We believe in the future of allogeneic CAR-T therapy

	Candidate	Preclinical	IND-Enabling	Phase 1	Phase 2	Phase 3
CAR-T FOR ONCOLOGY						
MULTIPLE MYELOMA	P-BCMA-101	Auto				
	P-BCMA-ALLO1 ★	Allo				
	Dual CAR (BCMA/CD19) ★	Allo				
PROSTATE CANCER	P-PSMA-101	Auto				
	P-PSMA-ALLO1 ★	Allo				
SOLID TUMOR	P-MUC1C-ALLO1 ★	Allo				
	Dual CAR (Undisclosed) ★	Allo				
B - CELL	Dual CAR (CD19/CD20) ★	Allo				
GENE THERAPY						
LIVER DIRECTED GENE THERAPIES	P-OTC-101	GT				
	P-MMUT-101	GT				

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Our unique platform generates a unique CAR-T product

piggyBac Transposon + piggyBac Transposase

ITR | CARGO | ITR

"Cut"

ITR | CARGO | ITR

"Paste"

Genomic DNA — ITR | CARGO | ITR

- High **efficiency** cargo delivery into genome

- Long-lived **stem cell** composition of CAR-T

LESS DIFFERENTIATED → MORE DIFFERENTIATED

Stem Cell Memory — T_{SCM} Cell
- Self-renewing
- Long-lived
- Multipotent

Central Memory — T_{CM} Cell

Effector Memory — T_{EM} Cell

Effector T-Cell — T_{EFF} Cell

THE POTENTIAL BENEFITS
Products with High% of T_{SCM} Cells:
- More gradual tumor killing with less toxicity
- Key to CAR-T success in solid tumors
- Better duration of response
- Potential for re-response

Typically 70-90% Tscm

T_{EFF} | T_{SCM}
T_{CM} | T_{CM}

CD45RA / CD62L

T_{EFF} Cell | T_{SCM} Cell
T_{EM} Cell | T_{CM} Cell

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Why pursue CAR-T treatments via an allogeneic approach?

AUTOLOGOUS	ALLOGENEIC

Anti-BCMA CAR

Patient

Healthy Donor

Patients

MHC I knock-out   TCR knock-out

CHALLENGES
- Variable potency & manufacturability of patient material (high potential for low **quality**)
- Long vein-to-vein **time**
- High **cost** of an individualized product

ADVANTAGES
- **Quality**: Healthy donor material for consistent potency & manufacturability
- **TAT**: Universal, off-the shelf product for increased patient access & decreased cost
- **Scalability/cost**: Large number of doses for numerous patients from a single manufacturing run

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Cas-CLOVER™ Efficiently Knocks-out TCR in T Cells *Ex Vivo*

- For **off-the-shelf** allogeneic CAR-T products, **efficient TCR KO** is critical to **prevent graft versus host disease** (GvHD)

- Cas-CLOVER™ allows for **highly efficient TCR KO** across wide range of healthy donors with editing rates of up to 99%

- Residual TCR+ cells are removed resulting in a **highly pure TCR-negative CAR-T product** with up to 99.9% TCR KO

