BACKGROUND

Transplant is the only proven curative therapy for many diseases, but up to half of the 23,000 patients requiring an allogeneic transplant will not find a well matched donor with sufficient cell dose. Low numbers of CD34+ cells results in delayed neutrophil recovery and a risk of graft failure. MGTA-456 is a cell therapy that consists of CD34+ cells expanded (a median of 327-fold from a single cord blood unit well matched to the patient) in a 15-day culture in the presence of an aryl hydrocarbon receptor antagonist (AHRa) and the CD34 depleted fraction obtained from the same CB unit. To date, 41 patients with hematological malignancies (n=36) and non-malignant diseases (n=5) have received MGTA-456 with a median follow-up of 2.5 years (range 0.1 to 5 years) and 75 days (80 to 203 days), respectively. All patients engrafted at a significantly faster rate as compared to similarly treated historical controls (p<0.01). The aim of the current study was to fully characterize the expanded CD34+ cell fraction of MGTA-456 phenotypically and functionally and identify the cell population that correlates with time to neutrophil recovery, to establish product potency. We found that the expanded CD34+CD90+ population of MGTA-456 were the cells responsible for engraftment in NOD-scid IL2Rgammanull (NSG) mice. We hypothesized that the dose of CD34+CD90+ cells/kg would have the strongest correlation with time to neutrophil recovery.

METHODS

The manufacturing process involves selection of CD34+ cells which contain the stem cells, from an umbilical cord blood unit matched to the patient to create a CD34-enriched cell fraction and a CD34-depleted cell fraction. The CD34-depleted fraction is cryopreserved following selection and later thawed and infused at the time of transplant. The CD34-enriched cells are expanded ex vivo for 15 days to produce MGTA-456 **(Figure 1)**. The MGTA-456 manufacturing process results in a median increase in the number of CD34+ cells of 327-fold (range: 67-fold to 848-fold) across all studies **(Figure 2)**. After culture, the cells are washed, characterized, and re-suspended in human serum albumin solution for infusion. Neutrophil recovery was determined as the number of days after transplant until the patient achieves an absolute neutrophil count (ANC) >0.5 x 10^9/L for 3 consecutive days.

To fully characterize the expanded CD34+ cell fraction of MGTA-456 phenotypically and functionally, the CD34 expanded product was evaluated for the cell surface markers CD34, CD90, CD133, CD41, CD71, CD235a, CD3, CD4, CD8, CD14, CD15, CD16, CD11b, CD33, CD19, CD56, and CD10 **(Figure 3)**, as well as colony-forming unit (CFU) capacity and engraftment potential in NOD-scid IL2Rgammanull (NSG) mice in a subset of products **(Figure 4)**. The dose of each cell population within the expanded CD34+ product were correlated with time to neutrophil recovery **(Figure 5)**.

Figure 1: The treatment schematic for the manufacturing of MGTA-456.

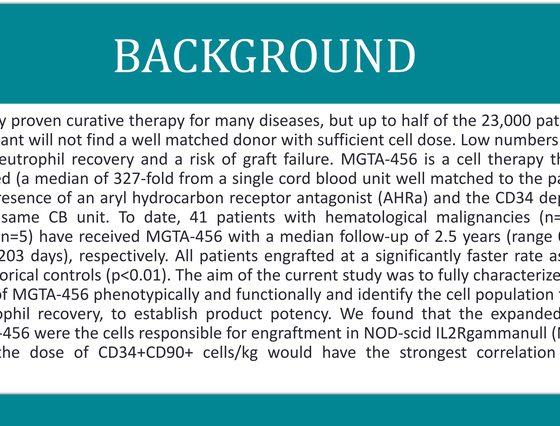
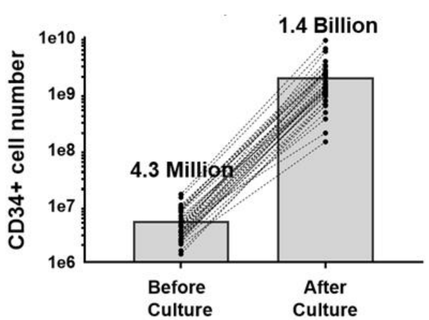


