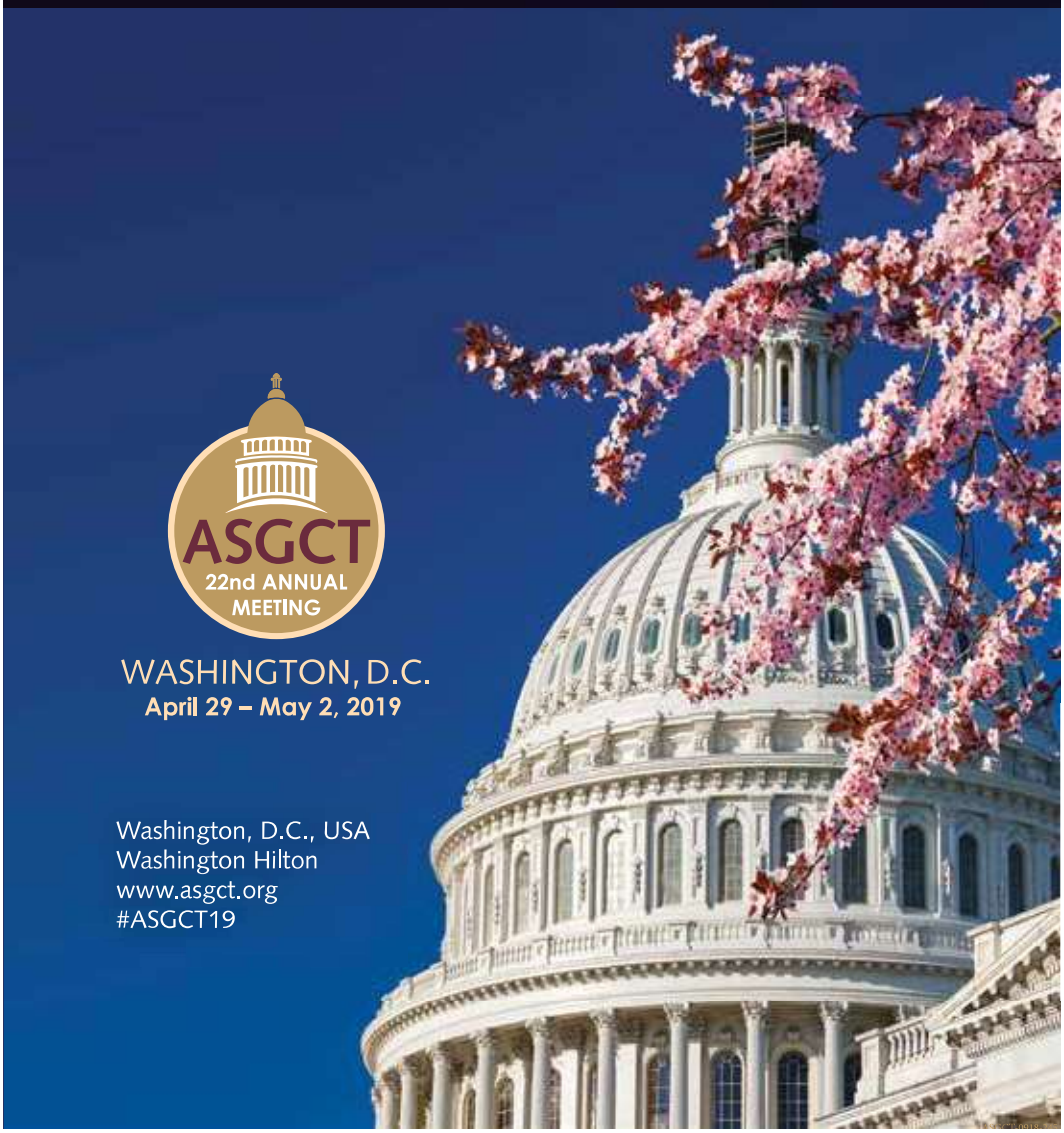
AMERICAN SOCIETY of GENE & CELL THERAPY

American Society of Gene & Cell Therapy

22nd Annual Meeting

Final Program Guide



ASGCT
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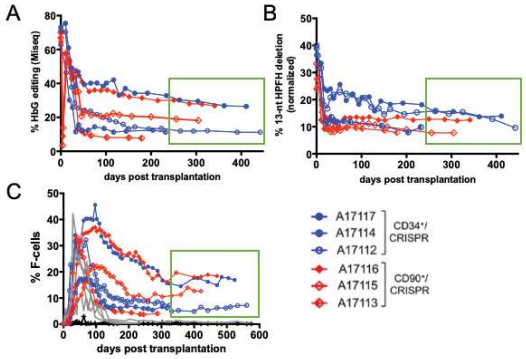
and *HBG2*, which results in a deletion spanning over 4kbp upon simultaneous cleavage of both sites. This deletion was detected in the infusion product at approximately 27% but frequency gradually decreased post-transplant, and was undetectable by 300 days. In conclusion, the levels of in vivo gene editing described here using bulk CD34+ or the CD34+CD90+CD45RA- subpopulation should be within a therapeutically relevant range for a number of genetic diseases. The conservation of the CD34+CD90+CD45RA- phenotype and the *HBG* CRISPR/Cas9 gRNA target site between NHP and human, combined with the use of a pre-clinical large animal model for stem cell gene therapy and transplantation, should permit the direct translation of this approach to patients.



