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

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Background: Expression of Factor VIII (FVIII) from a FVIII cDNA that has been integrated into the genome of hepatocytes has the potential to provide a life-long cure for Hemophilia A (HemA)
Aims: Determine if the CRISPR/Cas9 nuclease system can promote non-homologous end joining (NHEJ) mediated insertion of a Factor VIII (FVIII) cDNA into intron 1 of the albumin gene of mice and thereby generate therapeutic levels of FVIII
Methods: A human FVIII cDNA lacking the signal peptide and flanked by a splice acceptor and polyadenylation signal was packaged in AAV8. Streptococcus pyogenes Cas9 (spCas9) mRNA and a single guide RNA (sgRNA) targeting mouse albumin intron 1 were encapsulated in a lipid nanoparticle (LNP). Cohorts of 5 adult HemA mice or adult NOD *scid* gamma (NSG) mice were injected with 2e12 or 2e13 vg/kg respectively of this AAV8-FVIII donor and 2mg RNA/kg of the LNP. FVIII levels in the blood of HemA and NSG mice were measured with the Coatest® activity assay or a human FVIII specific capture-Coatest® assay, respectively. Droplet Digital PCR was used to quantify the frequency of integration of the FVIII gene in the forward orientation into albumin intron 1 in the liver
Results: Mice injected with the AAV8-FVIII donor alone had no detectable FVIII in their blood. In HemA mice injected with both the AAV8-FVIII donor and the LNP, 30% of normal human FVIII levels were measured at 2 weeks. NSG mice injected with the AAV8-FVIII donor and LNP had 70% of normal human FVIII levels that were stable through the longest time point measured at 4 months. The frequency of FVIII cassette integration in albumin intron 1 was between 0.5% to 3% of the murine albumin alleles
Conclusions: CRISPR/Cas9 mediated integration of a FVIII cDNA into albumin intron 1 at low frequency generated therapeutic levels of FVIII in mice