Successful Plerixafor-Mediated Mobilization, Apheresis, and Lentiviral Vector Transduction of Hematopoietic Stem Cells in Patients with Severe Sickle Cell Disease

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BACKGROUND

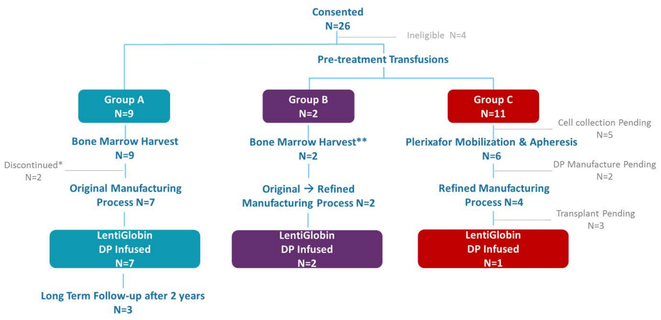
- Patients with severe sickle cell disease (SCD) may benefit from β-globin (*HBB*) gene transfer into autologous hematopoietic stem cells (HSC), which could enable production of adult Hb (HbA) or an anti-sickling HbA variant adequate to prevent RBC aggregation and correct hemolytic anemia.
- Successful β-globin gene transfer requires vector-mediated transduction of a patient's own HSCs. HSC collection for transplantation is generally accomplished by mobilizing HSCs into peripheral blood (PB) and collecting them via apheresis. However, the primary mobilization agent, G-CSF (filgrastim), can cause life-threatening complications and is contraindicated in SCD.
  - Both fatal and non-fatal extremely severe VOCs (Adler 2001, Abboud 1998, Wei and Grigg 2001) have been reported in people with S/S, S/C, and S/β+ sickle cell disease after G-CSF.
- Steady-state bone marrow (BM) is the default HSC source in patients with SCD. However, it has significant limitations.
  - BM harvest (BMH) is an invasive procedure usually performed under general anesthesia.
  - CD34+ cell yields from BMH are typically lower than those after mobilization and apheresis. Multiple rounds may be required to obtain adequate cell doses for autologous gene therapy (GT) protocols.
  - Normal human BM contains up to 30% CD34+CD19+ pro-B cells and other lineage-committed cell types ($CD34^{dim}$) that will not contribute to improved long-term erythropoiesis via gene therapy
  - $CD34^{dim}$ cells mobilize at low rates, so the phenotypic distribution of CD34+ cells collected via mobilization and apheresis are expected to include a lower proportion of $CD34^{dim}$ cells than cells collected by direct BMH.
- Plerixafor, a CXCR4 receptor antagonist, may accomplish HSC mobilization in patients with SCD without the degree of neutrophil or endothelial activation observed with G-CSF that elicit severe vaso-occlusion.

METHODS

- HGB-206 is a phase 1 study of LentiGlobin gene therapy, which contains autologous HSCs transduced *ex vivo* with the BB305 (betibeglogene darolentivec) lentiviral vector, in patients aged 18 years or older with severe SCD (history of recurrent vaso-occlusive crisis [VOC], acute chest syndrome, stroke, or tricuspid regurgitant jet velocity of >2.5 m/s).
- The study protocol was modified to allow exploratory collection of HSCs by plerixafor-mediated mobilization and apheresis for patients in Group B. A portion of the cells collected were reserved as backup in the case of graft failure, and the remainder transduced with BB305 for research purposes only. Patients in this group then underwent BMH to collect additional cells to be used to manufacture LentiGlobin drug product (DP).
  - Patients in group B who underwent mobilization received 240 µg/kg plerixafor followed 4 to 6 hours later by apheresis, processing ~3 total blood volumes.
- After safety and feasibility of this cell collection method was established, the research-grade DP was analyzed to determine whether to move forward with mobilization and apheresis for manufacture of clinical-grade DP.
- Based on initial results, a third study group, Group C, was initiated, with HSCs collected only via plerixafor mobilization and apheresis. Treatment of Group C using LentiGlobin DP manufactured from the mobilized PB is underway.
- Mass cytometry (CyTOF) was used to analyze *ex vivo* cultured CD34+ cells with over 35 cell surface markers.

