

The work of Wang et al is consistent with the following scenario. In the hepatocyte, SMAD1 and SMAD5 cooperate to mediate iron-related signaling through hemojuvelin (HJV), BMP, and BMP receptor (BMPR) for the promotion of hepcidin transcription through phosphorylated SMAD4. Homozygous inactivation of HJV or other molecules in the hepcidin-activation pathway leads to hemochromatosis through decreased SMAD1 and SMAD5 signaling and lack of hepcidin. Stimulation of erythroblasts by erythropoietin (EPO), especially in the setting of ineffective erythropoiesis, leads to increased production of erythroferrone by erythroblasts. In turn, erythroferrone serves to decrease phosphorylation (P) of SMAD1 and SMAD5 in hepatocytes, leading to iron loading as a result of lack of hepcidin.

SMAD5, and SMAD8 to hepcidin regulation. Knockdown of *SMAD1* or *SMAD5* but not *SMAD8* inhibited hepcidin messenger RNA (mRNA) expression in Hep3B cells, so the investigators focused further on SMAD1 and SMAD5. They generated mice with hepatocyte-specific inactivation of *Smad1*, *Smad5*, or both to demonstrate that SMAD1 and SMAD5 have overlapping functions in regulating hepcidin expression but that the activity of both is necessary for optimal regulation.

Investigators recently discovered that erythroferrone, a factor secreted by erythroid progenitors in the bone marrow, is an important mediator of the suppression of hepatic hepcidin expression that is observed with increased erythropoiesis, especially ineffective erythropoiesis.^{9,10} Exactly how erythroferrone suppresses hepcidin expression and whether the mechanism involves the BMP-SMAD signaling pathway is uncertain. Wang et al found that erythropoietin robustly induced bone marrow erythroferrone mRNA in control mice and mice with hepatic inactivation of both *Smad1* and *Smad5* but suppressed liver hepcidin mRNA only in control mice. In keeping with this observation, erythropoietin and erythroferrone reduced the phosphorylation of SMAD1 and SMAD5 in parallel with decreasing the expression of hepcidin in the livers of control mice and in control Hep3B cells. Furthermore, erythroferrone failed to decrease hepcidin expression in hepatocytes with inactivation of *Smad1* and *Smad5* and in Hep3B cells with

knockdown of *SMAD1* and *SMAD5*. These observations are consistent with the possibility that erythroferrone acts through SMAD1 and SMAD5 signaling to suppress hepcidin production.

The report by Wang et al represents an important advance in our understanding of the details of BMP signaling in hepcidin regulation. The results indicate that SMAD1 and SMAD5, but not SMAD8, work cooperatively to control hepcidin expression. The evidence for a role of SMAD1 and SMAD5 in mediating hepatic hepcidin suppression in response to erythropoietin and in response to erythroferrone secreted by bone marrow erythroid progenitors is of particular interest to hematologists. Although more

details need to be worked out, answers are now forthcoming to the old question of how ineffective erythropoiesis leads to nontransfusional iron overload.

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

REFERENCES

1. Wang C-Y, Core AB, Canali S, et al. Smad1/5 is required for erythropoietin-mediated suppression of hepcidin in mice. *Blood*. 2017;130(1):73-83.
2. Pootrakul P, Kitcharoen K, Yansukon P, et al. The effect of erythroid hyperplasia on iron balance. *Blood*. 1988;71(4):1124-1129.
3. Cazzola M, Barosi G, Bergamaschi G, et al. Iron loading in congenital dyserythropoietic anaemias and congenital sideroblastic anaemias. *Br J Haematol*. 1983; 54(4):649-654.
4. Gordeuk VR, McLaren GD, Samowitz W. Etiologies, consequences, and treatment of iron overload. *Crit Rev Clin Lab Sci*. 1994;31(2):89-133.
5. Camaschella C. Iron and hepcidin: a story of recycling and balance. *Hematology Am Soc Hematol Educ Program*. 2013;2013:1-8.
6. Babitt JL, Huang FW, Wrighting DM, et al. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat Genet*. 2006;38(5):531-539.
7. Kautz L, Meynard D, Monnier A, et al. Iron regulates phosphorylation of Smad1/5/8 and gene expression of *Bmp6*, *Smad7*, *Id1*, and *Atoh8* in the mouse liver. *Blood*. 2008;112(4):1503-1509.
8. Wang RH, Li C, Xu X, et al. A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metab*. 2005;2(6):399-409.
9. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. *Blood*. 2006;108(12):3730-3735.
10. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet*. 2014;46(7): 678-684.

