323 TALEN®-based Gene Edited iPSC-derived NK (iNK) Cells Demonstrate Enhanced Antitumor Activity

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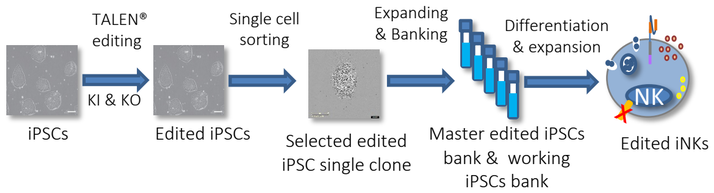
Background & Methods

Background: Natural killer (NK) cell therapies have shown a great promise for solid and liquid tumors in initial clinical trials. NK cells are innate immune cells with distinct potential safety and efficacy advantages compared to adoptive T-cell therapies. However, there are limitations with the persistence and immunosuppression of these cells in the tumor microenvironment. Furthermore, multiple sources of NK cells have been used in clinical trials and there are challenges with manufacturing homogeneous and high doses of these cells. Induced Pluripotent Stem Cell (iPSC)-derived NK cells offer an opportunity to generate unlimited and homogenous NK cells for allogeneic off-the-shelf therapies. We combined Cytovia's iNK cell platform with Cellectis TALEN®* gene editing technology to improve potency and the manufacturing process. Clonally edited iPSC lines were generated by Knocking In *IL-15* and Knocking Out *TGFβ2R* to improve the persistence and antitumor activity, respectively. Edited iPSCs were differentiated into iNK cells with high efficiency using Cytovia's proprietary platform.

Methods: iPSCs were edited at the *B2M* locus with TALEN® along with a template to Knock In *IL-15* by electroporation. Another TALEN® was sequentially used to Knock Out *TGFβR2*. Edited single cell iPSCs were printed, expanded, and screened for clone selection. Selected clones were sequence verified by NGS and samples were submitted for off-target assessment by GUIDE-seq. Expression of IL-15 and TGFβR2 was measured by ELISA and western blot, respectively. iPSCs were differentiated into iNK cells and analyzed by surface staining and flow cytometry. Cytotoxicity assay was performed against K562 tumor cells using iNK produced from Cytovia's proprietary platform.

Development of Cytovia's TALEN®-edited iPSC platform

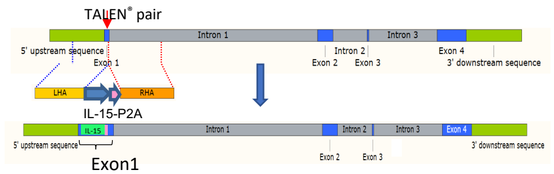
An exemplary workflow for manufacturing TALEN® edited iPSC-NK cells.



Strategies in generation of TALEN®-edited iPSC clones

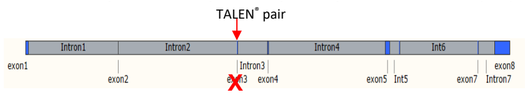
Step 1

Step 1: Gene structure of human B2M (beta-2-microglobulin) and Knock In (KI) strategy of IL-15 in exon1 locus.



Step 2

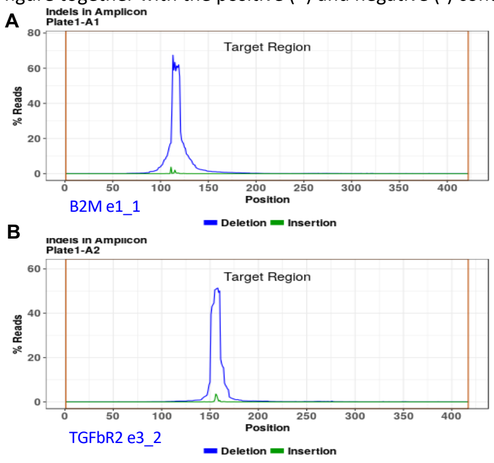
Step 2: Gene structure of human TGFβR2 and sequentially disruptive Knock Out (KO) strategy in exon3 locus.

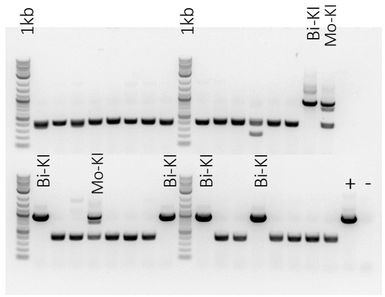


Results

1. TALEN® demonstrates high gene editing efficiency in iPSC cells

The KO editing efficiency at *B2M* or *TGFbR2* locus in iPSC cells were assessed by amplicon sequencing (A -B). The KI efficiency in iPSC cells were analyzed by PCR (C) for the sorted single clones. The 1kb ladder was labelled in the figure together with the positive (+) and negative (-) control.

