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In Advani et al, the biomarker experiments did not clearly delineate which patients were most likely to benefit or have treatment failure. Identifying predictive biomarkers of brentuximab-nivolumab and other novel targeted and immunotherapy combinations is vital for tailoring therapeutic intensity to risk and should be an integral component of any large study going forward.

In summary, Advani et al demonstrate tolerability and a high response rate of the brentuximab-nivolumab combination as salvage therapy before autologous SCT without compromising stem cell mobilization or engraftment. This regimen has the potential to provide an alternative to cytotoxic chemotherapy regimens such as ifosfamide, carboplatin and etoposide as a first salvage therapy, if the findings are confirmed in a randomized study. Optimal sequencing of the brentuximab-nivolumab regimen with standard salvage chemotherapy and other immunotherapies and the evolving role for SCT as a function of highly active second-line salvage regimens remain important questions for future studies.

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REFERENCES

1. Advani RH, Moskowitz AJ, Bartlett NL, et al. Brentuximab vedotin in combination with nivolumab in relapsed or refractory Hodgkin lymphoma: 3-year study results. *Blood*. 2021; 138(6):427-438.
2. Vose JM, Bierman PJ, Armitage JO. Hodgkin's disease: the role of bone marrow transplantation. *Semin Oncol*. 1990;17(6): 749-757.
3. Moskowitz AJ, Shah GL, Schoder H, et al. High complete response rate observed with second-line chemo-immunotherapy with pembrolizumab and GVD (gemcitabine, vinorelbine, and liposomal doxorubicin) in relapsed and refractory classical Hodgkin lymphoma [abstract]. *Blood*. 2019;134(suppl 1). Abstract 2837.
4. Herrera AF, Chen RW, Palmer J, et al. PET-adapted nivolumab or nivolumab plus ice as first salvage therapy in relapsed or refractory Hodgkin lymphoma [abstract]. *Blood*. 2019; 134(suppl 1). Abstract 239.
5. Bryan LJ, Smith SE, Allen P, et al. Safety and toxicity profile of pembrolizumab (PEM) in combination with ICE chemotherapy followed by autologous stem cell transplantation for relapsed/refractory classical Hodgkin lymphoma: no impairment in stem cell mobilization or engraftment [abstract]. *Blood*. 2019;134(suppl 1). Abstract 4029.
6. Bartlett NL, Herrera AF, Domingo-Domenech E, et al. A phase 1b study of AFM13 in combination with pembrolizumab in patients with relapsed or refractory Hodgkin lymphoma. *Blood*. 2020;136(21):2401-2409.
7. Diefenbach CS, Hong F, Ambinder RF, et al. Ipilimumab, nivolumab, and brentuximab vedotin combination therapies in patients with relapsed or refractory Hodgkin lymphoma: phase 1 results of an open-label, multicentre, phase 1/2 trial. *Lancet Haematol*. 2020;7(9):e660-e670.
8. LaCasce AS, Bociek RG, Sawas A, et al. Brentuximab vedotin plus bendamustine: a highly active first salvage regimen for relapsed or refractory Hodgkin lymphoma. *Blood*. 2018;132(1):40-48.
9. Carreau NA, Pail O, Armand P, et al. Checkpoint blockade treatment may sensitize hodgkin lymphoma to subsequent therapy. *Oncologist*. 2020;25(10):878-885.
10. Stamatoullas A, Ghesquieres H, Clement filliatre L et al. Brentuximab vedotin in first refractory/relapsed classical Hodgkin lymphoma patients treated by chemotherapy (ICE) before autologous transplantation. final analysis of phase II study [abstract]. *Blood*. 2019;134(suppl 1). Abstract 132.

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EMATOPOIESIS AND STEM CELLS

Comment on Flohr Svendsen et al, page 439

Mining old transcriptomes to predict HSC age

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In this issue of *Blood*, Flohr Svendsen et al propose to the research community a new and robust transcriptomic signature of aging and provide an online resource to help assess and understand the aging of hematopoietic stem cells (HSCs).[1]

What causes our hematopoietic system to age? Is aging of the hematopoietic system due to intrinsic changes in HSCs? To answer these burning questions about the immunosenescence of the elderly, many groups have used transcriptome profiling of HSCs from mice of different ages, a strategy with great potential. After almost 2 decades, we have discovered a plethora of age-associated genes and almost as many potential mechanisms to explain HSC aging.[2,3] However, due to the quantity and divergence of results, probably because of differences in experimental parameters (ages, aging conditions, mouse strains, and platforms), we still lack a complete understanding of HSC aging.[2,3]

In an attempt to reconciliate these studies, Flohr Svendsen et al took advantage of computational biology to revisit 16 transcriptomic datasets of aged HSCs that used a variety of designs and platforms, ranging from the first microarray study[4] to their latest RNA-sequencing study.[5] To overcome the complexity of interexperimental variations, the authors developed tools for calculating and flattening transcriptomic data, without losing key information. The strength of this strategy lies in using different approaches to validate the robustness of their age-related gene list and smooth out the platform-dependent bias of each platform. They succeeded in providing a list of ∼100 genes whose expression indicates aged HSCs, thus defining a unique and robust aging signature (AS) in mouse HSCs.

