B2M-KO iMSCs Better Suppress T Cell Proliferation by Upregulating IDO1 in Response to Proinflammatory Signals

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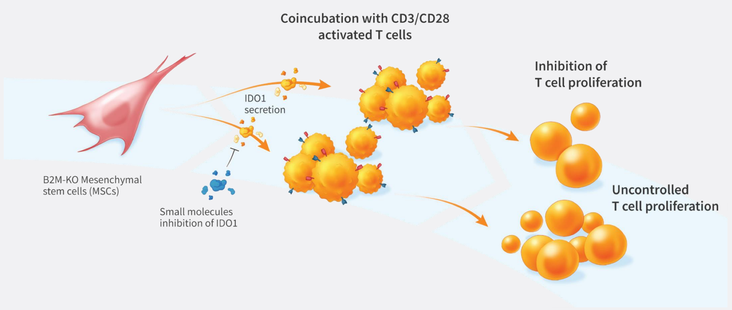
Summary

- Mesenchymal stem cells (MSCs) are multipotent, relatively non-immunogenic, and can differentiate according to environmental cues, making them an ideal candidate for numerous allogeneic cell therapies.
- MSCs have consistently demonstrated excellent safety profiles in clinical trials, but poor therapeutic responses have impeded their translational success.
- Induced pluripotent stem cell (iPSC)-derived MSCs (iMSCs) engineered with stealthing features are designed to address the shortcomings of traditional MSC therapeutics, including source heterogeneity, limited expansion potential, and suboptimal pharmacokinetics.
- Here, we report on the development and characterization of β-2-microglobulin-knockout iMSCs (B2M-KO iMSCs) derived from mRNA-reprogrammed iPSCs.



Conclusions

- B2M-KO iMSCs address the common shortcomings of tissue-derived MSC therapy candidates.
- B2M-KO iMSCs express more of the immunoregulatory enzyme, IDO1 than native iMSCs in response to IFNγ.
- The IDO1 expressed by B2M-KO iMSCs is critical for their ability to suppress CD4+ and CD8+ T cells.
- These results suggest that B2M-KO iMSCs may prove useful for the treatment of T cell mediated inflammatory conditions.

1. Development of B2M-KO iMSCs

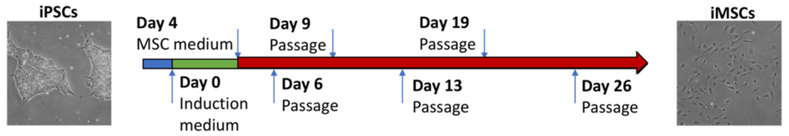


Figure 1. B2M-KO iMSCs were differentiated from B2M-KO iPSCs. The β-2-microglobulin (B2M) gene was first inactivated by introducing a biallelic, 14 base pair deletion at exon 2 of the B2M gene using mRNA encoding UltraSlice™, a proprietary gene-editing endonuclease. Following colony selection, knockout verification was confirmed by amplicon sequencing. B2M-KO iPSCs were then differentiated into iMSCs using our previously published protocols.

	ISCT-Defined MSC Markers			iPSC Marker	HSC Marker
	CD73	**CD105**	**CD90**	**TRA-1-81**	**CD34**
B2M-KO iMSCs	99%	99%	99%	<1%	<1%
Control Cells	<1% *iPSCs*	<1% *iPSCs*	<5% *PBMCs*	99% *iPSCs*	90% *iPSC-Derived HSCs*

