Therapeutic Levels of FVIII Generated by CRISPR/Cas9-mediated in vivo Genome Editing in Hemophilia A Mice

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Abstract:
Modification of the genome of somatic cells using sequence specific nucleases has the potential to provide lifelong correction of genetic diseases. We used the CRISPR/Cas9 system to insert a functional Factor VIII (FVIII) cDNA into intron 1 of the albumin gene of adult mice by non-homologous end joining (NHEJ) such that transcription of the exogenous FVIII gene was driven by the strong liver specific albumin promoter. To minimize off-target cleavage events we delivered Streptococcus pyogenes Cas9 (spCas9) mRNA to transiently express spCas9 nuclease, and a single guide RNA (sgRNA) targeting mouse albumin intron 1, by encapsulation in an optimized lipid nanoparticle (LNP). This LNP formulation enabled cleavage of up to 60% of murine albumin alleles in the liver after a single IV injection. A donor template composed of a codon optimized human FVIII cDNA lacking the signal peptide flanked by a splice acceptor and polyadenylation signal was delivered to mice using an adeno-associated virus (AAV). Because the FVIII cDNA lacks a signal peptide, the secretion of FVIII protein relies on fusion to the albumin signal peptide encoded in exon 1. In hemophilia A (HemA) mice that received 2e12 vg/kg of the AAV-FVIII donor and 2mg RNA/kg of the LNP, FVIII levels measured with the Coatest® activity assay averaged 30% of normal human levels at 2 weeks post dosing. About 25% of the HemA mice eventually developed antibodies against human FVIII associated with a loss of detectable FVIII activity. In immune deficient NOD *scid* gamma (NSG) mice dosed with 2e13 vg/kg of AAV donor and 2mg RNA /kg of the LNP, FVIII levels measured with a human FVIII specific capture-Coatest® assay averaged 70% of normal human levels and were stable over time. Integration of the FVIII cassette at the on-target site in albumin intron 1 in the liver was demonstrated by droplet digital in-out PCR, which revealed targeted integration frequencies in the range of 0.5% to 3% of the murine albumin alleles. As repeat dosing of the LNP-encapsulated spCas9 mRNA/sgRNA may lead to incremental increases in donor template targeted integration, we evaluated expression of a secreted alkaline phosphatase (SEAP) reporter gene lacking a signal peptide flanked by a splice acceptor and polyadenylation signal such that after integration into the same site in albumin intron 1 the expression and secretion of the SEAP protein is driven from the endogenous albumin promoter. No SEAP activity was detected when only the AAVSEAP virus was injected. Cohorts of 5 HemA mice that received the AAV-SEAP followed by 1, 2 or 3 doses of LNP encapsulated spCas9/sgRNA had mean SEAP levels in their blood (expressed as micro U/ml) of 3306 +/-848, 6900 +/-2120 and 13117 +/-1318, respectively. These results demonstrate a doubling of SEAP expression after each additional LNP dose. AAV and LNP dose response studies are currently in progress to elucidate the relationship between dose of AAV FVIII donor, frequency of on-target double strand breaks, targeted integration and FVIII levels. Further exploration of these concepts is ongoing and ultimately might provide a durable and curative treatment option for hemophilia patients.