RESULTS

Figure 1. HGB 206: Study Disposition



** 1 due to insufficient cell collection; 1 withdrew consent. **One patient also received a single mobilization cycle to collect cells for back-up.* Data as of Nov 30, 2017

Table 1. Patient baseline characteristics (patients with cells collected)

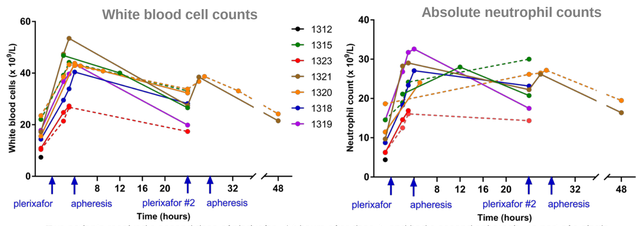
Parameter	Group A	Group B	Group C
Number	9	2	6
Age at enrollment, years *median (min-max)*	26 (18-43)	24.5 (22-27)	24.5 (19-35)
Regular pRBC transfusions for ≥2 months prior to harvest†	1 (11%)	2 (100%)	6 (100%)
Bone marrow harvests, n *median (min-max)*	2 (1-4)	2.5 (2-3)	n/a
Mobilization cycles, n *median (min-max)*	n/a	1*	1.5 (1-2)

All patients enrolled to date have β^s/β^s genotypes. †1 patient in Group A and 2 patients in Group C were receiving chronic pRBC transfusions prior to study enrollment. *For back-up only, in 1 patient only. n/a=not applicable

Safety with bone marrow harvest vs plerixafor mobilization and apheresis

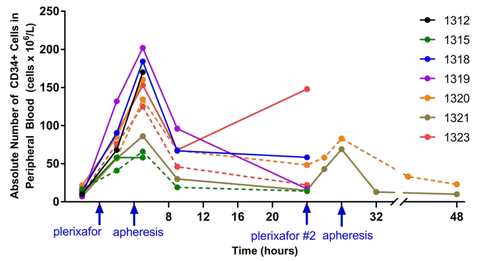
- In 26 BMHs in 9 patients, 17 ≥ grade 3 adverse events (AEs) were reported in 5 patients
  - 10 grade 3 AEs of procedural pain in 5 patients, including 1 SAE
  - 3 grade 3 AEs of anemia in 2 patients
  - 3 grade 3 SAEs of SCD-related pain crisis in 2 patients
  - 1 event of decreased lymphocyte count
- In 7 patients who underwent mobilization and apheresis, 5 ≥ grade 3 AEs were reported in 3 patients
  - 2 non-serious grade 3 AEs in 1 patient each: hypomagnesemia and non-cardiac chest pain
  - 3 grade 3 SAEs of SCD-related pain crisis in 3 patients
    - Pain crises were non-severe and were consistent with patients' histories of vaso-occlusive events. The affected patients were hospitalized, or hospitalization was prolonged, for standard management. All 3 patients recovered without sequelae.

Figure 2. WBC counts and ANC after plerixafor administration



Two patients received a second dose of plerixafor ~24 hours after dose 1, and had a second apheresis, as part of a single mobilization cycle. Three patients underwent a second mobilization cycle, represented by dashed lines.

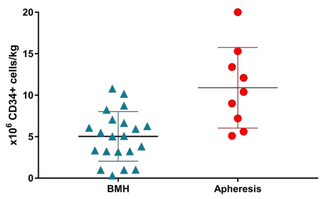
Figure 3. CD34+ cell counts after plerixafor dosing, and before and after apheresis



Two patients received a second dose of plerixafor ~24 hours after dose 1, and had a second apheresis, as part of a single mobilization cycle.

Three patients underwent a second mobilization cycle, represented by dashed lines.

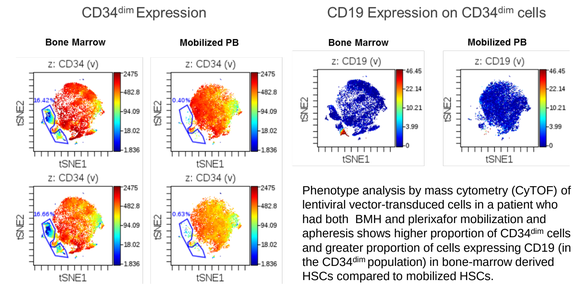
Figure 4. Total CD34 cells collected per collection cycle



A median of 5.0 (0.3 – 10.8) x 10^6 CD34+ cells/kg were collected per BMH. A median of 10.4 (5.1 – 20.0) x 10^6 CD34+ cells/kg were collected per apheresis cycle.