- Cas-CLOVER™ edited & purified cells do not exhibit alloreactivity/GvHD when mixed with donor-mismatched PBMCs
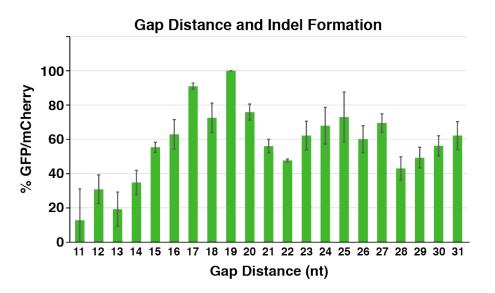
Composition: Clo51-dCas9 = Cas-CLOVER™ Nuclease
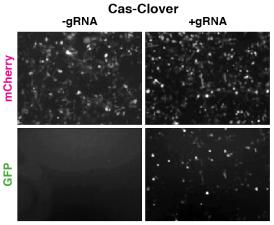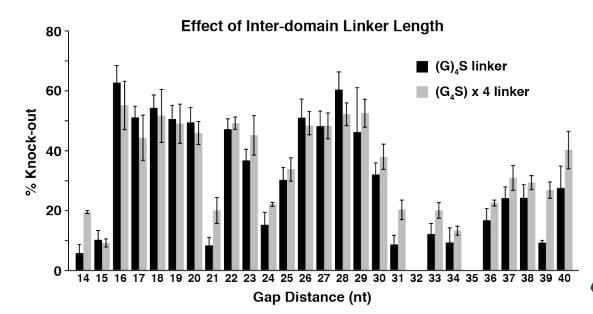
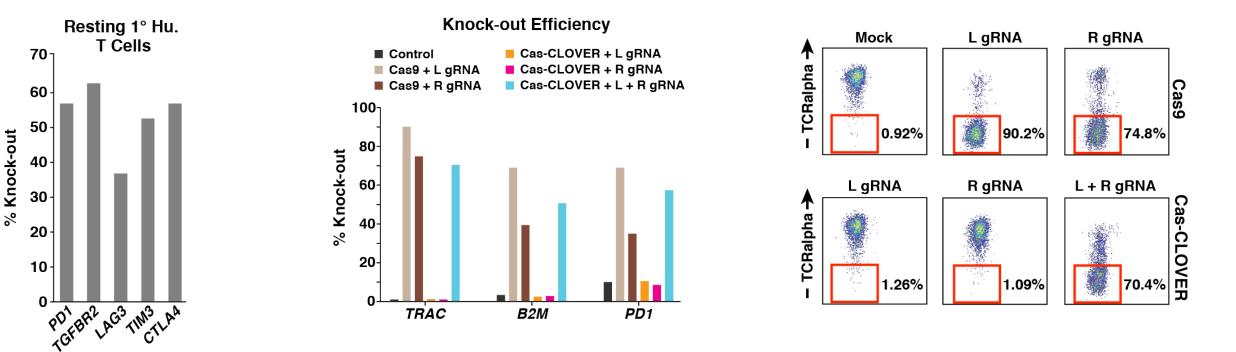# Spacing Requirements of Cas-CLOVER™

Optimal gap of 16-20 nt
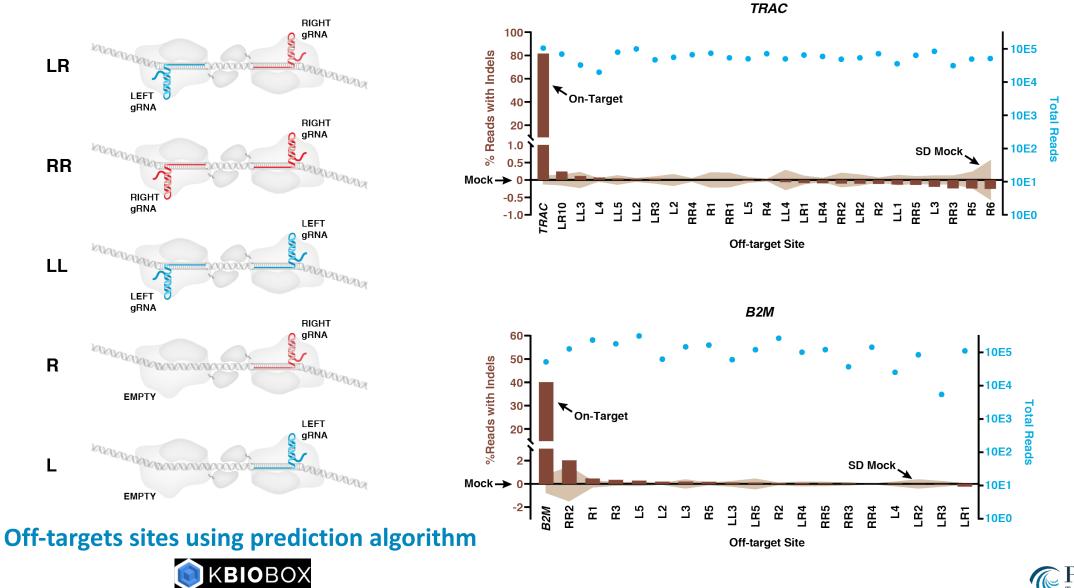
Extended flexible linker is largely unnecessary.

