blood® commentary

PLATELETS AND THROMBOPOIESIS

Comment on Tozawa et al, page 633

Megakaryocytes from fat: a new recipe for platelets

Alessandra Balduini | University of Pavia

In this issue of *Blood*, Tozawa et al report the production of platelets from human adipose–derived mesenchymal stem/stromal cells for use in transfusion medicine as donor-independent source (see figure).[1]

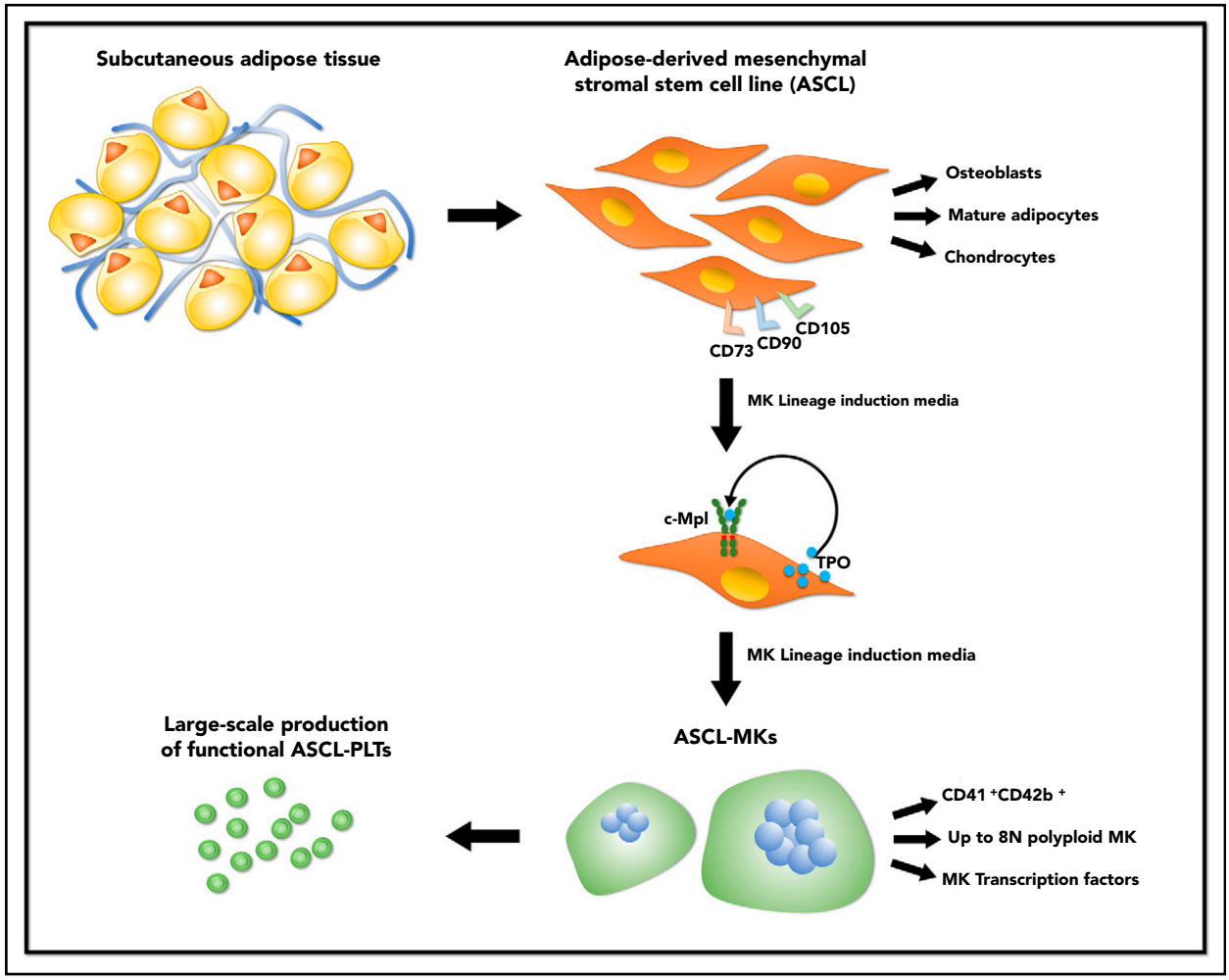
Circulating platelets are highly specialized cells produced by megakaryocytes that participate in hemostatic functions. Despite their critical role, very little is known about the mechanisms regulating their production from megakaryocytes and even less is known about therapeutic approaches to modulate their release. Thrombocytopenia often requires platelet transfusions for patient treatment; however, the supply is limited and is entirely dependent on allogeneic donations. In addition, platelet transfusions expose patients to risks of disease transmission and induce acute reactions and alloimmunization, making the patients resistant to further infusions. Thus, scientific and clinical communities are actively searching for new ways of generating functional platelets ex vivo for clinical use and mechanistic studies.