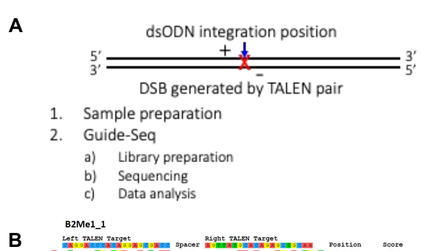


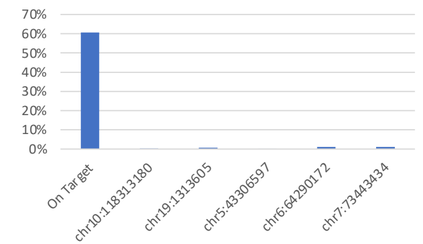


- The bi-allelic KI (Bi-KI) percentage is 15.63% (5/32), while the mono-allelic KI (Mo-KI) percentage is 6.3% (2/32).
- The bi-allelic KI efficiency is sufficient for sorting and generating the positive BI-KI iPSC single clones as starting material.

2. TALEN® demonstrates specificity of gene editing & optimum offsite profile

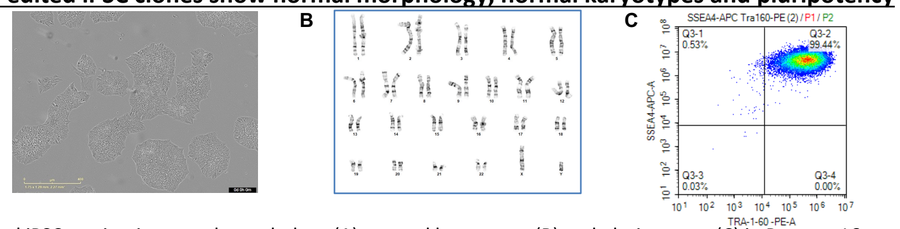
The basic workflow for TALEN putative off-target sites assay (A). TALEN® (B2M e1_1) putative off-target sites assay by company A (B). TALEN® (B2M e1_1) putative off-target sites assay by company B (C).





- The potential off-target sites identified by readout 1 and readout 2 in iPSC cells are completely non-overlapping. These results suggest that the TALEN offsites likely are background noise.
- Non-bias genome wide profiling with dsODN showed our TALEN® have optimal offsite profile.

3. TALEN® edited iPSC clones show normal morphology, normal karyotypes and pluripotency



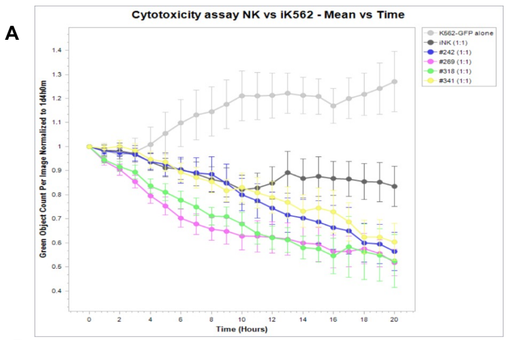
- Dual edited iPSCs maintain normal morphology (A), normal karyotypes (B) and pluripotency (C) in Passage 16 cells by our customized platform. These results pave the way for generating Cytovia's master edited iPSC cells bank.

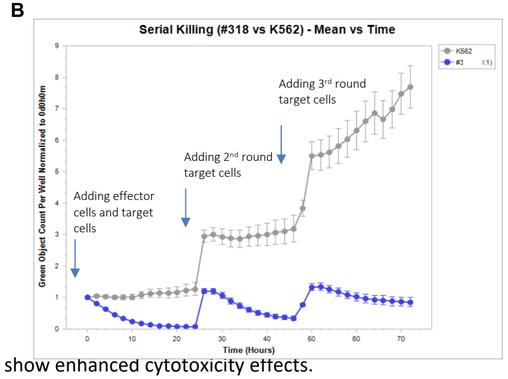
4. TALEN®edited iNK clones show high purity and specific phenotype

iNK Clone	% of CD56+ cells	IL-15 KI	TGFbR2 KO
242	98.28%	+	-
269	96.94%	-	+
318	96.72%	+	+
341	96.85%	+	+

5. TALEN® edited iNK cells show enhanced serial killing effects vs K562 tumor cells

The cell-killing ability of edited iNK cells was determined by ET ratio 1:1 using an IncuCyte S3 instrument with real-time and automated analysis. K562 alone and non-edited iNK cells were used as controls (A). Serial cytotoxic activity of edited iNK cells against K562 tumor cells was similarly analyzed using an IncuCyte S3 instrument (B).





- The edited iNKs show enhanced cytotoxicity effects.
- The edited iNK cells demonstrate functional persistence

Conclusions

- Cytovia's iPSC-NK platform combined with TALEN® gene-editing robustly and reliably generated single and double bi-allelic Knock-in cell edited iPSC clones with optimal offsite profile which were expanded and differentiated into functionally improved edited iNK cells.
- iNK cells edited with an *IL-15* Knock-In and *TGFβR2* Knock-Out resulted in enhanced antitumor activity compared to unedited iNK cells.
- The editing and manufacturing process will enable clinical evaluation of these product candidates.

*TALEN® is a trademark owned by Cellectis

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