months follow-up. These interim data suggest that gene therapy with LentiGlobin DP is a promising potential treatment for severe SCD and transfusion-dependent βT.

280. Lentiviral-Mediated Gene Therapy Restores B Cell Homeostasis and Tolerance in Wiskott-Aldrich Syndrome Patients

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Wiskott-Aldrich Syndrome (WAS) is a severe X-linked primary immunodeficiency characterized by micro-thrombocytopenia, eczema and increased risk of infections, autoimmunity and tumors. Allogeneic hematopoietic stem cell (HSC) transplantation is a recognized curative treatment for WAS, but when a matched donor is not available, administration of *WAS* gene-corrected autologous HSCs represents a valid alternative therapeutic approach. Since alterations of WAS protein (WASp)-deficient B lymphocytes contribute to immunodeficiency and autoimmunity in WAS, we followed the B cell reconstitution in 4 WAS patients treated by lentiviral vector-gene therapy (GT) after a reduced-intensity conditioning regimen combined with anti-CD20 administration. We analyzed the B cell subset distribution in the bone marrow and peripheral blood by flow cytometry and the autoantibody profile by a high-throughput autoantigen microarray platform before and after GT. Lentiviral vector-transduced progenitor cells were able to repopulate the B cell compartment with a normal distribution of transitional, naïve and memory B cells. The reduction in the proportion of autoimmune-associated $CD21^{low}$ B cells and in the plasma levels of B cell-activating factor was associated with the decreased autoantibody production in WAS patients after GT. Then, we evaluated the functionality of B cell tolerance checkpoints by testing the reactivity of recombinant antibodies isolated from single B cells. Before GT, we found a decreased frequency of autoreactive new emigrant/transitional B cells in WAS patients, suggesting a hyperfunctional central B cell checkpoint in the absence of WASp. In contrast, high frequency of polyreactive and Hep2 reactive clones were found in mature naïve B cells of WAS patients, indicating a defective peripheral B cell checkpoint. Both central and peripheral B cell tolerance checkpoints were restored after GT, further supporting the qualitative efficacy of this treatment. In conclusion, WASp plays an important role in the regulation of B cell homeostasis and in the establishment of B cell tolerance in humans and lentiviral-mediated GT is able to ameliorate the functionality of B cell compartment contributing to the clinical and immunological improvement in WAS patients.

281. Enforced Expression of a Mutant *HMGA2* Gene Leads to Competitive Expansion and High Level Marking of Long-Term Hematopoietic Stem Cells in Transplanted Nemestrina Macaques

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An important goal in gene therapy and stem cell transplant is to expand hematopoietic stem cells (HSCs) without causing hematopoietic abnormalities. This is particularly challenging in humans and other primates, where expansion protocols that work in mouse models are often less effective in primate HSCs. One approach is to test genes that have led to clonal expansions by vector insertion site effects in human gene therapy trials. In this study, we recreated a mutant HMGA2 cDNA that was associated with a benign clonal expansion in a patient treated on a β-thalassemia gene therapy trial. A lentiviral vector was generated that expressed a cDNA for human *HMGA2* with a 3' UTR deletion that eliminates all seven let-7 microRNA binding sites and thereby avoids the known repressive effects of let-7 miRNAs on HMGA2 expression. A gamma-retroviral MSCV promoter was used to express this mutant HMGA2 cDNA along with an Ires-GFP cassette. Bone marrow CD34+ cells were collected for Nemestrina Macaques and half the cells were transduced with the lentiviral vector containing the HMGA2-GFP construct while the other half was transduced with a mCherry control vector. The transduction efficiencies were approximately the same prior to transplant. At three months post transplantation, the marking in the peripheral blood mononuclear cells was 2.9% GFP+ and 1.1% mCherry+ for animal #16, and was 2.7% GFP+ and 3.2% mCherry+ for animal #27, suggesting relatively equivalent and low levels of transduction of repopulating cells. The HMGA2-GFP marking progressively increased over 21 and 26 months in the peripheral blood leukocytes to 39% for #16 and 41% for #27 while the mCherry marked cells have decreased (Fig 1A). Equivalent levels of marking were seen in various mature peripheral blood lineages, including circulating erythyrocytes, suggesting that expansion had occurred in pluripotent HSCs. This HSC expansion was further demonstrated by a marking analysis in bone marrow cells that showed 44.6% and 75.2% GFP marking in the CD34+CD45RA- HSC compartment for animal #16 and #27 respectively, at the latest time point. Clonality analyses using vector integration sites (VIS) showed overall oligoclonal marking in both the GFP+ and mCherry+ cells in both animals (Fig 1B). The top most frequent VISs were present in all PB lineages indicating that expansion had occurred in multiple HSC clones. The WBC counts, lineage distribution in the peripheral blood, the percentage of CD34+ cells in the bone marrow and all the mature lineages in the peripheral blood are all within the normal range, demonstrating lack of any detectable hematopoietic abnormality. Gene expression microarray analysis of RNA from sorted BM CD34+GFP+ and CD34+mCherry+ cells showed sharp upregulation of several genes in the HMGA2-expressing cells, particularly the IGF2BP gene, a known downstream target of HMGA2. In summary, our data show that long-term HSCs from non-human primates can be progressively expanded in vivo over several years by overexpressing HMGA2. This could be useful for gene therapy, particularly for expanding and obtaining high numbers of transduced erythrocytes and granulocytes for diseases in which a naturally occurring selection advantage is not present.