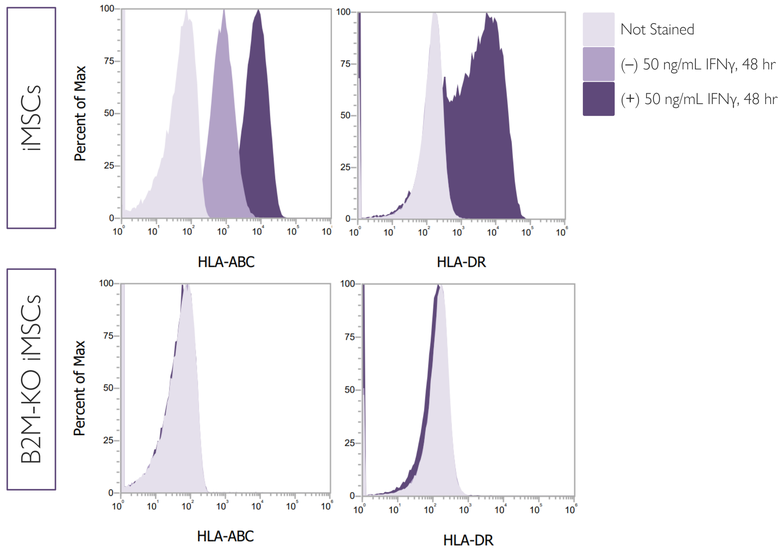


Figure 2. B2M-KO iMSCs Do Not Express HLA-I or HLA-DR. Flow cytometry confirmed that in native iMSCs, HLA-I was expressed and upregulated in response to activation. B2M-KO iMSCs had no expression of HLA-I both before or after activation. Native iMSCs did not express HLA-DR until activated, where it was highly upregulated. B2M-KO iMSCs did not express HLA-DR both before or after activation.

2. B2M-KO iMSCs Upregulate Treg Cells

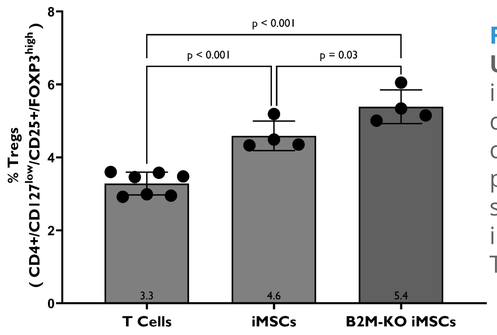


Figure 3. B2M-KO iMSCs Upregulate Treg Phenotype. iMSCs and T Cells were co-cultured 1:10 for 8 days. Flow cytometry revealed that the presence of B2M-KO iMSCs significantly upregulated the immunosuppressive Treg phenotype.

3. B2M-KO iMSCs Express More IDO1 than Native iMSCs

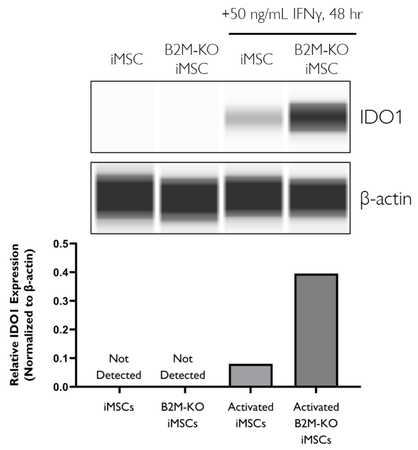


Figure 4. B2M-KO iMSCs Upregulated IDO1 Expression Following IFNγ Activation. Native and B2M-KO iMSCs were cultured for 48 hours in the presence or absence of 50 ng/mL IFNγ for activation. Cells were then harvested and protein was isolated for Western analysis. B2M-KO iMSCs were observed to have a higher relative IDO1 expression level, normalized to β-actin, than native iMSCs.

4. B2M-KO iMSCs Better Suppress PBMCs

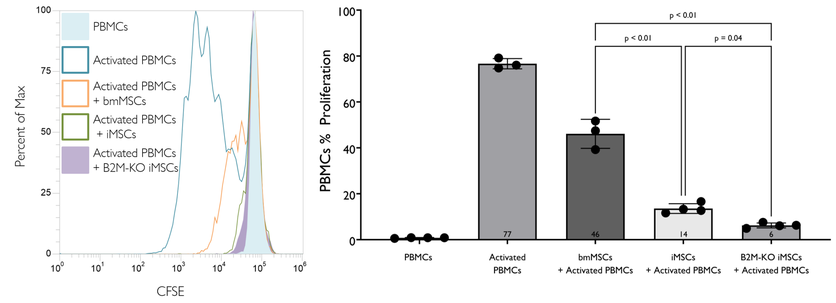


Figure 5. PBMC Suppression Assay. iMSCs and bone marrow-derived MSCs were each co-cultured with CFSE-labeled PBMCs in the presence of a T cell activator for 96 hours. While iMSCs showed greater PBMC suppression ability than bmMSCs, B2M-KO iMSCs showed the greatest PBMC suppression ability.