Cas-CLOVER™ Yields Knockouts with High Efficiency

Primary Resting Human T Cells

- **Highly efficient**, with rates comparable to WT SpCas9

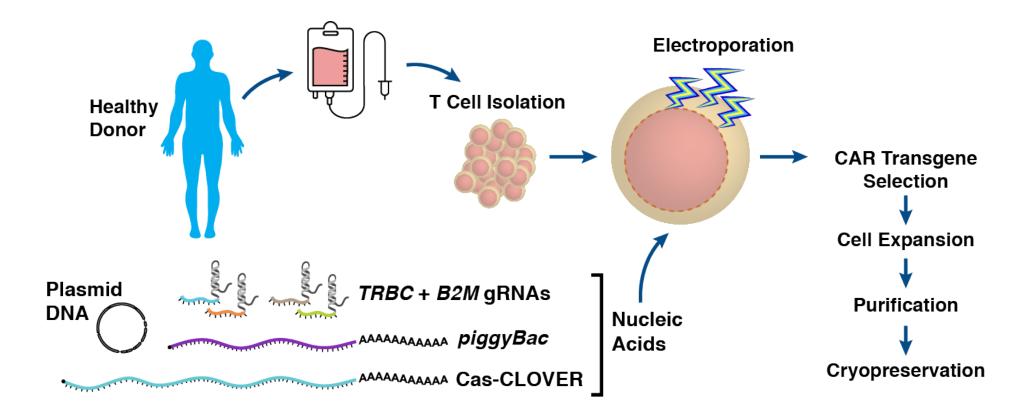- **Consistent** knock-out efficiency at **multiple** loci, with stringent parameters
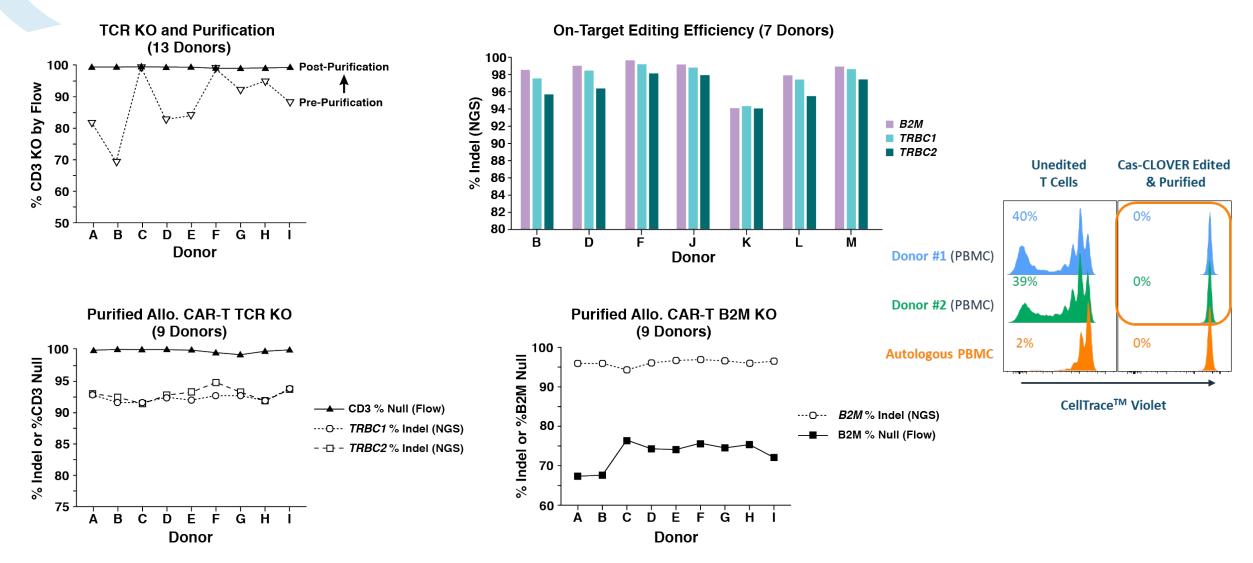
Cas-CLOVER™ Yields High Fidelity Targeted Knock-Outs

Off-targets sites using prediction algorithm

Allogeneic CAR-T Production with piggyBac™ & Cas-CLOVER™
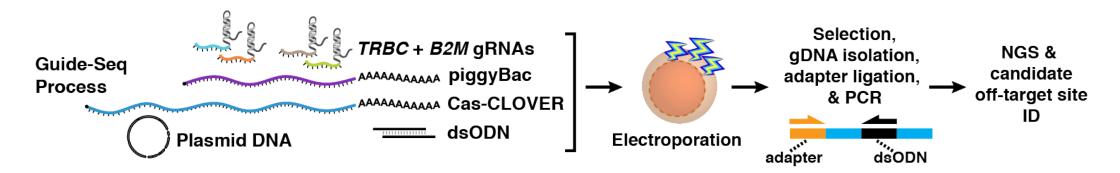
Efficient Inactivation of TCR with Cas-CLOVER™