A) *HBG* editing efficiency measured in peripheral total nucleated cells from transplanted animals. **B)** Levels of 13nt-HPFH deletion in the same animals as in (A) after normalization of all deletion frequencies to 100%. **C)** F-cells frequencies in transplanted animals as compared to historical transplant controls (grey) and one untransplanted control (black). Green rectangles focus on stabilization of editing or F-cell levels.

975. Efficient Genome Editing of the PKLR Locus in Human Long-Term Hematopoietic Stem Cells Using Specific CRISPR/CAS9 RNP and AAV6-Delivery of Donor Templates to Treat Pyruvate Kinase Deficiency

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Pyruvate kinase deficiency (PKD) is an autosomal recessive disorder caused by mutations in the *PKLR* gene that lead to a reduction of the activity of the erythroid pyruvate kinase (RPK) protein. This disease is associated with reticulocytosis, splenomegaly and iron overload, and may be life-threatening in severely affected patients. In selected and severe cases, allogeneic Hematopoietic Stem Cell Transplantation (HSCT) has been shown to correct the disorder; however this is associated with extensive toxicity and not considered a standard therapy in PKD. Autologous HSCT of genetically corrected cells will offer a durable and curative therapeutic option. Over the last years, gene editing has emerged as a promising gene therapy approach for blood cell disorders. The clinical application of gene editing to correct some genetic hematopoietic diseases is supported by the high level of correction got in Hematopoietic Stem and Progenitor Cells (HSPCs). With that in mind, we conducted a gene editing approach to correct PKD in human HSPCs. We developed a knock-in gene editing strategy at the genomic starting site of the *PKLR* gene by combining RNP electroporation and adeno-associated viral vector (AAV6) carrying donor sequences. Specific gRNAs generating up to 60% indels at the RPK starting site in human Cord Blood CD34$^+$ (CB-CD34$^+$) were designed. Two different AAV6 constructions were produced to deliver either a TurboGFP expression cassette or a promotor-less therapeutic codon optimized RPK cDNA (coRPK), flanked by specific homologous arms. Specific donor integration and stable expression of turboGFP and of coRPK driven by *PKLR* endogenous promoter was detected in edited human K562 erythroleukemia cells. Furthermore, up to 40% specific integration and stable expression of both donors were detected in colony forming units (CFUs) generated from gene edited CB-CD34$^+$cells, in the absence of toxicity related to the procedure. Moreover, these gene edited CB-CD34$^+$ cells engrafted efficiently in both primary and secondary NSG mice, demonstrating that gene editing of Long-Term HSCs has been achieved. These results demonstrate the feasibility of editing the *PKLR* locus in HSPCs at efficiencies that could be clinically applicable to treat PKD. Gene editing experiments using PKD patient derived CD34$^+$ cells are now being conducted to further prove the clinical relevance of this approach to correct PKD.

976. Gene Correction of Beta-Thalassemia Ex Vivo and In Vivo Mediated by PNA Nanoparticles

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Blood disorders caused by a single mutation such as β-thalassemia are attractive targets for gene editing and efforts to correct hereditary genomic mutations have advanced with the improvement of genome engineering technologies. Programmable RNA-guided Cas9 endonucleases enable efficient editing, but also exhibit high off-target effects. Moreover, *in vivo* delivery of CRISPR/Cas9 remains challenging. These drawbacks have motivated the development of non-nuclease-based platforms, such as peptide nucleic acids (PNAs). PNAs designed to bind to sites in genomic DNA and form PNA/DNA/PNA triplexes can initiate an endogenous DNA repair response mediated by nucleotide excision repair and homology-dependent repair pathways. Our recent work has demonstrated a safe and efficient method for site-specific gene editing to correct disease-causing mutations *in vivo* via intravenous (IV) administration of biodegradable polymeric nanoparticles (NPs) loaded with PNAs and single-stranded donor DNA. For example, we have mediated significant gene editing of the β-thalassemia-associated IVS2-654 mutation in the bone marrow (BM), leading to functional improvement with extremely low off-target effects using polymeric