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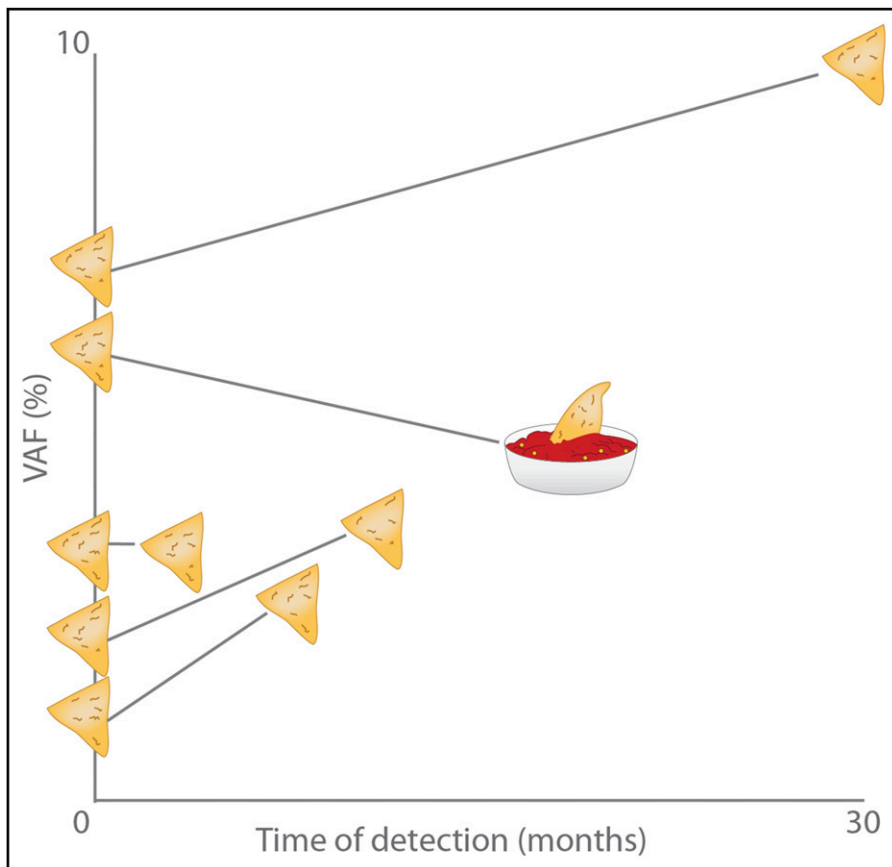
CHIPs and engraftment dips

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In this issue of *Blood*, Gibson et al provide provocative information suggesting that use of otherwise normal stem cell donors with clonal hematopoiesis of indeterminate potential (CHIP) results in impaired hematopoietic recovery following allogeneic hematopoietic stem cell transplantation (HSCT).¹

The authors retrospectively reviewed results of 552 patients who underwent allogeneic HSCT and identified 89 patients (16%) who

had at least 1 cytopenia beyond day 100 after HSCT. Among those 89 patients, a probable cause for the cytopenia was identified in 83



The x-axis represents months from the time of transplantation until detection of clonal hematopoiesis in the patient. The y-axis indicates the variant allele fraction (VAF). The VAF at time 0 is measured from the donor stem cell product. The VAF at later times is from the patient.

cases, while in 6 patients (~1% overall), no obvious cause could be found. In these 6 cases, *DNMT3A* mutations were found in 5 patients, with confirmation as donor origin by sequencing archival donor stem cells. Twenty-four of the 83 cases with explainable cytopenia also underwent mutation testing, and none showed mutations. These data suggest a strong association between the development of otherwise unexplained cytopenias post-HSCT and use of donors with CHIP.

Allogeneic HSCT is increasingly used to treat patients in their sixth, seventh, and even eighth decade of life, resulting in a greater use of older related donors. Given the frequency of CHIP in otherwise normal individuals in this age group, the current study raises many questions: (1) How strong is the association between use of a CHIP-positive donor and impaired hematologic recovery? These data suggest that the association is almost absolute: 5 out of 6 patients with unexplained cytopenias had CHIP-positive donors, while none of the 24 patients without unexplained cytopenias

had such donors (however, only a handful of these 24 cases were older, so the difference may not be as striking as it appears). (2) How large does the CHIP clone have to be in the donor to be consequential? The usual methods for detecting such clones have a limit of detection of 2% to 3%. Many donors categorized as CHIP negative likely have small clones below the limits of detection. The median clone size in the 5 patients with unexplained cytopenia and mutations was ~4% (range, 2.6–11). Can a clone at a level of only 0.5% do the same? (3) Which donors are at highest risk for being CHIP positive? Is this strictly age related? Are first-degree relatives of patients with hematologic malignancies at higher risk? (4) What are the clinical consequences of using a CHIP-positive donor? While the development of a single cytopenia beyond day 100 post-HSCT is concerning, it is unclear what this means long-term, as few details about the duration of cytopenias or other transplant outcomes were provided in this report. Are the cytopenias of limited clinical consequence, or

