Check for updates

HEMATOPOIESIS AND STEM CELLS

Comment on Radtke et al, *page 33*

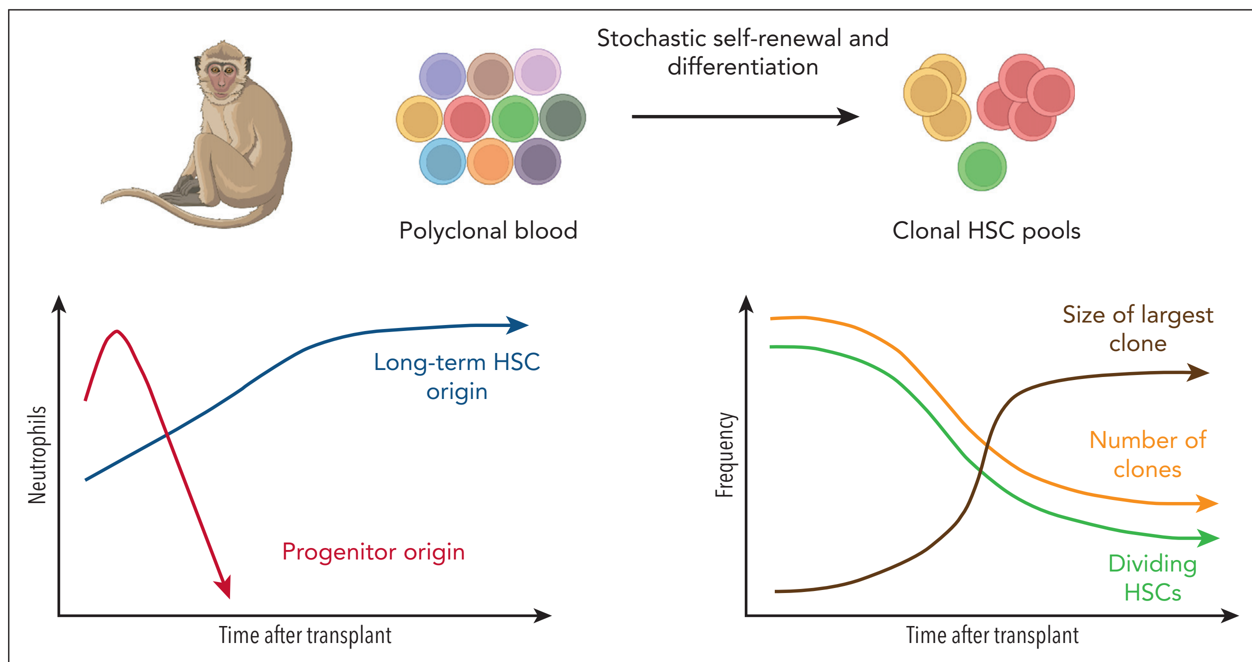
HSCs "shift and drift" during transplantation

Alejo E. Rodriguez-Fraticelli | Institute for Research in Biomedicine, Barcelona Institute for Science and Technology, and Catalan Institution for Research and Advanced Studies

In this issue of *Blood*, Radtke et al[1] demonstrate that transplanted hematopoietic stem cells (HSCs) produce mature cells earlier than expected and, consequently, stochastically drift into clonal pools.

The survival of patients who underwent hematopoietic transplantation depends on the timely recovery of blood and immune cells. However, the hematopoietic cells that are responsible for this lifesaving function at different times after the transplantation remain unidentifiable. The reigning view holds that hematopoietic regeneration after transplantation occurs in 2 phases, with HSCs principally contributing to the long-lived second phase that initiates after the first 6 months.[2,3] Radtke et al present evidence in nonhuman primate models that early and rapid blood cell recovery is also driven by long-term persisting stem cells (see figure). These findings are profoundly significant for stem-cell transplantation and gene therapy fields, in which determining the source of modified blood cells is essential.

To quantitatively assess the clonal dynamics of transplanted stem cells in nonhuman primate models, researchers used both DNA barcodes and deep vector integration-site analysis. Prior studies by these researchers showed that increasing the detection sensitivity was vital in detecting the early contribution of long-term repopulating clones.[4,5] With these more sensitive methods, Radtke et al now tracked transplanted HSCs across multiple lineages for up to 4.5 years. Although many stem-cell clones rapidly divide and disappear after the first year, about 50% of the early-contributing clones are still detectable at the last-assessed time points. These results suggest that past studies in humans and primates might have failed to detect early contribution of HSCs because of the small size and large number of clones during the first phase. But could HSCs be sufficient to explain everything? With these densely mapped data, Radtke et al generated a simple stochastic model that accounted for stem-cell self-renewal and differentiation. Indeed, this purely stem-cell driven model was sufficient to estimate the number of persistent clones from just the input population. Intriguingly, the model was insufficient to fit the kinetics and, especially, the sharp decrease in clonal abundance at the intermediate stages of recovery. To fit these early kinetics of clonal loss, investigators had to create a two-phase model in which the proportion of dividing stem cells (per day) was very high in the early phases of the transplantation (100%) and much lower in the late phase (10%). To validate this heterogeneity, researchers performed single stem-cell cultures, which showed a wide range of clonal expansion, roughly correlating with the percentage of $CD34^+CD90^+$



Long-term HSCs can contribute to multiple blood lineages during the early phases of posthematopoietic transplantation in nonhuman primates. Because of their faster proliferation rate in the early posttransplantation phase, clonal stem-cell pools are formed. Figure created using BioRender.