TCR KO and Purification (13 Donors)

On-Target Editing Efficiency (7 Donors)

Purified Allo. CAR-T TCR KO (9 Donors)

Purified Allo. CAR-T B2M KO (9 Donors)
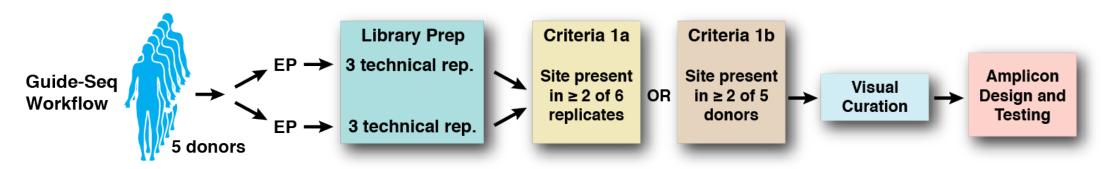
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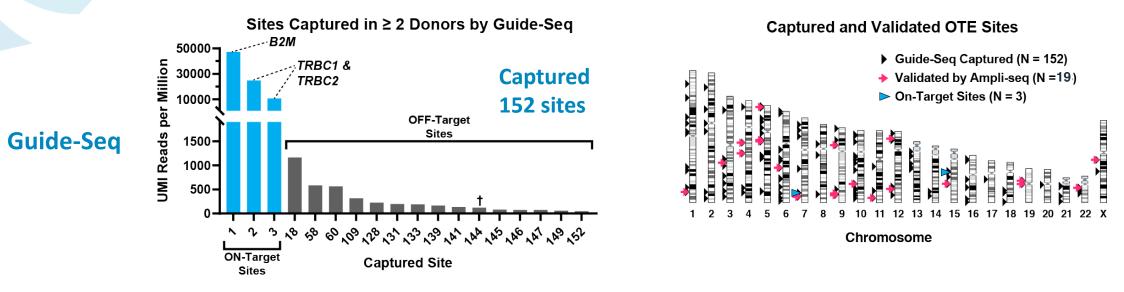# Off-Target Site Identification with Guide-Seq/Oligo Capture

- *In cellulo* approach
- Unbiased, widely accepted, with high reproducibility
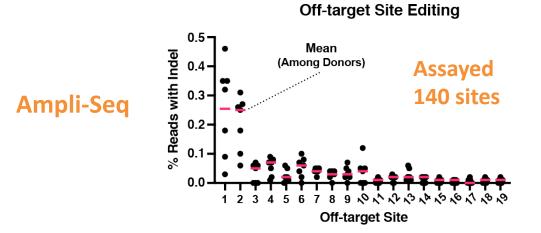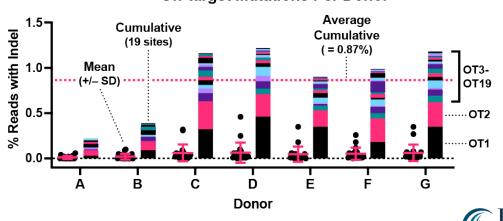- Discovers candidate off-targets in context of manufacturing process

Off-Target Site Identification with Guide-Seq/Oligo Capture

Sites Captured in ≥ 2 Donors by Guide-Seq

Captured 152 sites

Guide-Seq

Captured and Validated OTE Sites

▶ Guide-Seq Captured (N = 152)
➡ Validated by Ampli-seq (N = 19)
▶ On-Target Sites (N = 3)

No hits in oncogenes or tumor suppressors (COSMIC database)

Off-target Site Editing

Assayed 140 sites

Ampli-Seq
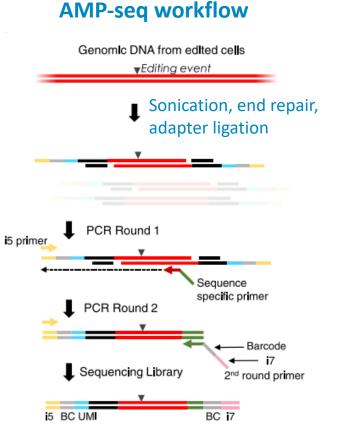
Off-target Mutations Per Donor

