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## G-CSF in stem cell mobilization: new insights, new questions

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In this article, Bernitz and colleagues (1) utilize a lineage tracing mouse model to show that G-CSF selectively mobilizes quiescent non-dividing hematopoietic stem cells (HSC) from bone marrow (BM) to peripheral blood (PB) and that G-CSF administration does not induce HSC cycling/proliferation. Mobilized non-dividing HSC can return to BM, again without dividing. Moreover, the non-dividing HSC represent the true long-term repopulating cells, and those HSC left in BM after mobilization, while expressing phenotypic markers of HSC, do not in fact have repopulating capacity. (Summarized in Figure 1)

G-CSF mobilized HSC from peripheral blood (PBSC) is the most widely used source of HSC for clinical transplantation. Recently though, some questions have been raised as to whether this shift is clinically justified. PBSC mobilization, particularly with G-CSF, yields greater donor cell CD34<sub>+</sub> numbers with faster engraftment and reduced incidence of graft failure; however, overall patient survival is not different from BM transplantation (2,3). This can be explained at least in part by increased graft versus host disease (GVHD) with PBSC transplants (2,3), but mobilized HSC may also have reduced function compared to those from the BM on a cell-to-cell basis (4).

Because G-CSF can induce proliferation within the HSC compartment (5–7) and proliferative history is inversely correlated to HSC function (8–10), Bernitz *et al.* hypothesized that a defect in mobilized HSC could result from G-CSF induced cell division (1). A division-tracking mouse model was employed wherein a GFP-tagged histone H2B (H2B-GFP) is incorporated into nucleosomes when expressed under the control of a tetracycline-response element, but only when the activator is available. The activator is itself controlled by a human CD34 promoter in the HSC, and can be sequestered by doxycycline treatment to halt H2B-GFP expression. After sustained doxycycline treatment, GFP intensity becomes reduced with each cell division while quiescent HSC retain high signal intensity.

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These label-retaining HSC (LTR-HSC) have been shown previously to contain all of the long-term repopulating HSC (LTR-HSC) potential in the BM (9).

Using this model, the authors provide several interesting findings regarding G-CSF mobilization. Significantly, the driving scientific question of this work received an unexpected and intriguing answer: G-CSF did not induce proliferation of LTR-HSC as previously thought. After a 6-week doxycycline chase, a G-CSF regimen, and a subsequent 8-week re-equilibration (with continuous doxycycline) the number of LTR-HSC found in the BM was the same as that of mice that did not receive G-CSF. This indicated that the LTR-HSC did not divide in response to G-CSF. Previous reports that G-CSF induces HSC cycling were likely detecting proliferation in the CD41+ portion of the phenotypic HSC pool [defined as Lin<sup>-</sup>, Sca1<sup>+</sup>, c-kit<sup>+</sup> (LSK), CD150<sup>+</sup>, CD48<sup>-</sup>], which was found not to contain the LTR-HSC according to label-retention in this model.

Another surprising finding from this work is that, following a G-CSF regimen, mouse BM was almost 90% depleted of LTR-HSC and the remaining BM cells were found to have very little regenerative potential in transplantation assays. This raises a clinical question; Are PBSC donors for allogeneic transplant compromised with respect to their repopulating potential following PBSC donation? In clinical PBSC collection one would expect almost all HSC in the periphery to be removed after apheresis of 5 blood volumes. If the current mouse model is representative, this would leave the donor with severely depleted and defective BM HSC repopulation capacity. However, it could be that the non-dividing HSC that localize to the spleen for several weeks, as seen in this model, can escape collection and are sufficient to later repopulate the donor BM stem cell pool—a question which could warrant further exploration. In this regard, studies performed in splenectomized mice might be informative.

Short-term side effects as well as hematologic changes after G-CSF application are generally well tolerated and do not pose serious risks for most healthy donors. While serious acute toxicities have been reported, they are rare. Based on limited long-term data from healthy donors who received filgrastim, no long-term risks for development of leukemia, lymphoma or other blood diseases have been found (11,12). The only statistically increased cancer incidence was for melanoma, the significance of which is unclear. Reports of donor follow-up of blood cell counts, mostly within 5 years, find a sustained decrease from baseline in certain PB counts—particularly neutrophils—throughout the follow-up period, and this is especially pronounced in ‘poor mobilizers’ (12). This data perhaps hints at impaired hematopoiesis following PBSC donation, but even donors with normalized blood counts could harbor compromised HSC that might only be revealed through a stress event such as myelotoxic therapy, severe infection, or aging. The results of Bernitz and colleagues may suggest a need for longer-term donor follow-up studies to fully assess risks that may not be currently understood.

