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Cas-Clover Editing Efficiency and Off-Target Activity in Human Hepatocytes at the *KLKB1* Locus

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Cutting-edge gene editing holds enormous promise for tackling devastating genetic diseases like hereditary angioedema (HAE). Here, we describe the efficient inactivation of the gene encoding pre-kallikrein, *KLKB1*, using our proprietary Cas-CLOVER™ high-fidelity nuclease with our non-viral, lipid nanoparticle (LNP) delivery system. Genetic inactivation of *KLKB1* is an alternative clinical approach that provides durable relief to both Type I and II HAE. HAE is a rare genetic disease characterized by subcutaneous and submucosal edema, with swelling of the upper respiratory tract posing a life-threatening situation. Type I and II HAE are the most common types and are caused by mutations in the *SERPING1* gene, which leads to compromised production or function of the C1 protease inhibitor. Strategies for treatment and prophylaxis include the restoration of C1 inhibitor function, or downstream antagonism of active plasma kallikrein. Safe and effective gene editing of *KLKB1* could be a viable alternative for patients not adequately responding to the current standard of care. However, gene editing approaches must demonstrate an exquisitely high level of fidelity for optimal safety.

To demonstrate such an approach with Cas-CLOVER, multiple guide RNAs (gRNA) targeting the human *KLKB1* gene were screened in human hepatoma cell lines to identify gRNA pairs with optimal editing. Next, we evaluated KLKB1 protein reduction in primary human hepatocytes (PHH) that were incubated with LNPs encapsulating Cas-CLOVER mRNA along with each gRNA pair. Lead candidate gRNAs showed robust *KLKB1* editing in a dose-responsive manner, achieving >65% editing and >85% reduction in KLKB1 protein secreted into culture medium at 0.5 ug/mL (EC90). To evaluate Cas-CLOVER off-target activity, oligo incorporation by iGUIDE was carried out by a licensed contract research organization. In this assay, double-stranded oligodeoxyribonucleotides (dsODNs) were co-electroporated with Cas-CLOVER mRNA, along with our lead *KLKB1* gRNA pair, in the Huh7 cell line, and candidate off-target sites were identified by Illumina next-generation sequencing. Off-target activity was assessed by amplicon-seq at the eight top sites nominated by iGUIDE. In PHHs treated with 0.5 ug/mL of Cas-CLOVER LNPs, off-target editing was detected in 3/8 sites at very low levels (<0.25%). Remarkably, this low level of off-target editing remained unchanged when PHHs were treated with 10-fold higher concentrations of Cas-CLOVER LNP.

For further evaluation of our platform, we sought to determine *KLKB1* editing efficiency and fidelity in a mouse model of liver humanization. TK-Nog mice engrafted with PHHs were treated with a single intravenous injection of an LNP formulation co-encapsulating Cas-CLOVER mRNA and our lead *KLKB1* gRNA pair. Amplicon-seq analysis demonstrated that 60% of *KLKB1* alleles in the liver were edited. Importantly, no off-target editing was detected among the top eight sites identified by iGUIDE, including the three off-target sites validated in cultured PHHs. Next, we evaluated efficacy and tissue specificity of our platform in wild type mice. C57BL/6 male and female mice were dosed with LNP encapsulating Cas-CLOVER mRNA and mouse *Klkb1*-targeting gRNAs. A single intravenous LNP injection achieved high *Klkb1* editing (>50% of haploid genomes) in the liver and >80% reduction in serum pre-kallikrein levels. No *Klkb1* editing was detected in gonads.

In summary, these results highlight the efficacy and specificity of our high-fidelity Cas-CLOVER gene editing platform that enables targeted and therapeutically relevant kallikrein reduction in a fully non-viral manner. These data provide a promising foundation for the development of a highly specific gene editing therapy for HAE.

Disclosures Alvarez: *Poseida Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Homa:** *Poseida Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Kearney:** *Poseida Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Hajj:** *Poseida Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Ma:** *Poseida Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Negron:** *Poseida Therapeutics:* Current Employment, Current equity holder in publicly-

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