Figure 2: Expansion characteristics of MGTA-456. A median of 4.3 million CD34+ cells were seeded into expansion culture. 1.4 billion CD34+ cells were manufactured following a 15 day culture. The median CD34+ cell expansion was 327-fold (67-848) and the median infused CD34+ cell dose was 17.5 x 10^6/kg (1-48)



RESULTS

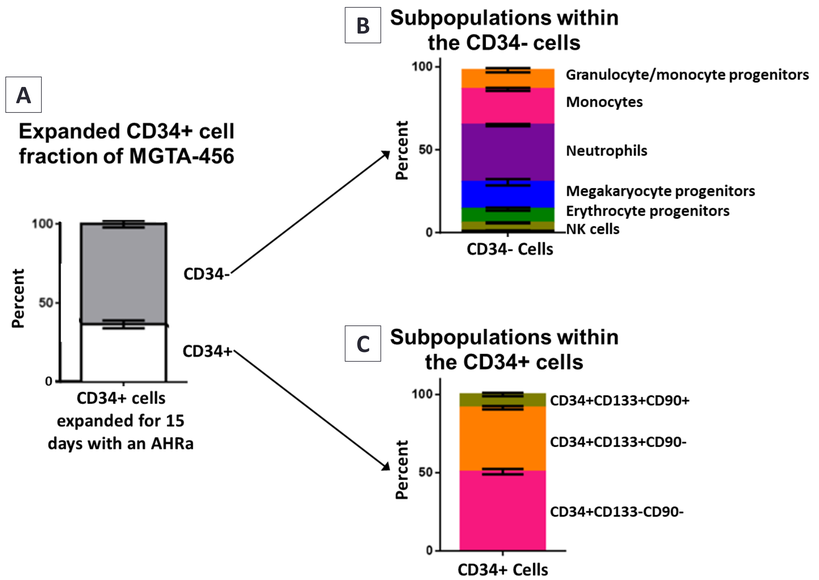


Figure 3: Outline of the cell populations present in the expanded fraction of MGTA-456. (A) The expanded fraction of MGTA-456 can be divided into two populations CD34+ (left, white bar) and CD34- (left, gray bar). **(B)** The CD34+ cells can be further subdivided into CD34+ (CD34+CD133-CD90-), CD133+ (CD34+CD133+CD90-) and CD90+ cells (CD34+CD133+CD90+, right bottom stacked bar chart). **(C)** The CD34- cells contain the cell populations outlined in the right top stacked bar chart.

