C) is presented as the mean frequency of cells ± SEM from 17 recipients of young and 23 recipients of old BM\(^{\text{BCR-ABL}}\) that developed MPDs analyzed in 5 independent experiments. (D) Immunostaining used to define populations enriched for leukemic HSC (top; GFP\(^+\)Lin\(^-\)Sca-1\(^{\text{Hi}}\)CD117\(^{\text{Hi}}\)), CMP (middle; GFP\(^+\)Lin\(^-\)Sca-1\(^-\)CD127\(^+\)CD16/32\(^+\)Lo\(\text{CD117}^{\text{Hi}}\)), and GMP (bottom; GFP\(^+\)Lin\(^-\)Sca-1\(^-\)CD127\(^-\)CD16/32\(^+\)Lo\(\text{CD117}^{\text{Hi}}\)) in the BM and spleen of BM\(^{\text{BCR-ABL}}\) recipients. (E) Recipients of young BM\(^{\text{BCR-ABL}}\) cells have more leukemic (GFP\(^+\)) HSC, CMP, and GMP in their BM (upper panel) and spleen (lower panel) compared to those transplanted with old BM\(^{\text{BCR-ABL}}\) cells. The frequency of GFP\(^+\) HSC, CMP, and GMP is presented as the mean ± SEM of 12 recipients of young and 13 recipients of old BM\(^{\text{BCR-ABL}}\) that developed MPDs with no B lineage involvement.

**Figure 5. Myelopoietic defects are present in old mice.**

(A) The frequency of CMP and GMP is increased in the BM of old (78-105 weeks) compared to young (5 weeks) mice both at steady state and at day 8 post 5-FU treatment. Steady state frequencies are presented as the mean ± SEM of 16 young and 12 old mice analyzed in 4 independent experiments. 5-FU frequencies are presented from the pooled BM of 9 young and 7 old mice analyzed in two independent experiments. (B) The number of Annexin V\(^+\) CMP and GMP is increased in the BM of old compared to young mice. Cell numbers are presented as the mean ± SEM of 12 young and 8 old mice analyzed in 3 independent experiments and normalized per 50,000 CMP or GMP respectively. Myeloid colonies derived from whole BM cells (C) and sorted CMP (D) isolated from young mice contained more cells than those derived from BM and CMP isolated from old mice. 5x 10\(^4\) whole BM cells and 250 CMP were plated per dish. Cell numbers in (C) are presented as the mean ± SEM of 24 colonies derived from young BM cells and 24 colonies derived from old BM cells picked in 2 independent experiments. Cell numbers in (D) are presented as the mean of 12 colonies derived from young CMP
and 12 colonies derived from old CMP ± SEM picked in 1 of 2 representative experiments. (E) 1.5 x 10^5 BM cells and 2 x 10^3 CMP (F) plated in liquid culture supplemented with myelopoietic cytokines isolated from young mice produce more Gr-1^CD11b^ myeloid cells when compared to their old counterparts. Numbers are presented as the mean ± SEM of 3 to 6 wells analyzed in 1 of 2 representative experiments.

**Figure 6. Age-related hematopoietic defects alter the leukemogenic potential of LSC.**

(A) Secondary recipients of splenocytes from leukemic mice grafted with young BM^{BCR-ABL} cells developed MPDs and B lymphoid leukemia. 5 x 10^6 splenocytes from primary young BM^{BCR-ABL} recipients were transplanted into secondary recipients (n=15). (B) Secondary recipients of 5 x 10^6 splenocytes from leukemic mice grafted with old BM^{BCR-ABL} cells (n=12) develop MPDs with no significant involvement of B lineage cells. (C) The frequency of total leukemic GFP^+ cells and leukemic myeloid GFP^+Gr-1^CD11b^ cells in the spleens of secondary recipients of splenocytes from tumors derived from young BM^{BCR-ABL} cells is increased compared to secondary recipients of tumors derived old BM^{BCR-ABL} cells. (D) The frequency of B lineage cells in the spleen of secondary recipients of tumors derived from young BM^{BCR-ABL} cells is decreased when compared to secondary recipients of tumors derived from old BM^{BCR-ABL} cells. In all transfers, donor splenocytes were transplanted into at least 3 secondary recipients. At least three primary recipients were analyzed in each experiment. Cell frequency in (C-D) is presented as the mean frequency of cells ± SEM. The mice analyzed in (C-D) developed MPDs with no B lineage involvement.
Table 1. Disease Characteristics of Mice Transplanted with Young or Old EGFP or BCR-ABL<sub>P210</sub> Transduced BM Cells In One Representative Experiment