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Detecting Translocations with AMP-Seq
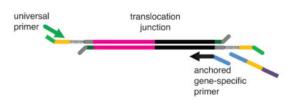
Anchored multiplex PCR (AMP) - NGS
- Previously used for detecting translocations in clinical samples
- Uses a high DNA input amt (2 ug)
- Shears DNA using ultrasonication
- Includes DNA end repair and adapter ligation steps

Zheng, Z. *et al. Nature Medicine* 20, 1479–1484 (2014)
Tsai, S. Q. *et al. Nat Biotechnol* 33, 187–197 (2015)
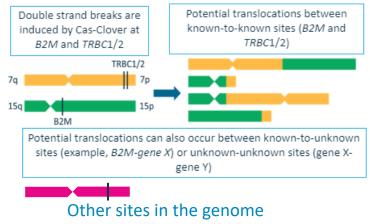Giannoukos, G. *et al. BMC Genomics* 19, 212 (2018)

AMP-seq workflow

Genomic DNA from edited cells
▼*Editing event*

Sonication, end repair, adapter ligation

i5 primer

PCR Round 1

Sequence specific primer

PCR Round 2

Barcode
i7
2nd round primer

Sequencing Library

i5 BC UMI   BC i7

Modified from
Giannoukos, G. et al. *BMC Genomics* 19, 212 (2018)

AMP-seq primers annealing around DNA fragments with translocation

universal primer
translocation junction
anchored gene-specific primer

From Tsai, S. Q. et al. *Nat Biotechnol* 33, 187–197 (2015)

Potential translocations

Double strand breaks are induced by Cas-Clover at *B2M* and *TRBC1/2*

Potential translocations between known-to-known sites (*B2M* and *TRBC1/2*)

TRBC1/2
7q   7p
15q   15p
B2M

Potential translocations can also occur between known-to-unknown sites (example, *B2M-gene X*) or unknown-unknown sites (gene X-gene Y)
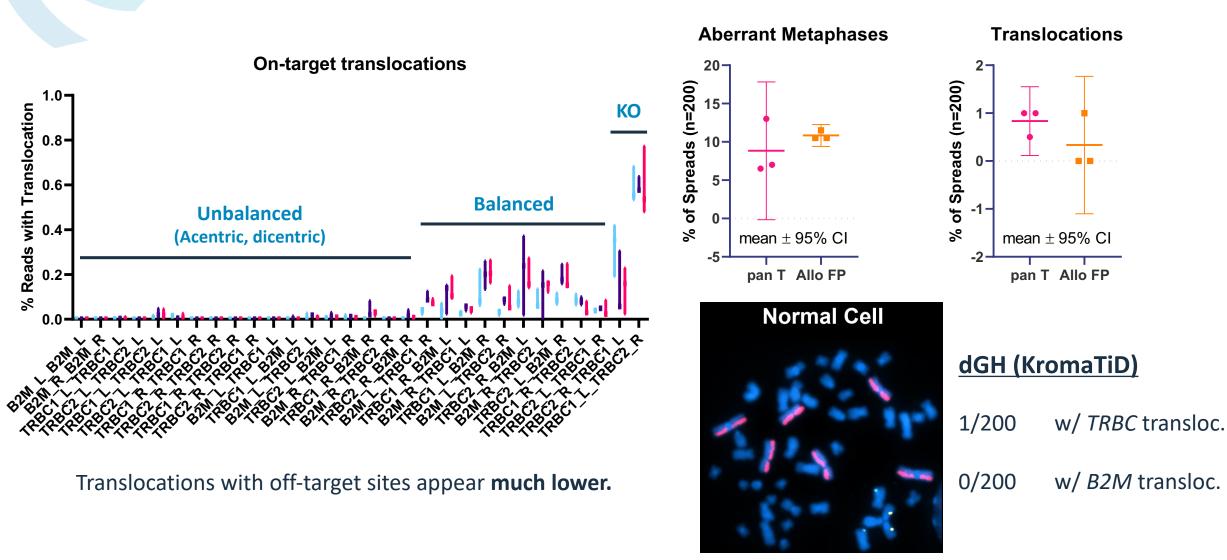
Other sites in the genome
(off-target, genomic hotspot, other sites)

