applications in the management of AA patients. Indeed, deep whole exome sequencing,[15] CyTOF[14] and deep TCR analysis[2] all help to better describe the pathogenic events underlying bone marrow failure syndromes. Even if none of them translates into immediate therapeutic decisions, they are all useful to confirm the diagnosis, to determine the prognosis and possibly to monitor the clinical course of AA patients. Indeed, this latter application may be useful for early identification of refractory or relapsing patients, paving the way for pre-emptive therapeutic interventions. Moreover, the deep dissection at the clonal and at the functional levels of the immune T-cell compartment (e.g. combining CyTOF and TCR analysis) may also answer some open questions in the field. For example, the differential depletion of some specific T-cell subsets might explain the different outcome seen with different ATG preparations.[3] These novel technologies may help identify the specific T-cell subsets which are crucial to the pathophysiology of AA (and possibly differentially depleted by distinct ATG brands), possibly driving the development of future targeted therapies.

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Hematopoietic stem cell mobilization with plerixafor in sickle cell disease

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After more than a half-century since the molecular basis for sickle cell disease (SCD) was described by Linus Pauling and colleagues, we now possess the molecular tools to contemplate a one-time cure through genetic modification of autologous hematopoietic stem cells (HSC). For these promising gene transfer and gene editing strategies to become a reality, a sufficient number of HSC of high purity must be obtained. Filgrastim, or granulocyte colony-stimulating factor, mobilization and apheresis is the standard method for HSC collection in healthy adult donors, yet this approach is associated with high rates of adverse events requiring hospitalization in SCD, including vaso-occlusive crises, multi-organ failure, and even death, prompting our call for a moratorium on its use for HSC mobilization in SCD.[1] Thus, bone marrow harvesting is the default approach, with evidence supporting its utility in both animal models and *in vitro* studies utilizing patients' material.[2-4] However, bone marrow harvest-

ing employed in an ongoing HSC gene therapy trial was recently recognized to result in suboptimal yields of high purity HSC at the end of collection and processing, along with substantial pain after each harvest, and most subjects required two or three harvests to yield sufficient cell doses for manufacturing.[5,6]

In this issue of the Journal, two groups of investigators report their results using a third approach to HSC collection in SCD through mobilization with an inhibitor of the CXCR4 chemokine receptor, plerixafor. Boulad *et al.* performed a dose escalation study of plerixafor among a total of 15 SCD patients at steady state.[7] Ten of the patients were receiving concomitant treatment with hydroxyurea. Only a minority of patients in each cohort achieved the target of ≥30 $CD34^+$ cells/μL at 12 h after the plerixafor injection: three out six at a dose of 80 μg/kg, one out of three at a dose of 160 μg/kg, and two out of six at a dose of 240 μg/kg. Two patients (15%) experienced a vaso-occlusive crisis during the study period – one each at 80 and 240 μg/kg. None of the patients underwent leukapheresis, thus attribution of these adverse events could be narrowed to plerixafor. On the other hand, Lagresle-Peyrou *et al.* reported the outcomes of three patients who received plerixafor at a dose of 240 μg/kg.[8] All three patients received at least 2 months of red cell exchange transfusion to target a sickle hemoglobin (HbS) near 30% while hydroxyurea was discontinued. The peak $CD34^+$ cell count reached >75 cells/μL at as early as 3 h after the injection. All three patients also underwent leukapheresis of 15 to 21 L, with a resulting total $CD34^+$ cell yield of 4.5 to 5.8 x 10^6 cells/kg and a purity of 80% to 95%. No pain, vaso-occlusive crises, or sickle-related events were observed in these three patients.

While the number of patients is relatively small in both studies, important lessons relevant to autologous HSC mobilization and collection in SCD with plerixafor can be gleaned. The first lesson regards preparation of the patients. Specifically, stopping hydroxyurea and utilizing red cell transfusions, simple or exchange, to target a HbS of 30% were likely key factors in the successful mobilization of the series reported by Lagresle-Peyrou *et al.* Conversely, the absence of these measures in the study by Boulad *et al.* may explain why the majority of their patients failed to reach the target $CD34^+$ concentration. This is consistent with prior work demonstrating a lower $CD34^+$ cell content in the marrow of SCD patients on hydroxyurea when compared to those not on the drug.[3] Discontinuation of hydroxyurea combined with scheduled red cell transfusion to keep the HbS near 30% may also have improved purity, which was 80% to 95% in the study by Lagresle-Peyrou *et al.*, while helping to minimize the risk of sickle cell-related adverse events while hydroxyurea treatment was interrupted. Secondly, leukocyte and neutrophil counts increased 2- to 3-fold just hours after a single injection of plerixafor, even at the lowest dose of 80 μg/kg tested. Although increases of a similar magnitude also occurred with filgrastim, the adverse events seen with filgrastim may have been related to the prolonged duration of 5 to 6 days from filgrastim that led to the high rates of adverse events in the earlier reports. We will need more patients to ascertain the contribution of leukocytosis alone and/or the duration of leukocytosis in developing sickle-related complications. Thirdly, only three patients underwent leukapheresis and though adverse events appear acceptable, expanded accrual could capture additional side effects. Furthermore, if patients with SCD do not meet the goal and need additional mobilization and collection, there could be cumulative side effects. Finally, the peak of mobilization of $CD34^+$ cells appeared to be much earlier, at 3-6 hours. This observation is distinct from that in healthy donors, in whom the peak is observed at 6-12 hours.[9] Perhaps the chronically hyperproliferative marrow in SCD partly explains this early release of HSC; there could be other factors at play. Regardless, this observation suggests that for optimal collection, apheresis should be started within 4-6 hours of dosing.

As clinical applications of gene transfer and gene editing strategies are being implemented in SCD, obtaining adequate numbers of HSC safely from patients could be the 'bottleneck', preventing broad dissemination of these exciting approaches. The early results provide optimism that mobilization with plerixafor could be a safer and more efficacious alternative for HSC collection to either filgrastim mobilization or bone marrow harvesting, and provide general confidence for the further development of these promising approaches to a one-time cure for SCD.

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