Since the first description of an in vitro culture for the generation of human platelets and the cloning of human thrombopoietin 20 years ago, significant effort has been spent in developing different systems for successful megakaryocyte differentiation starting from hematopoietic stem cells derived from umbilical cord blood, adult peripheral blood, or bone marrow.[2] All 3 cell sources have been used to provide insights into the basic mechanisms of human platelet production. However, the need for cells to overcome the limits of donor-derived platelet supply has also prompted several groups to develop efficient culture systems from human pluripotent stem cells, which can be divided into human embryonic stem cells and human-induced pluripotent stem cells (hiPSCs). hiPSCs are thought to be an inexhaustible source of megakaryocytes, able to produce human platelets duplicating peripheral blood in many aspects, including ultrastructure, surface antigen expression, and function. Nakamura et al developed an immortalized megakaryocyte progenitor cell line by the sequential introduction of c-MYC and BMI1 genes, followed by the BCL-XL gene into human hiPSC-derived hematopoietic progenitor cells,[3] whereas Moreau et al established forward programmed megakaryocytes by overexpressing 3 transcription factors: GATA1, FLI1, and TAL1. These 3 transcription factors were introduced into human hiPSCs as part of a forward programming strategy to promote megakaryocyte differentiation under feeder-free conditions.[4]

Manufacturing clinically meaningful numbers of platelets is still difficult due to low platelet release from hiPSC-derived megakaryocytes. Recently, Ito et al identified turbulence as a physical regulator of platelet production from hiPSC-megakaryocytes and developed a turbulence-controlled bioreactor to scale up platelet production to a sufficient level for clinical applications.[5] These data demonstrate that future advancement in the field will depend on the evolution of bioengineering techniques for reproducing physiologically relevant conditions to promote platelet formation ex vivo.[6-9]

Despite these important advancements, it is still unknown whether platelet production from megakaryocytes, derived from different progenitor sources, is regulated by alternative mechanisms. In other words, we still do not understand the regulation of platelet production. Until this is understood, it will be difficult to determine exact, safe triggers to promote a constant rate of platelet production. In addition, this uncertainty makes it difficult to determine the safety of the platelet products and the possible costs of the production process scale-up.

In searching for new strategies to produce platelets in a simple and cost-effective way, the group of Matsubara explored the use of adipose tissue–derived stromal cells (ASCs) as a source of megakaryocytes to produce platelets for clinical applications.[10] First, they demonstrated that megakaryocytes could be differentiated from ASCs without the need of any genetic manipulation. Next, they showed that endogenous thrombopoietin supported megakaryocyte differentiation from ASCs. Finally, they proved that the endogenous thrombopoietin was secreted in a transferrin-dependent manner as blockage of the transferrin receptor CD-71 on ASCs caused a decrease of the thrombopoietin levels and megakaryocyte differentiation. The limit of the technique presented was that the ASCs were too heterogeneous to be used as a source of donor-independent megakaryocytes and platelets. Based on these studies, Tozawa et al developed an ASC cell line (ASCL) that was able to proliferate for 2 months without any alteration in the karyotype. In a selected culture medium, ASCL differentiated into megakaryocytes after 8 days, and platelets were released after an additional 4 days. Platelets produced ex vivo resembled peripheral blood platelets and showed the same in vivo kinetics after infusion into irradiated immunodeficient NSG mice. However, these platelets had a tendency to be hyperactive in vitro as demonstrated by higher levels of PAC1 binding, P-selectin surface exposure, and ristocetin-induced



Manufacturing platelets from human adipose–derived mesenchymal stem/stromal cells. Human subcutaneous adipose tissue was used to generate adipose-derived mesenchymal stromal/stem cell line (ASCL) that fully satisfies the criteria for defining mesenchymal stem cells as determined by The International Society for Cellular Therapy. When cultured in Megakaryocyte (MK) Lineage Induction Media, ASCL produces and secretes thrombopoietin (TPO), which binds to c-Mpl, supporting MK differentiation. Once mature, MKs can be cultured within a cell expansion system and generate functional human ASCL-Platelets (PLTs).

