ENABLING THE ENGINEERING OF IPSC-DERIVED CELL THERAPIES USING MAD7, A NOVEL CRISPR NUCLEASE

Hunter Hoffman, Jill M. Carton, Buddha Gurung, Justin Bianchini, Shelby Keating, Michael F. Naso, Luis Borges
Century Therapeutics
Philadelphia, PA 19014

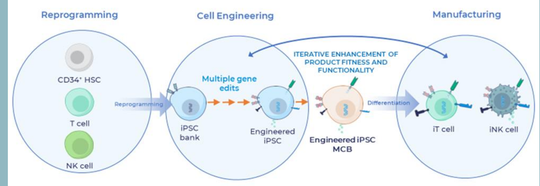
ABSTRACT

CRISPR nucleases have dramatically improved the process of precise genome engineering of mammalian cells, allowing for targeted gene deletions and insertions at high efficiencies. Although these advancements initially impacted the basic research community, they are now rapidly being applied to therapeutics, especially in engineered cell therapy programs utilizing primary cells and induced pluripotent stem cells (iPSCs).

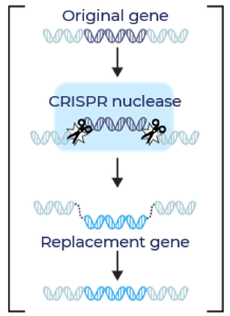
MAD7 is a class 2, type V-A CRISPR nuclease that utilizes a short gRNA without the requirement of a tracrRNA and utilizes a PAM site that is thymidine rich. Although MAD7 and Cpf1 share similar activity and structure, they only possess around 30% sequence identity.

We have developed a production process and biophysical analysis assays to produce and characterize recombinant MAD7 for use in ribonucleoproteins (RNPs) in our iPSC gene editing platform. Our process yields a protein that is homogenous and monomeric in solution after formulation. In addition, protein stability is maintained for 6 months at -80°C as measured by biophysical and functional characterization. The activity of recombinantly produced MAD7 is equivalent to Cpf1 with regard to knock-outs (KO) and homology directed repair (HDR) efficiencies at multiple loci in iPSCs. We have generated and tested multiple gRNAs targeting different sites in the genome and demonstrated that MAD7 does not induce any structural anomalies as determined by orthogonal genetic characterization assays. The data indicate that recombinant MAD7 CRISPR nuclease can be efficiently expressed, purified and formulated to enable robust and precise engineering of mammalian cells as a ribonucleoprotein (RNP). We are currently using our MAD7 optimized process to generate MAD7 RNPs to enable the genetic engineering of therapeutic iPSC-derived NK and T cell product candidates with multiple gene edits.

Century's end-to-end platform has the key components to realize the potential of iPSCs



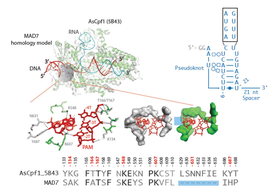
iPSC precision engineering



CRISPR-mediated HDR (MAD7)

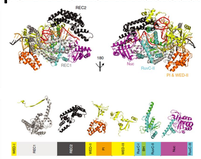
RESULTS

MAD7 structural attributes

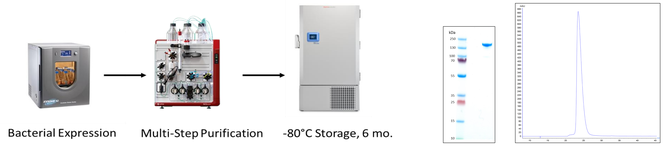


MAD7 has high structural similarity to AsCpf1, but low sequence identity (~30%). PAM recognition site comparison showing interaction, and crRNA structure

Domain structure of class 2 type V-A CRISPR nucleases

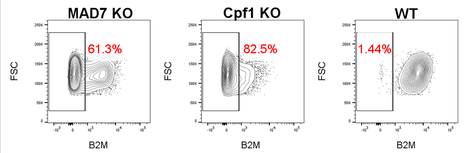


Class 2, type V CRISPR nuclease domain organization depicting the target DNA recognition and nuclease domains



Recombinant MAD7 bacterial expression followed by multi-step affinity, ion exchange, and size exclusion chromatography purification results in the generation of a highly monomeric, homogeneous product