The benefits of developing a consensual AS are multifaceted. The AS can serve as an "aging fingerprint" in searching for an aging phenotype in any mutant HSCs. To assist in this, they provide a user-friendly online resource (https://agingsignature.webhosting.rug.nl/) that allows any biologist to easily query their transcriptomic signature in relation to aging. As an extension, by testing the robustness of their AS on "rejuvenated HSCs" treated with β adrenaline, the authors opened an interesting application of their resource. They demonstrated the possibility of using it to test the efficacy of rejuvenation strategies, such as

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bone marrow niche change or pharmacological intervention, which are under development.[6]

A limitation of this type of signature is that it reflects the total cell population and fails to capture individual cellular differences. Single-cell approaches have highlighted the heterogeneity of the HSC population, and we can expect that most future transcriptomic analyses will be performed at single-cell resolution.[7] The authors were aware of this and, using machine learning algorithms, managed to extract from their global AS a shortened list of 20 genes that can predict whether an individual HSC is young or old. This age predictor list is unique and will be very important in assessing the aging heterogeneity of an HSC population after different treatments or stresses. Considering that this predictor indicates the biological age of a cell, it may be useful as a biomarker when evaluating the age of HSCs in relation to the clonal evolution of age-related myeloid diseases.

Beyond being a useful predictor of age, the AS is a goldmine for shedding light on functional changes in aged HSCs. Looking at gene enrichment, Flohr Svendsen et al pointed out that 20% of the AS genes encode membrane proteins. This implies that age-related changes impact the way an old HSC perceives its microenvironment.[8] The authors functionally validated one of their top genes, the *Selp* gene, encoding P-selectin, a platelet surface marker. They showed that re-expressing this aging marker on the surface of young HSCs blocked their erythroid differentiation potential. Altogether, these data demonstrate that the AS identifies genes that regulate aging-related processes. Their list certainly contains other nuggets, including the stress gene *Nupr1*, an interesting HSC aging candidate that has recently been described as an HSC quiescence regulator.[9]

By reanalyzing all these transcriptomic studies, Svendsen et al also made the interesting and surprising observation that the RNA content is higher and RNA polymerase II more active in aged HSCs. Do aged HSCs increase their transcriptional rate to compensate for altered proteolysis? Is it associated with an epigenetic drift toward an opening of the chromatin that occurs with aging?[10] The authors present convincing data in this direction. By assessing the relaxed state of chromatin as a function of age, they demonstrate that aged HSCs have many more accessible and, thus, open sites than young cells. It is therefore tempting to look for a correlation between the high RNA content and the relaxed chromatin state of aged HSCs. Although this certainly needs to be studied further, it may suggest that older cells are more transcriptionally active.

In an effort to reconcile years of transcriptomics, the authors have developed a powerful resource that will be very useful to the hematology community for not only understanding the mechanisms of aging but also assessing the biological age of our stem cells.

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REFERENCES

1. Flohr Svendsen A, Yang D, Kim KM, et al. A comprehensive transcriptome signature of murine hematopoietic stem cell aging. *Blood.* 2021138(6):
2. de Haan G, Lazare SS. Aging of hematopoietic stem cells. *Blood.* 2018;131(5):479-487.
3. Mejia-Ramirez E, Florian MC. Understanding intrinsic hematopoietic stem cell aging. *Haematologica.* 2020;105(1):22-37.
4. Rossi DJ, Bryder D, Zahn JM, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci USA.* 2005;102(26):9194-9199.
5. Renders S, Svendsen AF, Panten J, et al. Niche derived netrin-1 regulates hematopoietic stem cell dormancy via its receptor neogenin-1. *Nat Commun.* 2021;12(1):608.
6. Li X, Zeng X, Xu Y, et al. Mechanisms and rejuvenation strategies for aged hematopoietic stem cells. *J Hematol Oncol.* 2020;13(1):31.
7. Wilson NK, Kent DG, Buettner F, et al. Combined single-cell functional and gene expression analysis resolves heterogeneity within stem cell populations. *Cell Stem Cell.* 2015;16(6):712–724.
8. Verovskaya EV, Dellorusso PV, Passegué E. Losing sense of self and surroundings: hematopoietic stem cell aging and leukemic transformation. *Trends Mol Med.* 2019; 25(6):494–515.
9. Wang T, Xia C, Weng Q, et al. Loss of Nupr1 promotes engraftment by tuning the quiescence threshold of hematopoietic stem cell repository via regulating p53-checkpoint pathway [published online ahead of print 10 December 2020]. *Haematologica.* doi:10.3324/haematol.2019.239186.
10. Sun D, Luo M, Jeong M, et al. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. *Cell Stem Cell.* 2014;14(5):673-688.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Enns et al, page 486

Hepatocyte neogenin, another key actor in iron homeostasis

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In this issue of *Blood*, Enns et al[1] provide compelling evidence that hepatocyte neogenin (NEO1) is essential for systemic iron homeostasis. NEO1 function in bone morphogenetic protein (BMP) signaling and the regulation of hepcidin expression requires its association with hemojuvelin (HJV), the protein mutated in juvenile hemochromatosis.

Neogenin is a transmembrane receptor belonging to the immunoglobulin superfamily (see figure). It has crucial functions in diverse cellular processes ranging from cell motility and adhesion to survival and differentiation. To mediate these functions, neogenin binds different ligands such as repulsive guidance molecules (RGM) and Netrin-1.[2]

HJV (or RGMC) is 1 of the 3 members of the RGM family, and thus is a ligand for NEO1. It is also coreceptor for BMP ligands. In mice, hemojuvelin is indispensable for hepcidin expression and iron homeostasis.[3] Mutations in the *HJV* gene in humans reduce hepcidin expression in the liver and cause juvenile hemochromatosis.[4] Interestingly, the most common

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