Overcoming host histocompatibility barrier to create a renewable source of Off-the-shelf effector lymphocytes for adoptive immunotherapy

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ABSTRACT

Encouraging clinical outcomes in autologous cellular immunotherapy have garnered hope and excitement. However, limitations of patient-derived cancer immunotherapies remain to be addressed to deliver reliable and efficacious therapies with broader applicability. Induced pluripotent stem cells (iPSCs) are a unique, renewable source for overcoming the generation of cellular therapeutics and represent a highly promising approach for overcoming many of the limitations of autologous therapy. To advance the promise of iPSC technology as an “off-the-shelf” (OTS) source of cellular therapeutics, several considerations need to be addressed. Ensuring the persistence of allogeneic OTS therapies after adoptive cell transfer across histocompatibility barriers is a key requirement. Establishing a master cell line from genetically engineered clonal iPSC lines with the capacity to continuously generate homogenous populations of highly functional effector cells will also be necessary.

Here we demonstrate a comprehensive approach for the generation of immune tolerant effector cells derived from a genetically engineered iPSC master cell line. We successfully combined deletion of classical human leukocyte antigen molecules with expression of immunosuppressive proteins to generate clonal iPSC lines with the ability to escape immune rejection. Utilizing in vitro quantitative live cell analysis we show that OTS-iPSCs elicit a significantly decreased cytotoxic response from both peripheral blood (PB)-NK cells (67.9 vs. 91.4% survival at 3.1 E:T ratio) and PB-T cells (>2.7 fold greater number of OTS-iPSC derived cells remaining at 88 hrs). Additionally, mixed lymphocyte reactions employing unaltered PB mononuclear cells resulted in significantly decreased activation and proliferation of CD8+ T cells (63.4 vs. 29.6%). CD4+ T cells (70.9 vs. 17.3%) and NK cells (46.8 vs. 11.6%). In preclinical mouse models we demonstrate that OTS-iPSCs exhibit improved persistence in vivo. Bilateral engraftments were established in non-conditioned, fully immune-competent recipient mice using luciferized wildtype and OTS-iPSCs. Daily bioluminescence imaging revealed a significant increase in persistence of OTS-iPSCs during the 48-196 hour post injection window (>5.5 fold greater luminescence at 96 hrs). Using our potent chemically-defined stage-specific monolayer hematopoietic differentiation platform, we demonstrate that OTS-iPSC-derived CD34 expressing hematopoietic cells are reproducibly scaled and readily give rise to functional lymphocytes carrying the engineered targeted modality in a homogenous manner (95 +/- 5%). The outlined preclinical data illustrate that iPSCs are an ideal renewable source for OTS hematopoietic cell-based immunotherapies and represent a potentially exponential advancement in adoptive immunotherapy.

SUMMARY

- It is possible to extensively and accurately engineer multiple modalities into a single pluripotent stem cell to generate a master cell line for off-the-shelf immunotherapeutics.
- Increased persistence and immune evasion can be achieved via HLA class I modifications.
- Overexpression of immunosuppressive proteins can further improve the persistence of HiPSC-derived effector cells.
- Engineered clonal hiPSC lines can be differentiated into effector cell types such as T lymphocytes that can be matured and expanded in vitro for the generation of histocompatible off-the-shelf therapeutics.

REFERENCES


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RESULTS

Figure 1. Schematic representation of pluripotent cell platforms for off-the-shelf adoptive immunotherapy. Human induced pluripotent stem cells (hiPSCs) represent a rich source of immune effector cells for cell-based therapies. To create a master cell line that has an extended capacity for all patients, the key challenge of overcoming host alloreactivity needs to be addressed. (A) Platforms for the generation of patient-engineered iPSCs with multiple immunosuppression and immune suppression modules represents an optimal platform for an extended life cell immunotherapy.