

Cas-CLOVER Technology Enables Precise Gene Editing and Site-Specific Transgene Insertion in Mouse Liver

American Society of Gene & Cell Therapy 26th Annual Meeting

Oscar Alvarez, PhD

Associate Director, In Vivo Gene Therapy



Cas-CLOVER[™] – A High-Fidelity Gene Editing System



Potentially the Cleanest Gene Editing Platform

- Extensively vetted for off-target effects in peer-reviewed publication¹
- Key ability to efficiently edit single or multiple genes
- Fully non-viral approach for *in vivo* gene editing
- Diverse toolbox of variants for expanded targeting (e.g., PAM diversity)
- Currently utilized in clinical-stage CAR-T investigative product

- Low-to-no off-target cutting (non-nicking obligate dimer nuclease)
- High specificity
- High efficiency editing in liver with proprietary LNP
- Ease of use/design
- Multiplexing ability
- Lower potential costs



Combining Poseida Platforms to Enable Potentially Curative Therapies

Advantages of Fully Non-viral Cas-CLOVER for In Vivo Gene Therapies

- High fidelity
- High editing efficiency
- Multiplexing ability
- Multiple payload delivery
- Transient mRNA expression
- Low immunogenicity
- Redosing capability
- Delivery to multiple tissues with proprietary LNPs

Knock-out

- Precise editing of single or multiple genes
- Disruption of dysfunctional genes to reduce disease severity
- Single particle co-encapsulation



Knock-in

- Site-specific integration of a therapeutic transgene
- Functional disease correction
- Single particle co-encapsulation





Demonstration of Cas-CLOVER Editing in Liver with PCSK9 Knock-out





Efficient Cas-CLOVER Delivery and Editing in Mouse Liver

Cas-CLOVER edits liver *in vivo* with high efficiency

- Cas-CLOVER mRNA and gRNAs were delivered to WT mice using Poseida proprietary LNP
- Cas-CLOVER LNP efficacy is maximal at 2 mg/kg (65% indels) with tool LNPs
 - o 45% biallelic edits
 - o 19% monoallelic edits
- >80-85% decrease in PCSK9 protein with doses >1.5 mg/kg





Single-cell Genotyping Indicates Extremely Low Off-Target Editing

- Liver from control and edited mice examined at off-target (OT) sites via single cell/nuclei genotyping
- Among 148 OT sites queried, only 5 exhibited detectible editing at very low rates
- Examination of these 5 sites by bulk amplicon-seq revealed extremely low editing
- GUIDE-seq analysis in multiple additional targets showed similar ultra-high fidelity with Cas-CLOVER





Cas-CLOVER Demonstrates Favorable Reproductive Toxicology Profile

No Cas-CLOVER mRNA or Editing Detected in Gonads





Cas-CLOVER Lipid Nanoparticles Have Favorable Tolerability Profile

Cas-CLOVER LNPs exhibit very low hepatotoxicity / immunogenicity

- Single injection: transaminase levels maintained within normal range
- Multiple injections: Mice received 3 high dose Cas-CLOVER LNP injections (2 mg/kg; days 0, 8, and 16):
 - No histological or serum signatures of chronic liver injury
 - Max (>80%) PCSK9 KO after first dose
 - Serum transaminases and bilirubin at baseline levels on day 23





Non-viral Cas-CLOVER Enables Site-Specific Transgene Integration and Expression



Cas-CLOVER Demonstrates Potential as High-Fidelity Gene Editing System

Cas-CLOVER enables precise non-viral knockouts

- Cas-CLOVER is delivered using <u>Poseida's proprietary</u> <u>biodegradable mRNA LNP</u>
- <u>Gene editing efficiency (>60%) and protein reduction</u> (~85%) at *Pcsk9* locus following a single injection
- Single-cell genotyping indicates <u>very low off-target</u> <u>editing</u>
- Cas-CLOVER demonstrates <u>favorable reproductive</u> <u>toxicology and tolerability profiles</u>

Cas-CLOVER enables site-specific non-viral knock-ins

- Successful co-encapsulation of Cas-CLOVER mRNA, gRNAs, and donor DNA in a <u>single nanoparticle</u>
- Fully non-viral delivery enables <u>site-specific transgene</u> <u>integration</u> and expression in adult and growing liver
- Robust transgene activity persisted for 3 months



ACKNOWLEDGEMENTS

Stephanie Kearney Lucas Homa Cindy Negron **Kevin Briseno** Matt Nitzahn Nygel Oglesby Mauricio Barajas Khal Hajj Joe Kubicki Alicia Davis Marine Blum

Johannes Schwerk Haibin Xi Ming Lee Joe Lucas Fernando Espinoza Brian Truong Bernard Kok Mona Connerney **Arturo Barcenas Garret Arauz** Nick DeMarco



Thien Li Pranavanand Nyshadham Valerie Pasquetto Bonnie Jacques Mike Bennett Jack Rychak Pavan Battiprolu Brent Warner Blair Madison

...and All of the Poseida Gene Therapy Team