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605 Liver-Directed Gene Therapy for Hemophilia B with Immune Stealth Lentiviral Vectors

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Gene therapy is rapidly gaining renewed interest and is emerging as a realistic treatment option for several genetic and acquired diseases. Underlying this success is the development of improved gene transfer vectors. Among them, lentiviral vectors (LV) are emerging as versatile vehicles of relatively large capacity for stable transgene integration in the genome of target cells. Over the past years, we developed LV that achieve stable transgene expression in the liver and establish correction of hemophilia, in mouse and dog models of the disease, upon systemic administration. These LV are designed to stringently target transgene expression to hepatocytes through transcriptional and microRNA-mediated regulation. We previously evaluated portal vein administration of LV expressing canine factor IX (FIX) in 3 adult hemophilia B dogs and reported stable multiyear reconstitution of FIX activity up to 1 % of normal. We have more recently treated 4 dogs as pups (2-4 months of age) by peripheral vein administration of escalating doses of LV (5 to 10e9 T.U./kg) expressing a hyper-functional canine FIX and achieved reconstitution of FIX activity up to 32% of normal at the highest dose tested. We then modified the vector envelope by changing the protein composition of the producer cell plasma membrane through genome editing to reduce immunogenicity of LV particles. First, we performed genetic disruption of the β-2 microglobulin (B2M) gene, a required component for the assembly and trafficking of all class-I major histocompatibility complexes (MHC-I) to the plasma membrane in LV producer cells, exploiting the RNA-guided Cas9 nuclease. The resulting B2M-negative cells were devoid of surface-exposed MHC-I and produced MHC-free LVs. These LVs retain their infectivity on all tested cells *in vitro* and efficiently transduced the mouse liver upon intravenous administration. These MHC-free LVs showed significantly reduced immunogenicity in a T-cell activation assay performed on human primary T cells co-cultured with autologous monocytes exposed to LV, from several healthy donors, suggesting that conventional MHC-bearing LV may trigger allogeneic immune responses. Secondly, we generated LV with increased levels of CD47, a phagocytosis inhibitor, on the vector surface (CD47high LV), which show substantially decreased uptake by human macrophages *in vitro*. In order to evaluate the role of CD47 in LV biodistribution upon *in vivo* administration we took advantage of the non-obese diabetic (NOD) mouse model whose SIRPα (the CD47 receptor) is known to have high affinity for the human CD47. In this setting, CD47 proved to be a key player in reducing phagocytosis of LV, decreasing the inflammatory cytokine response following their administration, and increasing hepatocyte transduction and FIX output. We have now administered MHC-free or MHC-free/CD47high LV to 6 non-human primates (NHP, 3 for each LV version). We chose *Macaca nemestrina* as recipient, because of the lower restriction to HIV infection than other NHP species. Administration of 7.5e9 T.U./kg LV via peripheral vein was well tolerated, without significant elevation of serum aminotransferases or body temperature and only caused a transient self-limiting leukopenia. Remarkably, human-specific FIX activity reached up to 300% of normal and was nearly 3-fold higher in the CD47high-LV treated animals, showing a much more favorable LV dose-response than observed in mice and dogs. Upon necropsy, we measured vector copies in liver, spleen and major organs of treated animals and found between 0.5 and 1.5 LV copies in the liver accounting for 80-90% of all the retrieved LV copies, showing selective targeting and efficient gene transfer to the liver by LV in NHP. Overall, our studies support the efficacy and safety of these immune-stealth LV in NHP and position them to address some of the outstanding challenges in liver-directed gene therapy for hemophilia and conceivably other diseases.

Disclosures: Liu: *Bioverativ:* Employment, Other: Shareholder. **Peters:** *Bioverativ:* Employment, Other: Shareholder.

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