3. Berentsen S, Barcellini W, D'Sa S, et al. Cold agglutinin disease revisited: a multinational, observational study of 232 patients. *Blood*. 2020;136(4):480-488.

4. Berentsen S, Ulvestad E, Gjertsen BT, et al. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. *Blood*. 2004;103(8):2925-2928.

5. Berentsen S, Randen U, Vågan AM, et al. High response rate and durable remissions following fludarabine and rituximab combination therapy for chronic cold agglutinin disease. *Blood*. 2010;116(17): 3180-3184.

6. Berentsen S, Randen U, Oksman M, et al. Bendamustine plus rituximab for chronic cold agglutinin disease: results of a Nordic prospective multicenter trial. *Blood*. 2017; 130(4):537-541.

7. Röth A, Bommer M, Hüttmann A, et al. Eculizumab in cold agglutinin disease (DECADE): an open-label, prospective, bicentric, nonrandomized phase 2 trial. *Blood Adv*. 2018;2(19):2543-2549.

8. Shi J, Rose EL, Singh A, et al. TNT003, an inhibitor of the serine protease C1s, prevents complement activation induced by cold agglutinins. *Blood*. 2014;123(26): 4015-4022.

9. Jäger U, D'Sa S, Schörgenhofer C, et al. Inhibition of complement C1s improves severe hemolytic anemia in cold agglutinin disease: a first-in-human trial. *Blood*. 2019; 133(9):893-901.

10. Röth A, Barcellini W, D'Sa S, et al. Sutimlimab in cold agglutinin disease. *N Engl J Med*. 2021;384(14):1323-1334.

HEMATOPOIESIS AND STEM CELLS

Comment on Keyvani Chahi et al, page 992

HSCs: slow me down with PLAG1

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In this issue of *Blood*, Keyvani Chahi et al[1] have described the transcription factor PLAG1 as a positive regulator of human cord blood (CB) hematopoietic stem cell (HSC) dormancy and self-renewal by repressing the expression of translational machinery that is customarily activated in response to ex vivo cultivation stress.

HSCs are capable of generating all blood cell lineages throughout a person's lifetime.[2] To preserve life-long stem cell functionality, HSCs primarily reside in a state of dormancy and quiescence characterized by low levels of protein synthesis.[3,4] Under stress conditions, such as ex vivo cultivation or transplantation, dormant HSCs are forced into a state of activation.[5] However, little is known about the preventative machinery that suppresses activation and promotes quiescence in human HSCs.

Previously, PLAG1 was shown to bind to the promoter of MSI2 to enhance in vitro expansion of hematopoietic stem and progenitor cells (HSPCs).[6] In their study, Keyvani Chahi et al showed that PLAG1 expression is restricted to non-cycling HSCs, which suggests a role for PLAG1 in modulating human HSPC function that is independent of the PLAG1/USF2-MSI2 regulatory axis. There are 3 human PLAG1 transcript variants encoding 2 protein isoforms. The shorter isoforms of PLAG1 (B and S) are enriched in CB HSCs, while the longer isoform (A) is expressed at much lower levels.[6] Knockdown of PLAG1 reduced the output of human HSPCs and was accompanied by impaired long-term bone marrow reconstitution, whereas the ectopic overexpression (OE) of the PLAG1-S isoform enhanced in vivo self-renewal of human HSCs.

From genomic binding and transcriptomic profiling of PLAG1-S[OE] HSPCs, the authors found that PLAG1-S can act as both a positive and a negative regulator of gene expression. For instance, ribosomal protein expression was downregulated in PLAG1-S[OE], which pointed toward the ability of PLAG1-S to attenuate protein synthesis machinery and thus regulate stem cell
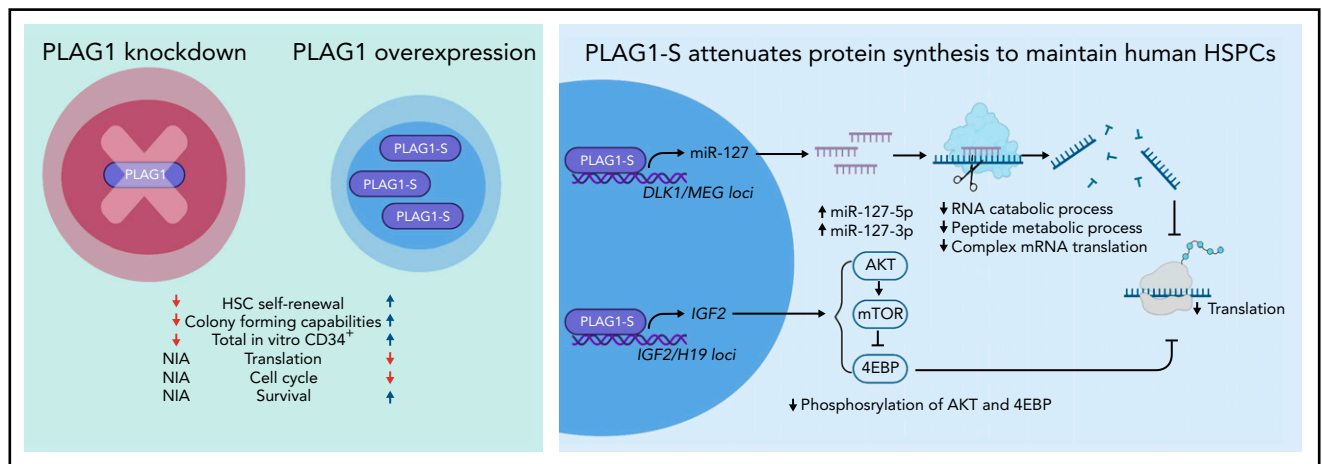
Loss and gain of PLAG1 function in human CB CD34+ HSPCs (left). Mechanisms by which PLAG1-S represses protein synthesis to maintain human CB HSC dormancy and self-renewal (right). mRNA, messenger RNA; NIA, no information available. Figure created by BioRender.com.

fate decisions. In agreement with this finding, the authors showed that PLAG1-S[OE] maintained low global translation rates in ex vivo cultivated HSPCs (see figure).

