cGMP Compliant iPSC Cell Genome Editing Platform Facilitates Its Therapeutic Application

Kai Wang, PhD and Ruby Yanru Chen-Tsai[1], PhD
Applied StemCell Inc., Milpitas, CA

Abstract

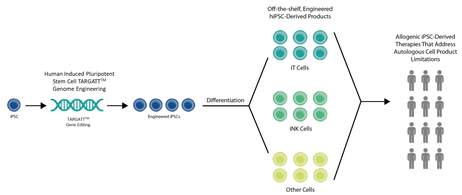
The advent of hiPSC technology (Human Induced Pluripotent Stem Cells) and genome editing technologies (e.g., CSRPR/CAS9 and TARGATT™) allow iPSC and its derived functional cells to be broadly used in gene/cell therapy and regenerative medicine. High-quality standard, such as cGMP/cGMP-compliant, is necessary to ensure the success of genome-edited iPSC-based therapeutic products in both preclinic and clinic phases. However, building cGMP capabilities in this field is challenging and needs intense investment, quality management system, and an experienced scientific and manufacturing team. In response to the needs of clients and market trends, we have established cGMP manufacturing processes for iPSC reprogramming, gene editing (CRISPR and TARGATT™), cell banking, and iPSC differentiated products. Our cGMP facility is fully certified with a drug manufacturing license from the State of California Food and Drug Branch. For immune-oncology cell products, we have established a manufacturing process using TARGATT™ master iPSC platform for the development of CAR-iNK products. A TARGATT™ master iPSC line containing a landing pad in a safe harbor genomic locus has been established and used to insert a CD19-CAR (>6kb in size). The CAR insertion efficiency was over 40% without selection (10 times better than CRISPR/Cas9 method). The CAR-iPS cells were differentiated ex vivo to iNK cells. Quantitative PCR analysis indicated that CD19-CAR was expressed in all stages of cells during differentiation as well as in the final iNK cells. The same process is now being repeated in the GMP facility. In conclusion, our cGMP compliant iPSC capability provides a necessary resource for our clients who are developing therapeutics cell products, with a focus in CAR-iNK products.

Current Limitation of CAR-NK/T Therapy

1. The development and manufacturing of an autologous CAR-NK or CAR-T product is expensive and can only be used by a single patient.
2. Random interion of CAR genes using lentiviruses or transposon methods can not be avoided and is unsafe.
3. Currently, lentivirus manufacturing of CAR-NK and CAR-T products is high-priced.
4. Increasing a company's capabilities to develop and manufacture cGMP iPSC or iPSC-derived products (e.g., CAR-iNK or CAR-iT) remains challenging and expensive.

TARGATT™ Master iPSC Platform Advantages

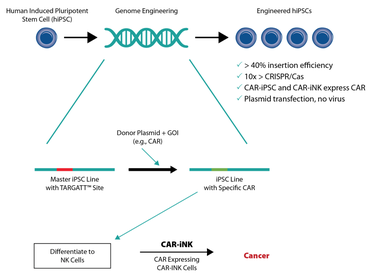
The site-specific knock-in technology, TARGATT™ and iPSC technology were combined to develop the TARGATT™ Master iPSC Platform for the manufacturing of CAR-iNK cell products. The TARGATT™ master iPS cells permit single-copy insertion of any gene of interest (up to 20kb). Using well established protocols, the modified iPSCs can be further differentiated into lineage committed cell types, including NK cells. The platform also includes cell banking to ensure a consistent manufacturing process.



1. TARGATT™ master iPSCs that contain a landing pad in a safe genomic locus for fast and efficient gene knock-in by TARGATT™ integrase.
2. iPSC differentiation of the knock-in TARGATT™ Master iPS cells to create an unlimited source for off-the-shelf allogeneic therapeutic cells.
3. Eliminate time consuming and costly viral manufacturing.

TARGATT™ Master iPSC Platform in CAR-iNK Therapy

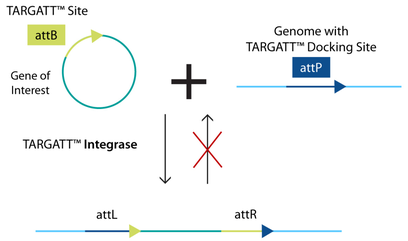
The TARGATT™ Master iPS cells were engineered to contain an integrase recognition landing pad at a safe-harbor locus. When transfected with a unique integrase plasmid and a CAR donor plasmid, integrase expression enables the safe knock-in of the CAR gene at the landing pad. The CAR-positive iPSCs can be further differentiated into CAR-iNK cells.



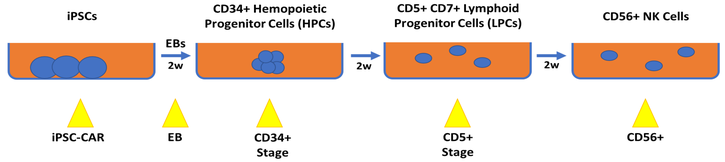
Experimental Design

TARGATT™ iPSC Knock-in Strategy

The TARGATT™ Master iPS cells were transfected with an integrase plasmid and the TARGATT™ CD19-CAR plasmid. Integrase expression enabled the site-specific integration of CD19-CAR at the "attP" docking site (landing pad).