do they signal major defects in multiple aspects of graft function? (5) Does the mutational basis of the clone influence outcome? Only *DNMT3A* mutations were represented in the report and, while these are the most frequent mutations associated with CHIP, other mutations, including *TET2* and *ASXL1*, have been identified.^{2,3} Are these other mutations likewise associated with impaired hematopoietic recovery? (6) Assuming the current results are confirmed, why does the use of a CHIP-positive donor increase the risk of post-HSCT cytopenias? Apparently, the donors had normal hematopoiesis at the time of collection, and there was no indication of any abnormality in the ability to mobilize and collect stem cells, at least as measured by CD34⁺ cell content. Following transplant and engraftment, the abnormal clone did not expand and continued to represent an allelic fraction very similar to that in the donor (see figure). Why then did this fraction apparently impair hematopoiesis in the recipient, but not in the donor? Does the abnormal clone behave similarly in the patient as in the donor, or does transplantation, immunosuppression, and other environmental features influence its behavior? (7) With longer follow-up, what will be the fate of the abnormal clone? In 1 of the 5 patients with CHIP, there was evidence of clonal evolution with the acquisition of additional mutations in *ASXL1* and *TP53* but without evidence of clinical progression to myelodysplasia or acute leukemia. It is reassuring that no case evolved into overt myelodysplasia or leukemia, but the follow-up period may be too brief. Donor cell leukemia occurs in ~0.1% of transplants, but how strongly this risk is impacted by the use of a CHIP-positive donor is unknown.^{4,5}

An obvious question is whether these findings should alter our current standard-care policies. The incidence of CHIP is rare in patients younger than 50 years, increases to 2% to 3% in patients 50 to 60 years, and then increases rapidly.^{2,3} Currently, the National Marrow Donor Program retires volunteer donors once they reach age 60 years. Should younger unrelated donors be used routinely instead of older matched siblings, or should older matched siblings be routinely tested for CHIP and excluded from donating if it is found? Several studies have retrospectively compared outcomes in older patients given younger allele-level 8/8 HLA-matched unrelated donor (MUD) vs older

HLA-matched sibling (MSD) transplants.⁶⁻⁸ A Center for International Blood and Marrow Transplant Research report of >2000 transplants found more acute and chronic graft-versus-host disease using younger MUDs than with older MSDs and, in good-risk patients, increased overall mortality with the use of MUDs. These data, combined with the longer delay in initiating transplantation and an increased burden on volunteer donors, argue against a wholesale move to the use of younger unrelated donors when older matched siblings are available. Screening older donors for CHIP is certainly feasible, but it is expensive. We agree with Gibson et al that a larger study of donor–recipient pairs, perhaps enriched for older patients with long follow-up, would be of enormous benefit in helping us better understand the frequency and

implications of CHIP among otherwise normal stem cell donors and in helping to set policy about how to approach issues of mutational screening and the use of older donors.

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REFERENCES

1. Gibson CJ, Kennedy JA, Nikiforow S, et al. Donor-engrafted CHIP is common among stem cell transplant recipients with unexplained cytopenias. *Blood*. 2017;130(1):91-94.
2. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
3. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498.
4. Kato M, Yamashita T, Suzuki R, et al. Donor cell-derived hematological malignancy: a survey by the Japan Society for Hematopoietic Cell Transplantation. *Leukemia*. 2016;30(8):1742-1745.

5. Dietz AC, DeFor TE, Brunstein CG, Wagner JE Jr. Donor-derived myelodysplastic syndrome and acute leukaemia after allogeneic haematopoietic stem cell transplantation: incidence, natural history and treatment response. *Br J Haematol*. 2014;166(2):209-212.

6. Alousi AM, Le-Rademacher J, Saliba RM, et al. Who is the better donor for older hematopoietic transplant recipients: an older-aged sibling or a young, matched unrelated volunteer? *Blood*. 2013;121(13):2567-2573.

7. Peffault de Latour R, Labopin M, Cornelissen J, et al; Acute Leukemia Working Party of EBMT. In patients older than 55 years with AML in first CR, should we search for a matched unrelated donor when an old sibling donor is available? *Bone Marrow Transplant*. 2015;50(11):1411-1415.

8. Rezvani AR, Storer BE, Guthrie KA, et al. Impact of donor age on outcome after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2015;21(1):105-112.

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