5. IDO1 is Critical for the Suppression of Inflammatory T Cells by B2M-KO iMSCs

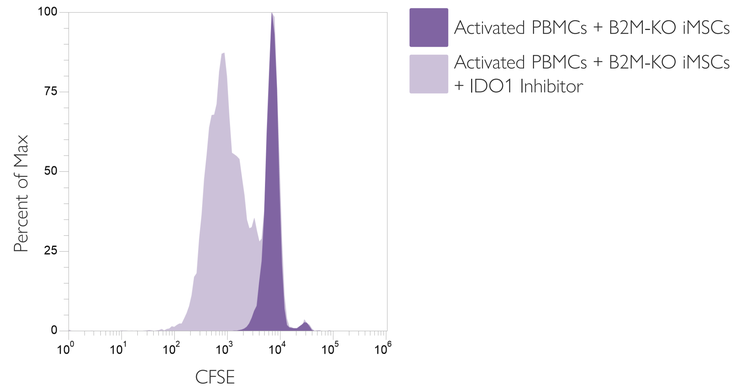


Figure 6. IDO1 Inhibition Negatively Affects the PBMC Suppression Ability of B2M-KO iMSCs. B2M-KO iMSCs were co-cultured with CFSE-labeled PBMCs in the presence of a T cell activator and a small-molecule IDO1 inhibitor for 96 hours. B2M-KO iMSCs exhibited significantly reduced suppression of PBMC proliferation when IDO1 was inhibited.

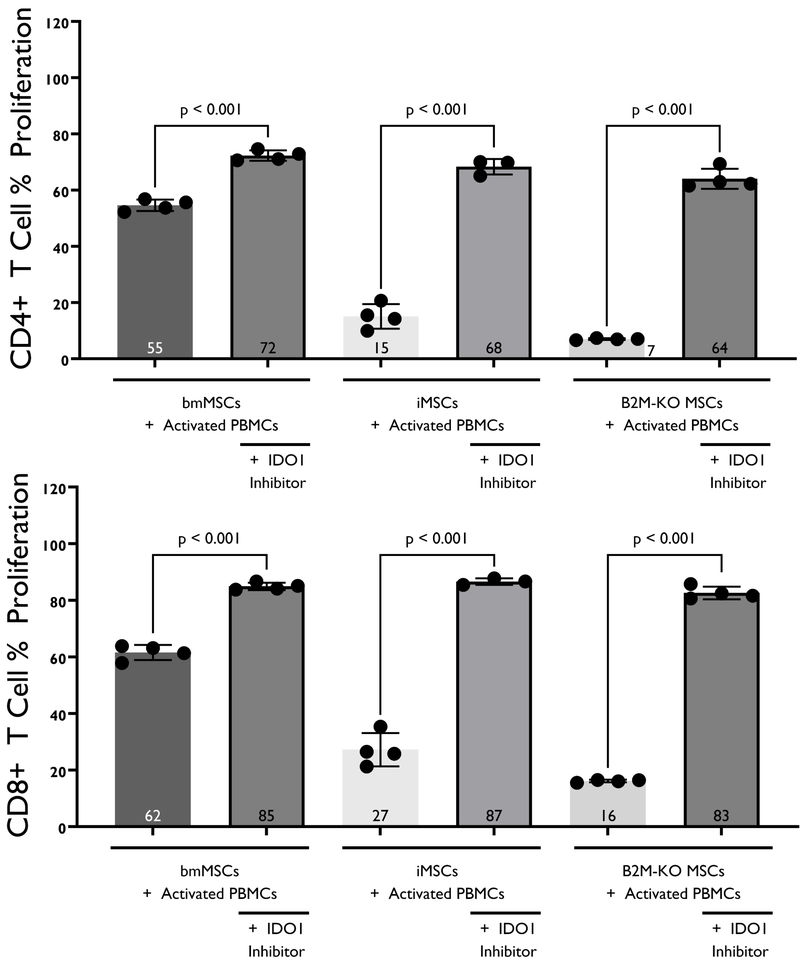


Figure 7. IDO1 is critical for bmMSC, iMSC, and B2M-KO iMSC mediated suppression of CD4+ and CD8+ T Cell Proliferation. Cells were co-cultured with CFSE-labeled PBMCs in the presence of a T cell activator and a small-molecule IDO1 inhibitor for 96 hours. For all MSC groups, IDO1 inhibition attenuated T cell suppression.