When considering surface area conversion of G-CSF dose from man to mouse (13) the model used by the Moore laboratory utilized a considerably higher dose than the 10 µg/kg/day typically used in humans for clinical mobilization. The mouse equivalent would be 125 µg/kg/day (14), while the current study injected 312.5 µg/kg/day for mobilization

(reported as 6.25 $\mu$ g/day; assuming an approximate mouse weight of 20 g). Thus, at doses of G-CSF used clinically, severe depletion of donor LTR-HSC may not be a concern. Further support for this comes from clinical studies in which grafts derived from the BM upon G-CSF mobilization, or “G-CSF primed” BM, can engraft with similar outcomes to mobilized PB or untreated BM (15,16). Thus, residual donor BM may not be quite as functionally compromised after clinical G-CSF mobilization as that of this model.

It is widely acknowledged that G-CSF mobilized PBSC restore white blood cell counts, particularly absolute neutrophil counts, faster than BM cells, likely because there are more LTR-HSC in the G-CSF mobilized graft. The current paper brings out some interesting points in this regard. First, quiescent HSC with high regenerative potential were preferentially mobilized. This helps explain PBSC graft potency and likely contributes significantly to PBSC transplant success. At the same time, the authors have confirmed an earlier observation by our group (4) of a competitive defect in G-CSF mobilized PB compared to unmanipulated BM, and have further elucidated in elegant fashion that this defect is independent of HSC divisional history. In our hands, comparison of G-CSF mobilized mouse PB MNC to unmanipulated BM over a 4:1 to 1:1 dilution range indicates that the G-CSF mobilized graft contains approximately one third the competitive repopulating unit (CRU) potential of BM. Here, the authors performed a direct competitive transplant with equal numbers of LSK CD48<sup>-</sup> cells purified from each source, normalized for proliferative history by label retention, and found that BM cells still outcompeted PB cells at a ratio of 4:1 in primary and secondary transplants. Thus, it appears that mobilized HSC may indeed be functionally less potent, and due to something other than proliferation. In any case, it is likely that the increased engraftment of G-CSF mobilized PB seen clinically is highly dependent upon the increased numbers of LTR-HSC transplanted.

The reason behind the decreased potency of mobilized HSC is an intriguing question that remains to be explained. A good start would be to determine whether other mobilizing agents such as cyclophosphamide, GRO-beta, or AMD3100, which have been shown to work through different mechanisms (4,17), would also yield HSC with reduced function. If this phenomenon is not specific to G-CSF, then it may be a function of the change in environment. Cells of the BM niche are intricately involved in the preservation of stem cell properties (18) and exposure to increased oxygen tension is known to negatively impact HSC when removed from the hypoxic BM environment (19,20). Ultimately, if the precise mobilization-induced deficiency can be elucidated and corrected by additional treatment or altered protocols, this could potentially optimize the PBSC transplant process to achieve higher levels of success for patients and donors alike.

Furthermore, since we see here that HSC can have differential potency despite an identical proliferation history, the initial observation of this paper could be taken a step further to determine whether this G-CSF-induced defect is long-term or short-term; is this effect permanent or reversible? Recall that after the doxycycline chase, G-CSF treatment or control, and re-equilibration, equivalent numbers of LR-HSC were found in the BM. The LTR-HSC did not divide, but are they just as functional? It would be interesting to take the BM cells at this point and compare repopulation efficiency. The answer to this question

could have bearing on both transplant donors and recipients regarding long-term effects of G-CSF on LTR-HSC.

Overall, is G-CSF positive or negative for stem cell function? We have seen here that G-CSF does not induce proliferation of the all-important LTR-HSC, contributing to their ultimate exhaustion as previously thought. However, G-CSF may leave donor BM more depleted of LTR-HSC than previously thought, potentially calling for longer-term donor follow-up studies. In addition, G-CSF mobilized HSC have lower repopulating potential per cell than untreated BM HSC, but can be more easily collected and in greater numbers. Ultimately, patient outcome is paramount and there are currently no significant survival differences between the two methods of HSC collection (2,3). The work in the Moore laboratory has provided important new insights towards understanding and improving the possibilities for HSC transplantation.