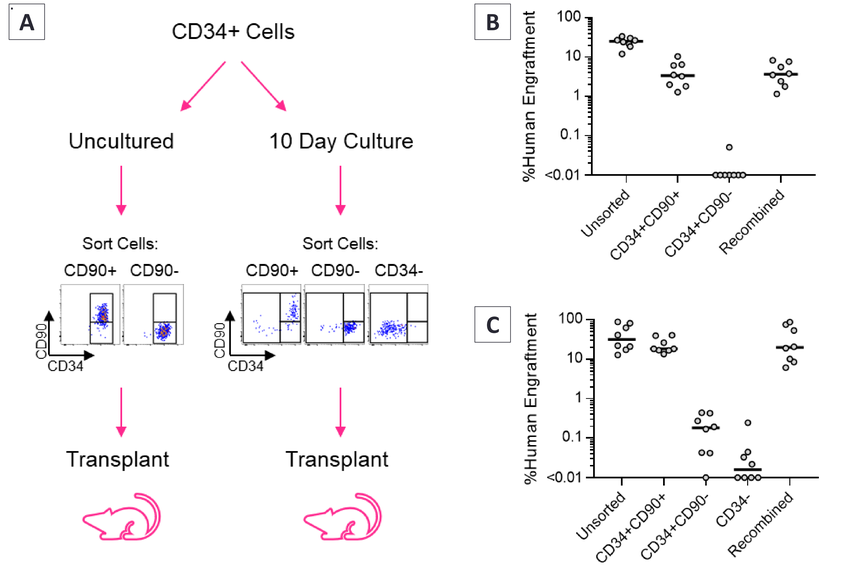


Figure 4: Cord Blood CD34+CD90+ hematopoietic stem cells contains all engraftment activity in NSG mice. (A) Experimental design schema. **(B)** Unmanipulated CD34+ cells were sorted and the indicated cell populations were injected into sub-lethally irradiated NSG mice: 20,000 unsorted cells; 315 CD34+CD90+ cells; 4,000 CD34+CD90- cells; and 315 CD34+CD90+ cells and 4,000 CD34+CD90- cells recombined. Human cell engraftment in NSG mice 13 weeks after transplant is shown. **(C)** CD34+ cells were cultured with cytokines and AHRa for 10 days and the indicated cell populations were sorted and the following populations were injected into NSG mice: 2 x 10^6 unsorted cells; 29,000 CD34+CD90+ cells; 265,000 CD34+CD90- cells; 1.2 x 10^6 CD34-CD90- cells; and 29,000 CD34+CD90+ cells, 265,000 CD34+CD90- cells and 1.2 x 10^6 CD34-CD90- cells recombined. Human cell engraftment in NSG mice 13 weeks after transplant is shown.

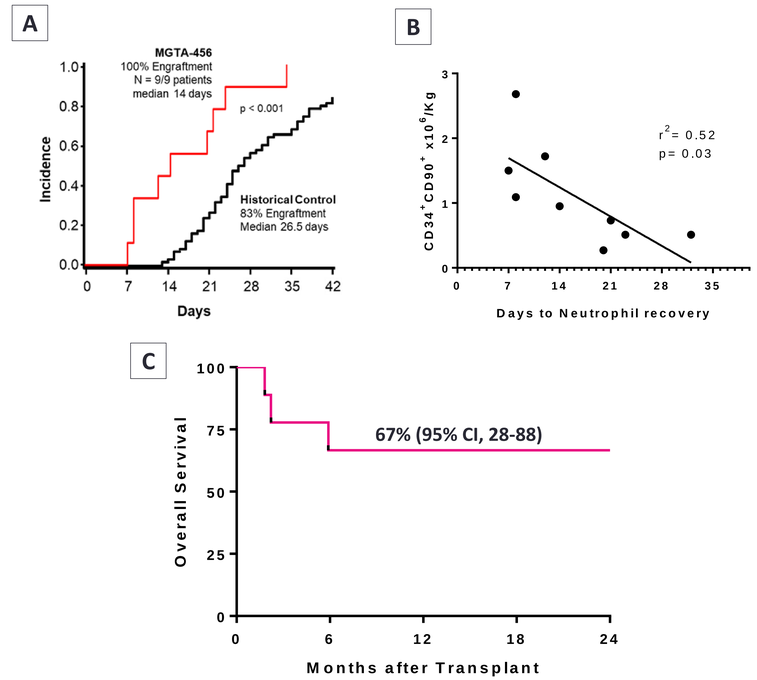


Figure 5: The dose of CD34+CD90+ cells/kg had the strongest correlation with time to neutrophil recovery. MGTA-456 was evaluated as a stand-alone graft after myeloablative conditioning (Cy 120 mg/kg, Flu 75 mg/m2, TBI 1320 cGy) in 9 patients with hematologic malignancy [AML CR1 (4) and CR2 (2), ALL CR1 (1) and CR2 (2), MDS (1), and NHL (1)]. All patients engrafted with median time to neutrophil recovery of 14 days. The effect of CD34+CD90+ cell dose on speed of recovery in the 9 patients was evaluated, as all human cell engraftment activity in NSG mice is found in the CD34+CD90+ subpopulation. (**A**) Time to engraftment, as defined by neutrophil recovery, for patients transplanted with MGTA-456 after myeloablative conditioning. (**B**) The CD34+CD90+ cell dose correlated with speed of neutrophil recovery (r^2 0.52, p=0.03). (**C**) Probability of survival of the 9 patients treated with MGTA-456 as a stand alone graft.

CONCLUSIONS

- **The expanded CD34+ cell fraction of MGTA-456 contains large doses of CD34+CD90+ HSC and progenitors**
- **The expanded CD34+CD90+ population of MGTA-456 are responsible for engraftment in NSG mice**
- **Consistent with the NSG results, the dose of CD34+CD90+ cells/kg had the strongest correlation with time to neutrophil recovery in patients**
- **100% engraftment at a median time of 14 days with 67% overall survival at 2 years was observed with MGTA-456 as a stand alone graft**
- **Based on these impressive results, an investigator-initiated study of MGTA-456 in patients with hematologic malignancies is on track to begin soon – this study will effectively triple the number of patients with blood cancers treated with MGTA-456 utilizing myeloablative conditioning**