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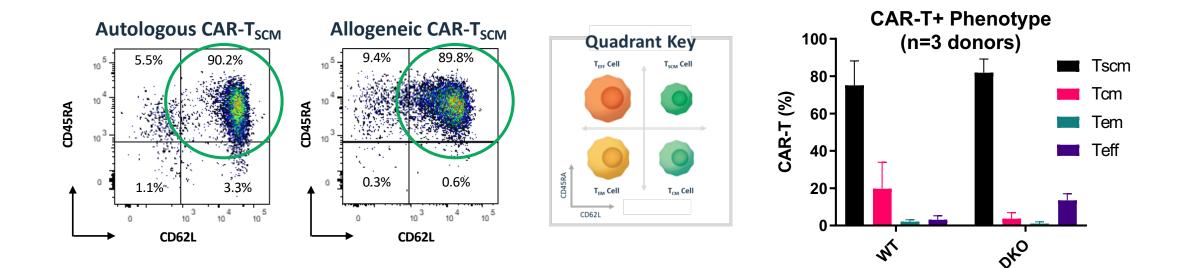
Cas-CLOVER^{TM} does not contribute to genome instability

On-target translocations

KO

Unbalanced (Acentric, dicentric)

Balanced

Translocations with off-target sites appear **much lower.**

Aberrant Metaphases

mean ± 95% CI

pan T   Allo FP

Translocations

mean ± 95% CI

pan T   Allo FP

Normal Cell

dGH (KromaTiD)

1/200   w/ *TRBC* transloc.

0/200   w/ *B2M* transloc.
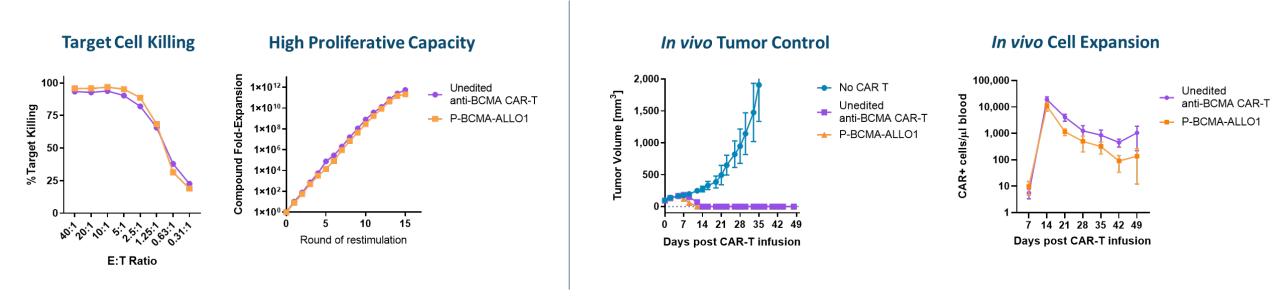
Cas-CLOVER™ Gene Editing in <u>Resting T Cells</u> for Generation of Fully Allogeneic CAR-T

- Cas-CLOVER™ editing in resting T cells <u>does not</u> adversely affect the **desirable T$_{SCM}$ cells** in the finished product

- A **high-percentage T$_{SCM}$, fully allogeneic CAR-T product** may confer **prodrug-like characteristics and a tolerability profile** similar to Poseida's autologous product candidate (P-BCMA-101)

Fully Allogeneic P-BCMA-ALLO1 Functions Equivalent to or Better Than Autologous (Unedited) Healthy Donor CAR-T *in vitro* & *in vivo*

- *In vitro*, P-BCMA-ALLO1 showed efficient target cell killing, cytokine secretion and high proliferative capacity

- *In vivo*, P-BCMA-ALLO1 exhibited efficient tumor control and robust CAR-T cell expansion in response to tumor in an animal model that has high positive predictive value of clinical efficacy

Where can I get Cas-CLOVER™?

Cell & Gene Therapeutics

Human therapeutics, including CAR-T and other cell and gene therapies

Karen Basbaum, VP Business Development

kbasbaum@poseida.com

Preclinical Discovery & Model Creation

- **Research reagents**
- **The SRG Rat**
- **OncoRat**
- **Cell line & animal model creation**

Mike Schlosser, CEO

mschlosser@herabiolabs.com

Biomanufacturing & Agriculture

- **Edited cells & plants for "bioprocessing"**
- **Engineered organisms (i.e. plants, animals & microbes) for agriculture & industrial applications**

Jack Crawford, CEO

jcrawford@demeetra.com

315-351-9115