iPSC-Derived Macrophages Engineered to Express IL-12 Enhance Cancer Cell Killing of a Multi-Cell Type Therapeutic Platform

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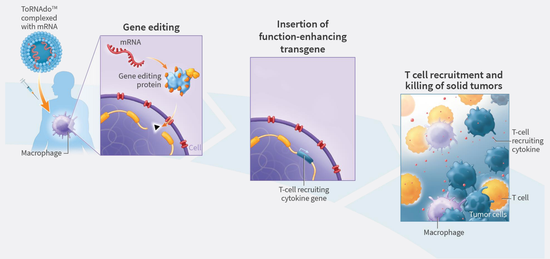
Summary

The immunologically cold microenvironment of solid tumors limits the ability of immune cells to infiltrate and attack cancer cells. The systemic injection of potent cytokines such as interferon gamma (IFNγ) and interleukin 12 (IL-12) have shown tumor shrinkage in mouse models, but have faced challenges in human clinical trials due to dose-limiting toxicities. Myeloid cells, including macrophages, can deliver cargo to the solid tumor microenvironment. However, autologous macrophages are limited by their resistance to genetic engineering, limited expansion potential, cost of characterization and the limited quantity of cells attainable via leukapheresis. Bioreactor-based differentiation of induced pluripotent stem cells (iPSCs) into macrophages can overcome the limitations of autologous macrophages, enabling the generation of large numbers of well characterized, genetically identical, engineered macrophages.



Conclusions

Engineered iPSC-derived macrophages offer a path to repolarize the tumor microenvironment and translate the clinical success of T cell immunotherapies targeting hematologic cancers to solid tumors, which comprise 90% of cancer cases. Here we show a scalable platform for producing iPSC-derived macrophages engineered to express the potent immunostimulatory protein IL-12. Engineered iPSC-derived macrophages may thus prove useful for the development of therapies designed to deliver biologics previously limited by toxicity or bioavailability directly to the tumor microenvironment.

1 Bioreactor Differentiation of iPSCs to iMacrophages

A 28-day 3-stage process

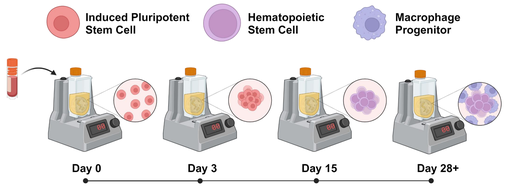


Figure 1. **Differentiation timeline from iPSC to iMacrophage.**

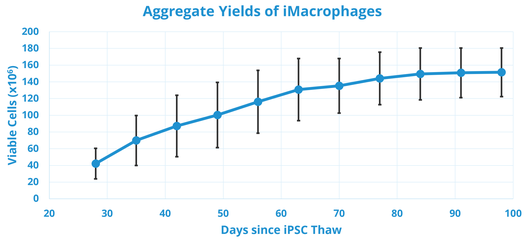


Figure 2. **Aggregate yields of iMacrophages from small scale bioreactors over time.** The differentiations were performed in 100mL vertical flywheel bioreactors, with harvests beginning on day 28. Harvested cells were strained through a 70µm strainer before counting to isolate single cells from embryoid bodies. 4x10^6 iPSCs were seeded on day 0.

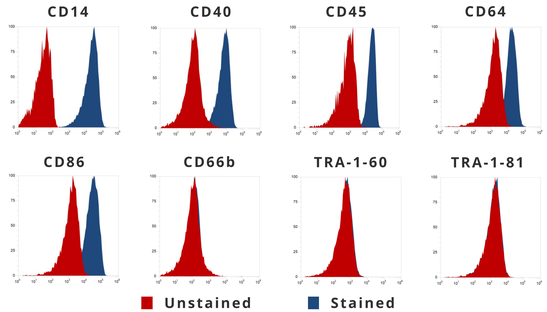


Figure 3. **Flow cytometry characterization of iMacrophages.** The isolated single cells were then analyzed for surface marker expression to confirm their iMacrophage identity using the Attune NxT flow cytometer. iMacrophages are characterized as CD14+/CD45/CD64+/CD66b-/TRA-1-60-/TRA-1-81-. iMacrophages were flowed on day 28 of the differentiation.

2 Transfection of iMacrophages with Functional mRNA Encoding IL-12

Comparing two construct designs

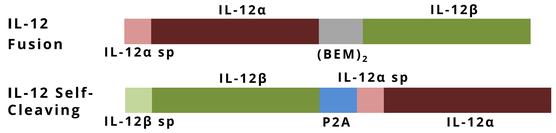


Figure 4. **Design of mRNA constructs encoding functional IL-12.** The IL-12 cytokine exists as a heterodimer of two subunits, IL-12α and IL-12β. These subunits can be placed in a single mRNA construct for expression following transfection by either linking them together with a flexible linker or by using a self-cleaving P2A peptide. sp: signal peptide, BEM: bovine elastin motif.