Downloaded from http://ashpublications.net/blood/article-pdf/142/1/3/2067171/blood_bld-2023-020189-c-main.pdf by guest on 29 May 2024

cells, suggesting that stochastic stem-cell expansions during the early phase are sufficient to explain the clone size heterogeneity observed in the late phase. Radtke et al further challenged their predictions using CellTag, an expressed DNA barcoding system, which allowed them to query the clonal dynamics of $CD34^+$ cells using single-cell transcriptomics.[6] The single-cell nature of the assay allowed them to reliably quantify the clonal sizes while simultaneously verifying their exact molecular identity. These results confirmed that HSCs contained a collection of clonally expanded stem-cell pools, which are the cause for the observed distributions in mature progenitors. Thus, the researchers conclude that HSCs are sufficient to drive all phases of cell recovery during hematopoietic transplantation and that the fast cell divisions required to drive early regeneration result in the stochastic expansion of clonal stem-cell pools, which stabilize after the first year after transplantation.

Although such simple stochastic models may be sufficient to explain clonal dynamics, previous work in mouse HSCs has suggested that various regenerative behaviors, including lineage bias and stem-cell expansion, are partly deterministic, driven by intrinsic and heritable properties.[7-9] Importantly, in these cases, stochastic models were also sufficient to fit distributions of individual samples. Still, clones acted more similarly than expected when the same clone was measured across multiple independent parallel functional tests, suggesting that both stochastic and deterministic mechanisms contribute to HSC dynamics. Further experiments in nonhuman primates should be able to determine whether heritable, deterministic programs also influence regenerative behaviors in this model. Finally, although HSCs are clearly sufficient to drive blood formation across all phases, the relative contribution of other progenitors in a real transplantation scenario is still uncertain. Solving this will require distinctly labeling and tracing multiple stem and progenitor populations simultaneously.[10] These and future findings will be of extreme importance to a growing community of clinical and translational HSC researchers who need to consider the hematopoietic populations that they need to target to achieve the most efficacious results for patients.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Radtke S, Enstrom M, Pande D, et al. Stochastic fate decisions of HSCs after transplantation: early contribution, symmetric expansion, and pool formation. *Blood.* 2023;142(1):33-43.
2. Biasco L, Pellin D, Scala S, et al. In vivo tracking of human Hematopoiesis reveals patterns of clonal dynamics during early and steady-state reconstitution phases. *Cell Stem Cell.* 2016;19(1):107-119.
3. Scala S, Basso-Ricci L, Dionisio F, et al. Dynamics of genetically engineered hematopoietic stem and progenitor cells after autologous transplantation in humans. *Nat Med.* 2018;24(11):1683-1690.
4. Radtke S, Adair JE, Giese MA, et al. A distinct hematopoietic stem cell population for rapid multilineage engraftment in nonhuman primates. *Sci Transl Med.* 2017;9(414):eaan1145.
5. Adair JE, Enstrom MR, Haworth KG, et al. DNA barcoding in nonhuman primates reveals important limitations in retrovirus integration site analysis. *Mol Ther Methods Clin Dev.* 2020;17:796-809.
6. Biddy BA, Kong W, Kamimoto K, et al. Single-cell mapping of lineage and identity in direct reprogramming. *Nature.* 2018;564(7735):219-224.
7. Rodriguez-Fraticelli AE, Weinreb C, Wang S-W, et al. Single-cell lineage tracing unveils a role for TCF15 in haematopoiesis. *Nature.* 2020;583(7817):585-589.
8. Naik SH, Perié L, Swart E, et al. Diverse and heritable lineage imprinting of early haematopoietic progenitors. *Nature.* 2013;496(7444):229-232.
9. Dykstra B, Kent D, Bowie M, et al. Long-term propagation of distinct hematopoietic differentiation programs in vivo. *Cell Stem Cell.* 2007;1(2):218-229.
10. Zonari E, Desantis G, Petrillo C, et al. Efficient ex vivo engineering and expansion of highly purified human hematopoietic stem and progenitor cell populations for gene therapy. *Stem Cell Rep.* 2017;8(4):977-990.

https://doi.org/10.1182/blood.2023020189

© 2023 by The American Society of Hematology

LYMPHOID NEOPLASIA

Comment on Jiang et al, page 44

Targeting the methylome to improve CLL outcome

John G. Gribben | Barts Cancer Institute

In this issue of *Blood*,[1] Jiang et al demonstrate that KDM1A is upregulated in malignant B cells and that this is associated with aggressive disease as well as adverse outcome in samples from the CLL8 clinical trial[2] (see figure panel A). They further show that knockdown of Kdm1a in a murine model altered the epigenome.

Epigenetic changes are known to drive clonal evolution and diversity in chronic lymphocytic leukemia (CLL), making CLL a good model to study epigenetic evolution over time.[3,4] However, the molecular mechanisms that drive these changes are poorly understood. To understand the mechanisms better, the authors generated a doxorubicin (Dox)-inducible *Kdm1a* knockdown in Eμ-TCL1 mice (*Kdm1a*-KD). *Kdm1a*-KD mice had reduced tumor burden, prolonged survival, upregulation of p53 and proapoptotic pathways, and changes in the tumor microenvironment (TME), indicating an important role for KDMIA. So, how does this occur and what, if anything, does this have to do with the CLL epigenome? In elegant and comprehensive experiments, the authors analyzed differences in global transcriptome by RNA sequencing as well as H3K4me3 marks by chromatin immunoprecipitation sequencing between Eμ-TCL1 and *Kdm1a*-KD mice and then confirmed their findings in human CLL samples. Their findings demonstrate that in CLL cells, KDM1A alters histone methylation patterns in pathways regulating cell death and motility, thereby acting as an oncogenic transcriptional repressor in this disease. More important, they were able

Downloaded from http://ashpublications.net/blood/article-pdf/142/1/3/2067171/blood_bld-2023-020189-c-main.pdf by guest on 29 May 2024