480. In Vivo Gene Targeting for HIV Immunoprophylaxis using Promoterless AAV Without Nucleases

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Expression of broadly neutralizing antibodies (bNAbs) from AAV can provide protection against infections by different pathogens, including: HIV, Influenza and Plasmodium falciparum. Wide use of such vectored immunoprophylaxis will depend on the safety profile and on the duration of the immune effect. Alarmingly, AAV injection in neonatal mice has been associated with subsequent development of hepatocellular carcinoma (HCC) and adenoma (HCA) in a manner that is strictly dependent on the vector borne promoter. In addition, when the antibody is expressed from an AAV episome, the duration of prophylaxis might be limited by vector dilution when transfected cells divide. Alternatively, nucleases can be used to promote stable transgene expression, but these may be associated with adverse effects such as immunogenicity and genotoxicity.

Recently, we have shown that AAV could be used for promoterless gene targeting without nucleases for stable expression of coagulation factor IX in the context of hemophilia treatment (Barzel et al. Nature 2015). Here, we apply a similar methodology to target the integration of a gene coding for a bNAb against HIV to be driven upon integration by the strong Albumin promoter in the liver. Tail vein injection of our vector, coding for the VRC01 anti HIV bNAb, at 1E12 vector genomes per mouse into adult B6 mice leads to high (250 ng/ml) and stable levels of the bNAb in the plasma of the mice. We further show that the bNAb from the plasma is highly effective in binding the HIV envelope glycoprotein: gp120. In order to assess the potential of our approach in HIV prophylaxis and cure we have transplanted neonatal NOD/SCID/ gc null (NSG) mice with human CD34+ HSC cells (1E6 cells/pup). Mice that are effectively reconstituted with human immune cells will be challenged with HIV (100-200ng p24 units) either prior to injection with our bNAb coding AAV (to assess therapeutic potential) or following such an injection (to assess prophylactic potential).

For HIV and other infections where conventional vaccination is challenging, stable immunoprophylaxis using a promoterless AAV coding for a bNAb may constitute a safe and efficient alternative.

481. Targeted Genome Editing in Mouse Hematopoietic Stem/Progenitor Cells (HSPC) To Model Gene Correction of SCID-X1

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Targeted genome editing by artificial nucleases has brought the goal of gene correction within the reach of gene therapy. A candidate disease for HSPC gene correction is SCID-X1, because gene therapy with early generation integrating vectors showed robust clinical efficacy even from few corrected cells but also the occurrence of adverse events due to insertional mutagenesis and unregulated transgene expression. We recently reported a strategy that enabled targeted integration of a corrective cDNA into the *IL2RG* gene in 6% of human HSPC with high specificity. Gene corrected HSPC generated polyclonal lymphoid cells that express the IL2RG protein and have a selective growth advantage over those carrying disruptive *IL2RG* mutations *(Genovese, Nature, 2014)*. Here, to model SCID-X1 disease correction, we developed a mouse model carrying the *IL2RG* human gene including a common disease-causing mutation in place of the murine *Il2rg* gene, allowing to use the same reagents utilized for gene correction of human cells. These mice have impairment in lymphoid development which phenocopies that reported for *Il2rg-/-* mice. To assess the minimal level of corrected HSPC required to achieve immune reconstitution we first performed competitive transplants with wild-type (WT) and *Il2rg-/-* HSPC and found that 1% of WT cells are sufficient to reconstitute at least in part the T and B cell compartments. We then developed a protocol to obtain gene correction in murine Lin- HSPC based on the delivery of donor DNA template by IDLVs followed by transfection of ZFN mRNAs. This protocol was associated with high on-target nuclease activity (40%) and a mean of 6% transgene integration by HDR, but also with high levels of acute cytotoxicity (65% cell loss). The surviving cells remained capable of expansion in culture and preserved their clonogenic potential. Importantly, upon transplant into lethally irradiated mice, only the gene corrected cells were able to generate lymphoid lineages (B and T cells), showing a clear selective advantage over un-corrected cells. These data indicate functional correction of the *IL2RG* gene by our strategy. Yet, measuring percentage of correction within myeloid cells at long-term we found that it was almost undetectable. Despite the lack of HSC marking, gene corrected lymphoid cells stably persisted in the mice up to 7 months post transplantation within all the hematopoietic organs. Furthermore, upon challenging the transplanted mice with a murine pathogen (LCMV Arm.) we observed viral-specific γIFN production by CD8+ gene corrected cells at a similar extent as for WT mice, proving in vivo the functionality of corrected T cells. These results suggest that our protocol achieves biologically relevant levels of gene correction in progenitors that sustain long-term lymphopoiesis but is limited in multipotent HSC. Ongoing studies aim to improve murine HSC gene targeting and to compare safety and efficacy of gene correction vs gene replacement in our disease model.

482. Editing of the CD40L Gene Restores Regulated CD40L Expression in X-HIGM Patient T Cells

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X-linked hyper-IgM syndrome (X-HIGM) is a primary immunodeficiency syndrome characterized by insufficient CD40 Ligand (CD40L) expression, resulting in defective T-cell help, impaired immunoglobulin class-switching and recurrent opportunistic infections. Previously, constitutive CD40L expression in murine bone marrow cells and thymocytes was achieved by retroviral gene transfer and successfully corrected immune function. However, treated animals developed severe lymphoproliferative disease, likely due to constitutive CD40L surface expression. This demonstrated the importance of preserving endogenous gene regulation in this and other gene therapy endeavors. Here, we report efficient, on-target Homology Directed Repair (HDR) editing of the CD40L locus in primary human T cells using a combination of TALEN gene targeting and a donor template delivered transiently by recombinant Adeno-Associated Virus (rAAV). Our TALEN and donor template reagents were designed to insert a coding sequence (GFP or CD40L) upstream of the endogenous translation start site within Exon 1, thus allowing transgene expression to be regulated by the endogenous CD40L promoter, and included the CD40L 3'UTR to preserve known post-transcriptional regulation features. The kinetics of GFP and edited CD40L surface expression after PMA-Ionomycin