Mechanistically, PLAG1-S directly binds and activates the imprinted loci IGF2/H19 and DLK1/MEG3. IGF2 stimulates the PI3K-AKT-mTOR signaling pathway, whose activation in turn facilitates protein synthesis. Here, phosphorylated AKT activates mTOR to phosphorylate 4EBP. In the phosphorylated state, 4EBP cannot inhibit protein synthesis. However, intracellular flow cytometry revealed reduced phosphorylation of AKT and 4EBP, which suggests that PLAG1-S represses phosphorylation of 4EBP and thus contributes to reduced protein synthesis. Indeed, the pharmacological inhibition of AKT and mTOR decreased total cell count but increased the proportion of HSPCs. However, only mTOR inhibition, but not AKT inhibition, attenuated protein synthesis. The DLK1/MEG3 locus encodes four microRNAs (eg, miR-127)[7] that were upregulated in PLAG1-S[OE]. The targets of these miRNAs included genes involved in complex cap-dependent translation and RNA and peptide metabolic processing, which were downregulated in PLAG1-S[OE]. In the presence of an inhibitory miR-127-5p sponge, PLAG1-S[OE] reduced HSPC output and increased protein synthesis, whereas overexpression of miR-127 enhanced HSPC output and reduced protein synthesis (see figure).

Intriguingly, MYC expression, a major transcriptional activator of cytoplasmic translation and ribosome biogenesis,[8] was not reduced in PLAG1-S[OE]. PLAG1-S[OE] instead upregulates MYC-regulated nuclear ribosome assembly targets, suggesting that PLAG1-S acts independently of MYC repression. PLAG1-S[OE] in the presence of c-MYC[OE] was able to reduce protein synthesis and increase HSPC output, which was previously negatively impacted by c-MYC[OE].

Keyvani Chahi et al identified PLAG1-S as a novel enforcer of human CB HSC dormancy and self-renewal via its dampening of protein synthesis upon ex vivo culture-induced stimulation. These results further highlight the physiological importance of low translation rates and suggest targeting proteostasis to manipulate ex vivo human CB HSCs for therapeutic benefits (eg, gene therapy). Furthermore, it has been demonstrated that translational inhibitors can eliminate primitive leukemic cells while sparing healthy HSCs.[9] Targeting components of the PLAG1-S signaling pathway could provide novel therapies for patients with leukemia.

It is worth highlighting the great effort the scientific community is making to understand how human stem cells are regulated, which will pave the way for the discovery of new ways to maintain and genetically modify these rare and precious blood cells.

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

REFERENCES

1. Keyvani Chahi A, Belew MS, Xu J, et al. PLAG1 dampens protein synthesis to promote human hematopoietic stem cell self-renewal. *Blood.* 2022;140(9):992-1008.

2. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell.* 2008;132(4):631-644.

3. Cabezas-Wallscheid N, Buettner F, Sommerkamp P, et al. Vitamin A-retinoic acid signaling regulates hematopoietic stem cell dormancy. *Cell.* 2017;169(5):807-823.e19.

4. Signer RAJ, Magee JA, Salic A, Morrison SJ. Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature.* 2014;509(7498):49-54.

5. Kruta M, Sunshine MJ, Chua BA, et al. Hsf1 promotes hematopoietic stem cell fitness and proteostasis in response to ex vivo culture stress and aging. *Cell Stem Cell.* 2021;28(11):1950-1965.e6.

6. Belew MS, Bhatia S, Keyvani Chahi A, Rentas S, Draper JS, Hope KJ. PLAG1 and USF2 co-regulate expression of Musashi-2 in human hematopoietic stem and progenitor cells. *Stem Cell Reports.* 2018;10(4):1384-1397.

7. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. *Nucleic Acids Res.* 2018;46(D1):D239-D245.

8. van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer.* 2010;10(4):301-309.

9. Callahan KP, Minhajuddin M, Corbett C, et al. Flavaglines target primitive leukemia cells and enhance anti-leukemia drug activity. *Leukemia.* 2014;28(10):1960-1968.

LYMPHOID NEOPLASIA

Comment on Brink et al, page 1009

To transplant or not to transplant: that is the question in PTCL

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Peripheral T-cell non-Hodgkin lymphoma (PTCL) represents a heterogeneous group of lymphomas with historically poor prognosis for most subtypes, with the exception of ALK[+] anaplastic large-cell lymphoma (ALCL).[1] Although the chemotherapy used for B-cell lymphomas, such as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), has been the standard of care for many years, the long-term disease-free survival (DFS) and overall survival (OS) are poor.[2] One strategy used to improve these outcomes has been the addition of etoposide to CHOP (CHOEP), which has improved the DFS in some subtypes of PTCL in some smaller studies[3]; and another strategy includes the use of high-dose chemotherapy and autologous peripheral stem cell transplantation (ASCT) in the first complete remission (CR1).[4] A large randomized trial in a homogenous patient population to confirm these modifications has not been undertaken. In this issue of *Blood*, Brink et al[5] analyze data from a nationwide population-based Netherlands Cancer Registry to evaluate this point.

In an extensive analysis, Brink at al evaluated patients 18 to 65 years of age with stage II to IV ALCL, angioimmunoblastic T-cell lymphoma (AITL), or PTCL and grouped the patients into 5 broad categories: (1) chemotherapy followed by