CAR-Positive iPSC Differentiation to Natural Killer Cells



The CD19-CAR-positive iPSCs were further differentiated into NK cells. iPSC samples were collected throughout the differentiation process (iPSC-CAR, EB6, CD5+, and CD56+) for Oct4, CD56, and CD19-CAR (iPSC-CAR, EB6, CD34+, CD5+, and CD56+) expression quantitative analysis.

CD19-CAR iPSCs Express CAR

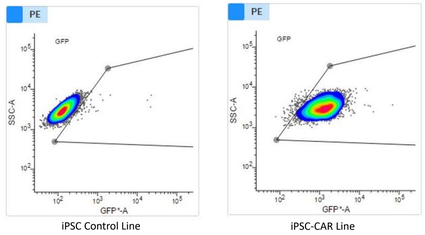


Figure 1: Flow cytometry analysis of the CD19-CAR iPSCs for surface expression of CAR. Flow cytometry results suggest that the CD19-CAR iPSCs express CD19-CAR. Expression of CAR was detected using monoclonal antibodies labeled with a fluorescent molecule.

CAR-iNK Cell Characterization

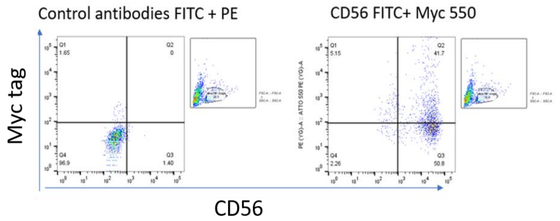


Figure 2: Flow cytometry analysis of the CD19-CAR iNK cells for biomarker expression. The iPSC-derived CD19-CAR iNK cells expressed NK cell biomarker CD56.

Oct4 or CD56 Expression At Various Cell Stages of Differentiation

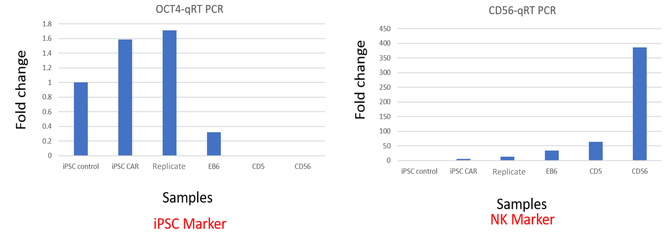


Figure 3: Quantitative analysis of Oct4 and CD56 marker expression throughout the differentiation process. qRT-PCR was used to evaluate the expression of the pluripotency marker Oct4 and the NK biomarker CD56 at different cell stages of differentiation (stages: iPSC, EB6, CD5, and CD56). Early in the differentiation process, the cells expressed high levels of Oct4. High levels of CD56 expression were detected in the final iNK stage.

CAR Expression Throughout Differentiation

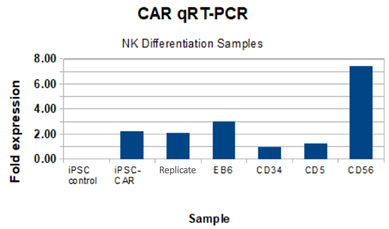


Figure 4: CD19-CAR expression at all cell stages of differentiation. qRT-PCR results suggest that CD19-CAR was expressed throughout differentiation, including the initial iPSC and the final iNK cell stages.

cGMP Compliant iPSC Cell Genome Editing Platform

This study in now being replicated in our new GMP facility. With our cGMP-compliant protocols in place, cell banking of our cGMP-grade iPSC Line (ASE-9280: from CD34+ Cord Blood, Male) was completed. Next steps have begun and will continue throughout the remainder of 2022.

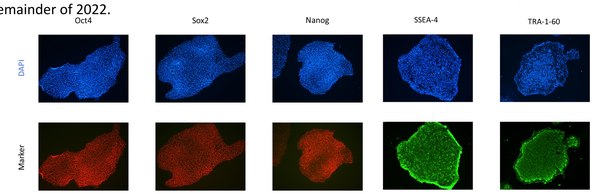


Figure 5: Pluripotency marker characterization of Applied StemCells's cGMP Line (ASE-9280). The cGMP cells expressed common iPSC biomarkers (Bottom Row: Oct4, Sox2, Nanog, SSEA-4, and TRA-1-60; Top Row: Corresponding DAPI nuclear staining).

Conclusion

1. In this study we successfully knocked-in a single-copy of CD19-CAR at the safe harbor locus of our TARGATT™ Master iPS cells and differentiated the CAR-positive iPSCs into iNK cells while maintaining CD19-CAR expression throughout all cell stages of the differentiation process.
2. cGMP manufacturing processes for iPSC reprogramming, gene editing (TARGATT™ and CRISPR), iPSC differentiation, and cell banking are now in place allowing us to move this study into our GMP facility.
3. We completed the cell banking and full characterization of ASC's cGMP-grade iPSC Line (ASE-9280: from CD34+ Cord Blood, Male), and we are moving forward with the development of a cGMP-grade TARGATT™ master iPSC line for therapeutic cell product development.