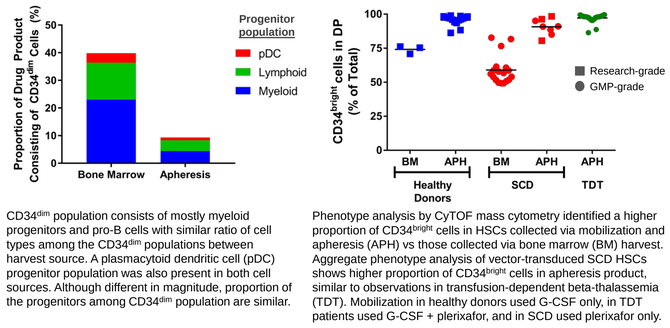
Sufficient cells for both DP manufacture and back-up were collected in 1 apheresis cycle in 3/6 patients. A median of 2 (1 – 4) BMH were required to collect adequate cells for both DP manufacture and back-up.

Figure 5. Cell phenotyping



Phenotype analysis by mass cytometry (CyTOF) of lentiviral vector-transduced cells in a patient who had both BMH and plerixafor mobilization and apheresis shows higher proportion of $CD34^{dim}$ cells and greater proportion of cells expressing CD19 (in the $CD34^{dim}$ population) in bone-marrow derived HSCs compared to mobilized HSCs.

Figure 6. $CD34^{dim}$ and $CD34^{bright}$ populations



$CD34^{dim}$ population consists of mostly myeloid progenitors and pro-B cells with similar ratio of cell types among the $CD34^{dim}$ populations between harvest source. A plasmacytoid dendritic cell (pDC) progenitor population was also present in both cell sources. Although different in magnitude, proportion of the progenitors among $CD34^{dim}$ population are similar.

Phenotype analysis by CyTOF mass cytometry identified a higher proportion of $CD34^{bright}$ cells in HSCs collected via mobilization and apheresis (APH) vs those collected via bone marrow (BM) harvest. Aggregate phenotype analysis of vector-transduced SCD HSCs shows higher proportion of $CD34^{bright}$ cells in apheresis product, similar to observations in transfusion-dependent beta-thalassemia (TDT). Mobilization in healthy donors used G-CSF only, in TDT patients used G-CSF + plerixafor, and in SCD used plerixafor only.

Figure 7. Peripheral blood stem cells transduce comparably to bone marrow-derived cells, while enabling higher cell doses

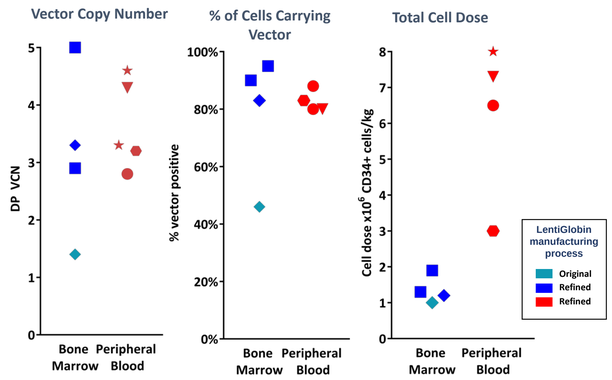
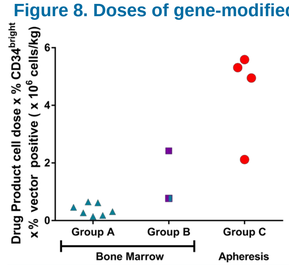


Figure 8. Doses of gene-modified early progenitor cells



We estimated the dose of cells with the phenotype considered likely to contribute most to long-term gene-modified hemoglobin production in patients for whom LentiGlobin has been manufactured. For each patient treated, we calculated the proportion of cells in the drug product that were CD34 bright cells, and then multiplied by the proportion of cells in the overall DP lot that were vector-positive. This may help inform interpretation of future clinical results in patients treated with gene therapy.

SUMMARY

- It is feasible to use single-agent plerixafor mobilization and apheresis to collect adequate volumes of CD34+ cells from patients with severe SCD to enable treatment with gene therapy
  - Total median CD34+ cells collected in a single mobilization and apheresis cycle were more than double those collected in a single bone marrow harvest
- Safety in patients with severe SCD appears acceptable with plerixafor doses of 240 µg/kg
  - Moderate and temporary increases in WBC were observed
  - Fewer Grade ≥3 or higher AEs per patient were reported with mobilization and apheresis than with BMH
  - Three of 7 patients (43%) experienced grade 3 VOCs shortly after mobilization or apheresis, however severity was milder than has been reported with G-CSF and patients recovered with no sequelae
- PB-derived CD34+ cells may be a preferred substrate for β-globin gene addition therapy
  - PB-derived CD34+ cells may contain lower proportions of lineage-committed CD34+ cells than BM-derived cells from patients with SCD
  - Cells collected by BMH and PB mobilization/apheresis appear to have an equivalent transduction efficiency

Dr. Tisdale has no conflicts of interest to report.

ASH 2017 Abstract 990