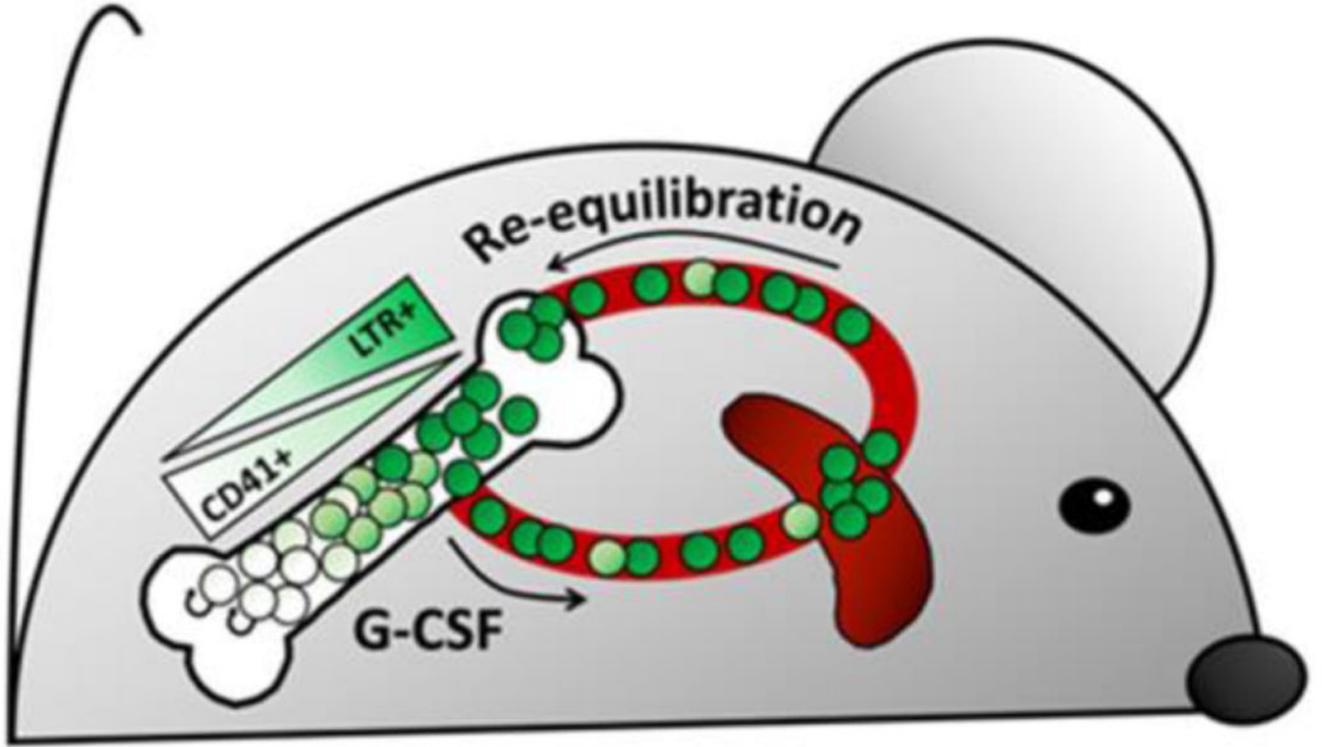
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**Figure 1.** Phenotypic HSC in the BM and their response to G-CSF treatment as observed by Bernitz *et al.* (1). Quiescent HSC retain the H2B-GFP label (green), which becomes diluted in dividing cells. LT-HSC contain all of the LTR potential in the BM, are mostly CD41–, and are preferentially mobilized by G-CSF. The remaining BM HSC population is enriched for CD41+, non-quiescent cells with poor repopulating potential, and is induced to proliferate by G-CSF. Some mobilized LT-HSC reside in the spleen for several weeks, and eventually return to the BM without dividing. LT-HSC, label retaining HSCs; LTR, long-term repopulating.