

## Immuno-Engineering

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**DEVELOPMENT OF B CELL MATURATION ANTIGEN (BCMA)-SPECIFIC CD8<sup>+</sup> CYTOTOXIC T LYMPHOCYTES USING INDUCED PLURIPOTENT STEM CELL TECHNOLOGY FOR MULTIPLE MYELOMA**

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**Background** A strategy for reversal of T cell exhaustion is reprogramming of antigen-specific CTL to early lineage memory T cells with selective anti-tumor activities. To accomplish this goal, we epigenetically reprogrammed BCMA-specific CD8<sup>+</sup> CTL to a pluripotent state through key defined transcription factors, established “induced Pluripotent Stem Cells (iPSC)” exhibiting transcriptional and epigenetic features, re-differentiated them back into antigen-specific CTL and evaluated their properties and functional activities against multiple myeloma (MM).

**Methods** Functionally active IFN- $\gamma$  producing HLA-A2 heteroclitic BCMA<sub>72-80</sub> (YLMFLLRKI)-specific CD8<sup>+</sup> CTL were applied for iPSC via transduction of four reprogramming factors (OCT3/4, SOX2, KLF4, c-MYC). Upon characterization of the BCMA-specific iPSC with high pluripotency potential, embryoid body was formed from the iPSC and further polarized into mesoderm layer development as evidenced by upregulation of transcriptional regulators (ABCA4, BMP10, CDH5, FOXF1, HAND1, PLVAP, SNAI2, TBX3). Next, BCMA-specific embryoid body-derived hematopoietic progenitor cells (HPC; CD34<sup>+</sup> CD43<sup>+</sup>/CD14<sup>-</sup> CD235a<sup>+</sup>) were sorted and induced to undergo T cell differentiation in the presence of Fc-DLL4 signaling and retonectin.

**Results** Our RNAseq analyses demonstrated unique transcriptional profiles of HPC from different iPSC clones committing to CD8<sup>+</sup> T cells or other cell lineages (monocytes/granulocytes, B lymphocytes/NK cells). Principal component analyses demonstrated a high similarity and low variability of transcription profiles within the replicates of HPC committed to the same cell lineage. In addition, distinct genome-wide shifts and differential gene expression profiles were detected in HPC committed to each specific cell differentiation pathway. Specifically, the HPC commit to CD8<sup>+</sup> T cells utilized a diverse repertoire of modulators promoting development of T cell maturation, specific immune response regulation, memory T cells, cytotoxicity and interferon induction, which were significantly higher than shown in HPC that differentiate to other cell lineages. In parallel, specific repression genes were identified in the HPC commit to CD8<sup>+</sup> T cells, which develop TGF- $\beta$  receptor, rearrangement of Ig heavy chain genes and inhibitory receptors. The T cells differentiated were mainly CD45RO<sup>+</sup> memory CTL and fully rejuvenated without immune checkpoints expression and regulatory T cells and with high anti-MM activities.

**Conclusions** These findings identify genetic and epigenetic mechanisms and regulatory elements, which play key roles during lineage specific commitment of HPC developed in iPSC into CD8<sup>+</sup> CTL and help to further design a next generation of regenerative medicine that provide the appropriate signals for T cell lineage commitment from progenitor cells.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.1220>