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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Platinum Talen-Mediated Knock-in of Single-Stranded DNA Templates Facilitates Manufacturing of Clinical-Scale Non-Virally Gene-Edited T Cells from Peripheral Blood

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Background:
During the past decade, technologies that manufacture gene-modified immune cells, such as chimeric antigen receptor T cells, have been rapidly evolved and introduced in human clinics. T cells reprogrammed to express antigen-specific T-cell receptors (TCR-T) are an up-and-coming tool to treat intractable infection or malignant neoplasm in a personalized manner. However, several caveats still exist in the production of TCR-T for clinical use, because competitive surface expression of endogenous and transduced TCRs might hamper desired expression of transduced TCR. Moreover, mispairing of TCR subunits could mediate unexpected harmful immune complications. Therefore, in this study, we aimed to develop a method to introduce any desired TCRs in human primary T cells independent of interference with endogenous TCR by way of targeted knockout of TRA and TRB gene loci using highly-efficient transcription activator-like effector nuclease (TALEN), named Platinum TALEN (Sakuma T, Sci Rep 2013).

Methods:
We designed and synthesized two pairs of 5'-Cap-1 Platinum TALEN mRNA targeting the gene sequences that encode constant regions of human *TRA* (*TRAC*) and *TRB* (*TRBC*). TCR knockout efficiencies by these TALEN pairs were assessed by flow cytometric analysis using anti-CD3/TCR antibodies. Next, we constructed single-stranded DNA (ssDNA) homology-directed repair templates for the knock-in of desired TCR transgene into the TALEN target sequence of the *TRAC* locus. As a model TCR, we selected a 1G4 clone that reacts with NY-ESO-1-derived peptide in an HLA-A*02-restricted manner. We delivered these TALEN mRNA pairs and 1G4-encoding ssDNA by electroporation into primary human T cells collected from healthy volunteer adult donors after 72 hours of anti-CD3/CD28 beads activation. Genome-edited T cells were subsequently cultured in Therapeak ^{TM} X-VIVO15 (Lonza) supplemented with 10% human AB serum in the presence of 2-mercaptoethanol and interleukin-2 followed by second anti-CD3/CD28 beads activation. Density of T cells were maintained approximately at 1.0-3.0 x10 ^{6}/mL and transferred to larger flasks with additional fresh media as appropriate according to cell counts throughout the culture.1G4-transduced T cells were detected and counted by HLA-A*02:01 NY-ESO-1 tetramers at 17-22 days after electroporation. Additionally, we performed optical genome mapping analysis (Bionano Genomics) of Platinum TALEN-treated cells to screen genome-editing-associated large chromosomal structural variations (SVs) including unintended translocation.

Results:
Platinum TALEN mRNA pairs targeting *TRAC* and *TRBC* yielded a high proportion (>70%) of CD3/TCR negative T cells without the cost of compromised cell viability. By co-electroporation of 1G4 ssDNA and these Platinum TALEN mRNA pairs, we successfully obtained an average of 10.5 (range 2.2-19.0) million (n=5)NY-ESO-1-tetramer-positive cells with high viability from 3 millionprimary T cells until 3 weeks after electroporation. By flow cytometric analysis, a proportion of 1G4-tetramer positive cells that express Vβ chains other than Vβ13 (Vβ of 1G4) was approximately 10%. The PD-1-positive fraction in the

expanded 1G4-expressing T cells was less than 10%, while levels of TIM-3 expression were 40-60%. These tetramer-positive cells showed specific cytotoxicities against HLA-A*02-positive cells pulsed with cognate NY-ESO-1 peptides. In an optical genome mapping analysis of genomic DNA obtained from TALEN-treated T cells, we found unique SVs with relatively small sizes and low coverage but no large unique SVs with high coverage when compared with control genomic DNA. Additionally, no interchromosomal translocation was observed.

Conclusions:

We have successfully designed Platinum TALEN mRNA pairs targeting *TRAC* and *TRBC* which facilitate the production of genome-edited human primary T cells. We also developed an electroporation and expansion culture protocol that enables us to produce viable genome-edited 1G4-transduced T cells at a large cell dose equivalent to a clinical scale. Collectively, these results suggest that targeted orthotopic knock-in of antigen-specific TCR-encoding ssDNA into the TCR locus by the use of Platinum TALEN is a promising strategy that can be applied for the clinical manufacturing of therapeutic TCR-T cells.

Disclosures Magoori: *Repertoire Genesis Inc.:* Current Employment. **Sato:** *Repertoire Genesis Inc.:* Current Employment. **Fukunaga:** *Repertoire Genesis Inc.:* Current Employment. **Budirahardja:** *Repertoire Genesis Inc.:* Current Employment. **Suzuki:** *Repertoire Genesis Inc.:* Current Employment, Current equity holder in publicly-traded company. **Yamamoto:** *Pt-Bio Inc.:* Consultancy. **Ichinohe:** *AsahiKasei Pharma Co.:* Honoraria, Research Funding; *Novartis:* Honoraria; *Kyowa Kirin:* Research Funding; *Abbvie Co.:* Honoraria, Research Funding; *Nippon Shinyaku Co.:* Honoraria, Research Funding; *Sumitomo Pharma Co:* Honoraria, Research Funding; *Ono Pharmaceutical Co.:* Honoraria; *Takeda Pharmaceutical Co.:* Honoraria; *Nippon Kayaku Co.:* Honoraria; *Wakunaga Pharmaceutical Co., Ltd.:* Research Funding; *Chugai:* Honoraria, Research Funding; *Repertoite Genesis Inc.:* Research Funding.

OffLabel Disclosure: Platinum TALEN for T cell genome editing

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