EMPOWERING IPSC-DERIVED INK CELLS WITH MULTIPLE GENE EDITS TO IMPROVE PERSISTENCE AND ANTI-TUMOR EFFICACY

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BACKGROUND
A unique attribute of iPSCs is the ability to perform sequential gene edits thus enabling development of highly engineered cell therapies. Here, we describe our next generation platform for iPSC-derived NK cells (iNK cells) that include genetic enhancements for improved allo-evasion, cell fitness, tumor targeting, in vivo imaging, and safety. By adopting a common progenitor strategy, an engineered iPSC master cell bank will be used as the starting point for future therapies across diverse indications.

METHODS
Clinical grade iPSC lines were engineered using Mad7 CRISPR endonuclease. β2 microglobulin was disrupted and a bicistronic transgene encoding both HLA-E and HLA-G was inserted. The gene encoding CIITA was disrupted and a bicistronic transgene encoding HSV-TK and PSMA ectodomain was inserted. A transgene encoding IL-15/IL-15Ra was delivered into the NKG2A locus, and a bicistronic transgene encoding CD16 and NKG2D was delivered into the CD70 locus.

RESULTS
iNK cells exhibited uniform expression of each transgene. Functional studies demonstrated that HLA-E/G enabled evasion of allogenic NK cells; IL-15/IL-15RA enhanced persistence of iNK cells both in vitro and in vivo; PSMA expression on the cell surface was confirmed through binding of a PSMA-specific fluorescent tracer; HSV-TK enabled the elimination of iNK cells in culture in the presence of ganciclovir; NKG2D enhanced elimination of tumor lines expressing the stress ligands MICA/MICB; and high-affinity CD16 enabled ADCC-mediated killing of CD20-expressing target cells in the presence of rituximab. HLA-A/B/C, HLA-DR/DP/DQ, NKG2A, and CD70 were absent from iNK cells.

CONCLUSIONS
A clonal iPSC line was derived with eleven total genetic modifications to improve allogeneic cell therapy for cancer. Future iNK cell therapies will be derived from the common progenitor by addition of a CAR(s) for indication-specific tumor targeting.