<table>
<thead>
<tr>
<th>Donor</th>
<th>BM</th>
<th>Spleen</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Cells</td>
<td>%GFP&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Gr-1&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFP1</td>
<td>9.4</td>
<td>1.9</td>
<td>1.1</td>
</tr>
<tr>
<td>EGFP2</td>
<td>26.0</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>EGFP3</td>
<td>13.2</td>
<td>2.1</td>
<td>0.9</td>
</tr>
<tr>
<td>EGFP4</td>
<td>18.8</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td>BCR1</td>
<td>9.2</td>
<td>62.0</td>
<td>39.1</td>
</tr>
<tr>
<td>BCR2</td>
<td>N/A</td>
<td>64.1</td>
<td>12.7</td>
</tr>
<tr>
<td>BCR3</td>
<td>11.0</td>
<td>76.3</td>
<td>52.3</td>
</tr>
<tr>
<td>BCR4</td>
<td>10.2</td>
<td>65.4</td>
<td>25.7</td>
</tr>
<tr>
<td>BCR5</td>
<td>12.0</td>
<td>55.7</td>
<td>44.7</td>
</tr>
<tr>
<td>BCR6</td>
<td>7.2</td>
<td>32.0</td>
<td>10.9</td>
</tr>
<tr>
<td>BCR7</td>
<td>11.6</td>
<td>60.7</td>
<td>1.3</td>
</tr>
<tr>
<td>BCR8</td>
<td>10.2</td>
<td>82.0</td>
<td>56.9</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFP1</td>
<td>17.8</td>
<td>2.4</td>
<td>1.7</td>
</tr>
<tr>
<td>EGFP2</td>
<td>23.4</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>BCR1</td>
<td>8.8</td>
<td>68.9</td>
<td>27.5</td>
</tr>
<tr>
<td>BCR2</td>
<td>20.0</td>
<td>17.8</td>
<td>12.9</td>
</tr>
<tr>
<td>BCR3</td>
<td>15.6</td>
<td>34.4</td>
<td>26.6</td>
</tr>
<tr>
<td>BCR4</td>
<td>9.4</td>
<td>56.0</td>
<td>23.1</td>
</tr>
<tr>
<td>BCR5</td>
<td>15.2</td>
<td>45.4</td>
<td>24.0</td>
</tr>
<tr>
<td>BCR6</td>
<td>14.8</td>
<td>30.7</td>
<td>25.2</td>
</tr>
<tr>
<td>BCR7</td>
<td>20.6</td>
<td>22.7</td>
<td>18.4</td>
</tr>
<tr>
<td>BCR8</td>
<td>20.8</td>
<td>2.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Numbers of BM cells are from 2 femurs and 2 tibias.
Figure 1

A Young (5-7 Weeks) or Old (90-104 Weeks) RETROVIRUS
   BCR-ABL
   OR
   IRES
   EGFP
   IL-11 + SCF + Flt-3L
   500 Rads
   2x10^5 Cells

B BM Cells (x10^9)
P < 0.00074
Young
Old

C Spleen Cells (x10^9)
P < 0.00022
Young
Old

D SSC
GFP
CD11b
Spleen Cells
GFP
CD11b

E Disease Incidence in B6 Recipients (%)
Myeloid
B
Erythroid
T
Young
Old

F Disease Incidence in RAG-1 Recipients (%)
Myeloid
B
Erythroid
Figure 3

A

Incidence of B Lymphoid Leukemia (%)

Young   Old

B

GFP+ B Lineage Cells (x10^4)

EGFP   BCR-ABL

P<0.003

C

GFP+ B Lineage Cells (x10^4)

EGFP   BCR-ABL

P<0.006

D

S5C

GFP

CD45R

CD19

99%

77%

89%

6%

E

GFP+ B Lineage Cells (x10^4)

Day 0  Day 5

Young   Old
Figure 4

A

B

C

D

E

For personal use only.on October 3, 2017. by guest

www.bloodjournal.org

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
Figure 5

A

Day 8 S-FU

Steep State

P<0.0003

P<0.002

CMP

GMP

CMP

GMP

B

Anemia Yr. Cells

P<0.012

P<0.13

CMP

GMP

C

Cells Per Colony x 10^4

P<0.03

Young BM

Old BM

D

Cells Per Colony x 10^4

P<0.04

Young CMP

Old CMP

E

Myeloid Cells x 10^6

P<0.002

Young BM

Old BM

F

Myeloid Cells x 10^6

P<0.04

Young CMP

Old CMP
Figure 6

A
Disease Incidence in Secondary Recipients (%)

Myeloid
B

B
Disease Incidence in Secondary Recipients (%)

Myeloid
B

C
Frequency in Spleen (%)

P<0.00002
YOUNG
OLD

GFP+
GFP+ Gr-1+ CD11b+
P<0.02

D
Frequency of B Lineage Cells
in Spleen (%)

P<0.0002
Young
Old

32
Age-related defects in B lymphopoiesis underlie the myeloid dominance of adult leukemia

Robert A.J. Signer, Encarnacion Montecino-Rodriguez, Owen N Witte, Jami McLaughlin and Kenneth Dorshkind