and ADP-induced platelet aggregation, although they showed similar levels of fibrinogen binding, collagen-induced platelet aggregation, and lower epinephrine-induced platelet aggregation compared with peripheral blood platelets. Although encouraging, these data raise the question on how to define platelets produced ex vivo that can be considered safe to be transfused from both a morphologic and a functional point of view. Further studies are needed to assess the cause of this increased response to platelet agonists and whether this preactivated state could impact the possibility of safely transfusing them in patients.

Finally, an intriguing point is that only 63% of ASCL differentiated into megakaryocytes. A possible explanation is that there are megakaryocyte-primed ASCLs among the ASCL population, and current studies are focused on understanding the role of mesenchymal markers in driving ASCL differentiation into megakaryocytes. These studies will be instrumental in understanding platelet production efficiency from these megakaryocytes and regulation of megakaryocyte maturation.

The study of Tozawa et al demonstrates a simple and inexpensive method to produce platelets from adipose tissue without the need for genetic manipulation and exogenous growth factors. In conclusion, substantial advances have been made in the field; however, bona fide generation of blood cells and platelets is still an unmet need requiring a definition of functional progenitors, megakaryocytes, and platelets.

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

REFERENCES

1. Tozawa K, Ono-Uruga Y, Yazawa M, et al. Megakaryocytes and platelets from a novel human adipose tissue–derived mesenchymal stem cell line. *Blood*. 2019;133(7):633-643.

2. Di Buduo CA, Wray LS, Tozzi L, et al. Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies. *Blood*. 2015; 125(14):2254-2264.

3. Nakamura S, Takayama N, Hirata S, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell*. 2014;14(4):535-548.

4. Moreau T, Evans AL, Vasquez L, et al. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. *Nat Commun*. 2016;7(1):11208.

5. Ito Y, Nakamura S, Sugimoto N, et al. Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. *Cell*. 2018;174(3):636-648.e18.

6. Di Buduo CA, Soprano PM, Tozzi L, et al. Modular flow chamber for engineering bone marrow architecture and function. *Biomaterials.* 2017;146:60-71.

7. Shepherd JH, Howard D, Waller AK, et al. Structurally graduated collagen scaffolds applied to the ex vivo generation of platelets from human pluripotent stem cell-derived megakaryocytes: Enhancing production and purity. *Biomaterials.* 2018;182:135-144.

8. Thon JN, Mazutis L, Wu S, et al. Platelet bioreactor-on-a-chip. *Blood.* 2014;124(12):1857-1867.

9. Blin A, Le Goff A, Magniez A, et al. Microfluidic model of the platelet-generating organ: beyond bone marrow biomimetics. *Sci Rep.* 2016;6(1):21700.

10. Ono-Uruga Y, Tozawa K, Horiuchi T, et al. Human adipose tissue-derived stromal cells can differentiate into megakaryocytes and platelets by secreting endogenous thrombopoietin. *J Thromb Haemost.* 2016;14(6):1285-1297.