Knock-out efficiency of MAD7 is comparable to Cpf1



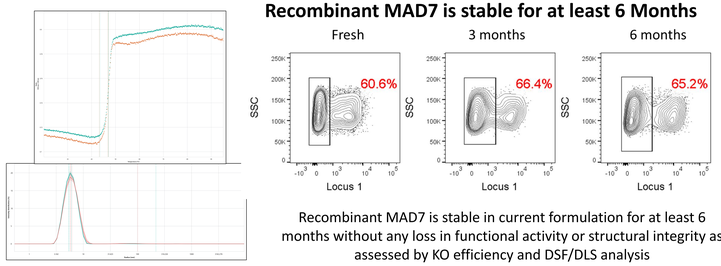
MAD7 RNP knock-out efficiency is comparable to Cpf1 in IPSC cells at the β2M locus determined by flow cytometry

MAD7 RNP genetic analysis

Assay	Quality
SNP microarray	Copy number variation (CNV)
Guide-seq	Off-target cutting

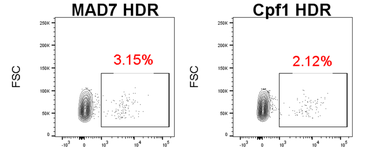
Guide-seq analysis and SNP microarrays CNV analysis were performed on MAD7 RNP engineered iPSCS and did not identify any new abnormalities

Recombinant MAD7 is stable for at least 6 Months



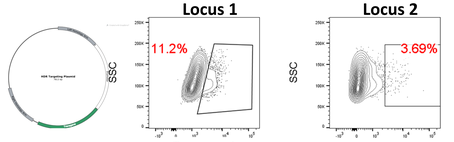
Recombinant MAD7 is stable in current formulation for at least 6 months without any loss in functional activity or structural integrity as assessed by KO efficiency and DSF/DLS analysis

HDR efficiency with MAD7 is comparable to Cpf1



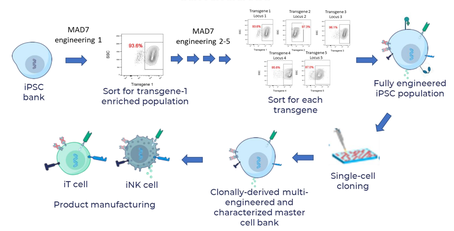
MAD7 RNP facilitates HDR rates comparable to Cpf1 in IPS cells

High HDR efficiency with MAD7 at multiple loci



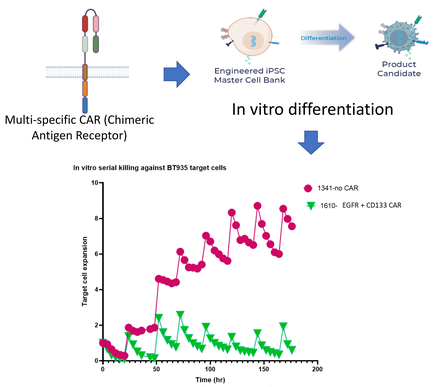
MAD7 RNPs facilitate efficient HDR in IPS cells at two loci with two different transgenes

Derivation of MAD7 multi-engineered product candidates



MAD7 RNPs were used to engineer an iPSCs population with multiple transgenes at multiple loci. After single cell cloning, genetic and functional characterization is performed to select a clone to move forward to generate an MCB and product manufacturing

CAR activity of MAD7 triple-engineered iPSCs differentiated into iNK cells



In vitro functional activity

MAD7 triple-engineered iPSCs cells with a multi-specific CAR differentiate efficiently into iNK cells and function in vitro at eliminating tumor cell lines in a serial killing assay

Supporting our iPSC-derived therapeutic pipeline

	Product	iPSC Platform	Targets	Indications	Expected IND Submission	Discovery	Preclinical	Clinical	Collaborator
MAD7 Engineered	CNTY-101	iNK	CD19	B-Cell Malignancies	Mid 2022				
	CNTY-103	iNK	CD133 + EGFR	Glioblastoma	2023				
	CNTY-102	iT	CD19 + CD79b	B-Cell Malignancies	2024				
	CNTY-104	iNK/iT	Multi-specific	Acute Myeloid Leukemia	2024				Bristol Myers Squibb
	CNTY-106	iNK/iT	Multi-specific	Multiple Myeloma	2024				Bristol Myers Squibb

Solid Tumors Hematologic Tumors

CONCLUSIONS

- We developed a robust process to produce recombinant MAD7 CRISPR nuclease for gene editing
- Recombinant MAD7 RNPs have comparable gene editing activity to Cpf1 RNPs for gene knockout (KO) and homology directed repair (HDR)
- Recombinant MAD7 is stable for at least 6 months at -80°C without any loss in activity
- MAD7 has little to no off-target activities based on SNP CNV analysis and guide-seq analysis
- iPSCs, engineered with multiple transgenes using MAD7, were differentiated into iNKs with superior function in vitro and in vivo
- MAD7 is being used to engineer our future iNK and iT cell product candidates