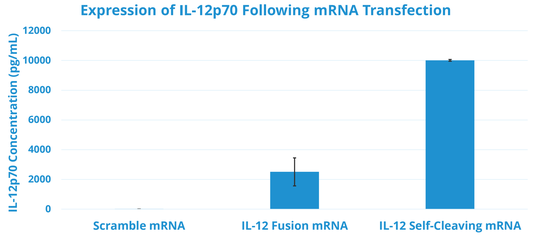


Figure 5. **Production of IL-12p70 following mRNA Transfection.** Only the heterodimer of IL-12α and IL-12β forms IL-12p70, the pro-inflammatory cytokine that stimulates lymphocytes. Functional IL-12p70 was detected using an IL-12p70 ELISA. 1x10^6 iMacrophages were plated and subsequently transfected with 1ug of mRNA encoding the IL-12 constructs or scramble RNA as a control. The culture supernatant was harvested 48 hours after transfection.

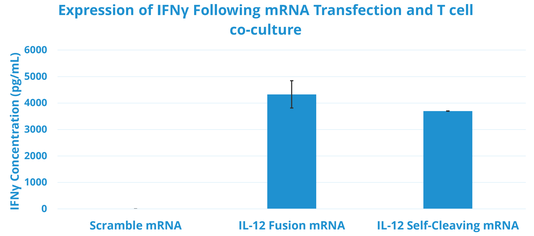


Figure 6. **Production of IFNγ following mRNA Transfection and T cell co-culture.** 1x10^6 iMacrophages were plated and subsequently transfected with 1ug of mRNA constructs encoding IL-12, or scramble RNA as a control. The media was changed 4 hours later, with 5x10^5 T cells added per well. The culture supernatant was harvested 48 hours after transfection. Functional IFNγ was detected using an IFNγ ELISA.

3 Enhanced Cancer Cell Killing *in vitro* of SK-OV-3 and MDA-MB-231 Cell Lines

Synergistic anti-tumor effect from iMacrophages and T cells

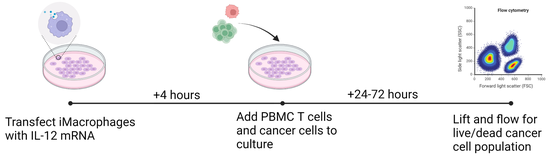


Figure 7. **Experimental workflow of cancer cell cytotoxicity assay.**

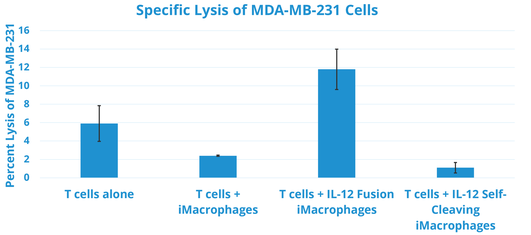


Figure 8. **Cytotoxicity assay towards MDA-MB-231 cells.** iMacrophages were plated and transfected with mRNA encoding IL-12. 4 hours later, MDA-MB-231 triple negative breast cancer cells and T cells were added to the culture for 24 hours at a 5:1 effector-to-target ratio. The cells were then lifted and stained for CD45 and Live/Dead Near IR, and flowed on the Attune NxT flow cytometer. Lysis percentage was calculated as the percentage of CD45- Dead+ cells minus MDA-MB-231 only control wells.

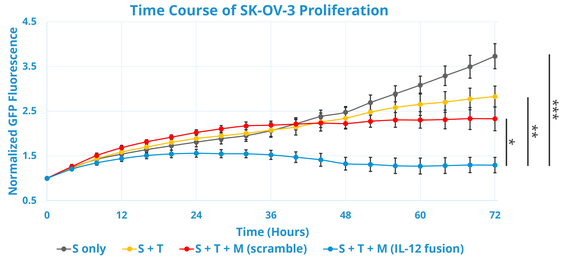


Figure 9. **Cytotoxicity assay towards GFP-SK-OV-3 cells.** iMacrophages were plated and transfected with mRNA encoding IL-12. 4 hours later, GFP-SK-OV-3 ovarian adenocarcinoma cells and PBMC T cells were added to the culture for 72 hours at a 10:1 effector-to-target ratio. The cells were cultured on an Operetta CLS and analyzed for GFP expression every 4 hours. S: GFP-SK-OV-3 cells, T: PBMC T cells, M: iMacrophages. Statistical significance evaluated using a 2-tailed student's T test. P-value: *=0.011, **=0.0006, ***=0.00008.

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