DOI 10.1182/blood-2018-12-889766

CLINICAL TRIALS AND OBSERVATIONS

Comment on Stein et al, page 676

IDH2 inhibition: another piece to the puzzle

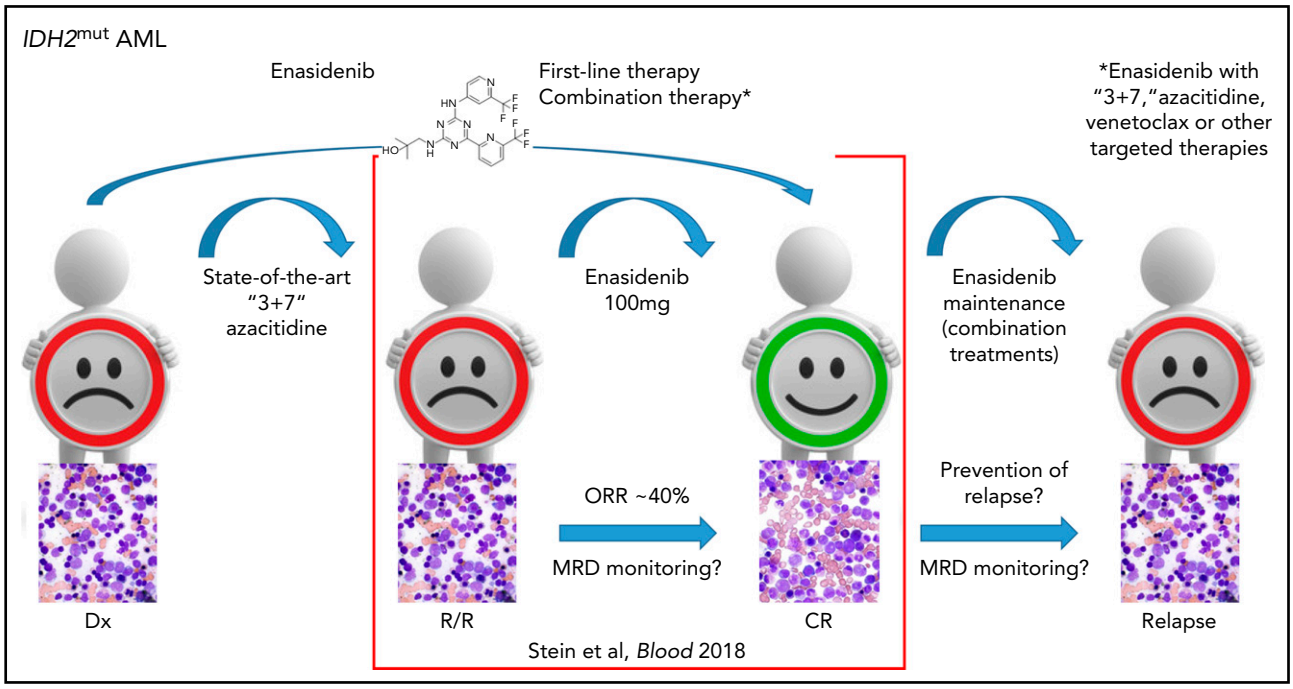
Lars Bullinger | Charité University Medicine

In this issue of *Blood*, Stein et al[1] report the outcomes for all isocitrate dehydrogenase-2 (IDH2) mutant relapsed or refractory acute myeloid leukemia (R/R AML) patients treated with enasidenib in the first phase 1/2 study including data on molecular clearance and molecular relationships to response or resistance.

Following a long dry spell, novel targeted therapeutic options are changing AML management, most notably midostaurin added to standard chemotherapy with anthracycline and cytarabine for *FLT3* mutant AML.[2] Technological advances are improving the molecular characterization of AML. Studies in large well-characterized patient cohorts could provide further evidence that AML with *IDH2* R172 mutation might represent a distinct disease class, based on the mutual exclusivity of *NPM1* mutation and other class-defining lesions.[3]

Mutated *IDH2* is found at position R172 in ~1% to 4% of AML patients. Mutations at R140 are found in ~5% to 15% of AML cases, which are often accompanied by *NPM1* mutations.[4] Both *IDH2* mutation positions represent early events in leukemogenesis and both positions are associated with clonal hematopoiesis in healthy elderly people. *IDH2* mutation incidence increases with age (~5% to 10% in patients <60 years; ~10% to 15% in patients >60 years).[2]

Recently, inhibitors have been developed for mutant cytoplasmic IDH1 and mitochondrial IDH2, providing important new pieces for the leukemia treatment puzzle. The first results for enasidenib, AG-221, a first-in-class orally available inhibitor of mutant IDH2, in R/R AML showed an overall response rate (ORR) of 40% last year.[5] Results from the first clinical study of the inhibitor of mutant IDH1, ivosidenib, AG-120, reported a similar ORR of 41.6% earlier this year.[6]



IDH2 mutant R/R AML responds to enasidenib monotherapy inducing a CR in ~20% of patients. Measurable residual disease (MRD) monitoring will be helpful in predicting treatment response because response may occur as late as after the sixth cycle. Using enasidenib in first-line therapy or in combination with other therapies might further improve outcome and avoid resistance. These strategies will be further evaluated in ongoing studies, including maintenance therapy. Dx, diagnosis; "3+7," standard chemotherapy with anthracycline and cytarabine. Bone marrow morphology images adapted from Stein et al.[5]

2019 133: 623-625
doi:10.1182/blood-2018-12-889766

Megakaryocytes from fat: a new recipe for platelets

Alessandra Balduini

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.