iPSC-derived γδT with Novel Combinatorial Knockout Demonstrated Significant Anti-tumor Activity and Extended Longevity Without Cytokine Support

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Abstract

The effectiveness of allogeneic cell therapy is hampered by the lack of persistence and durable anti-tumor activity. To overcome these limitations, we combined the advantages of iPSC-derived allogeneic cell therapy with a novel set of genetic modifications.

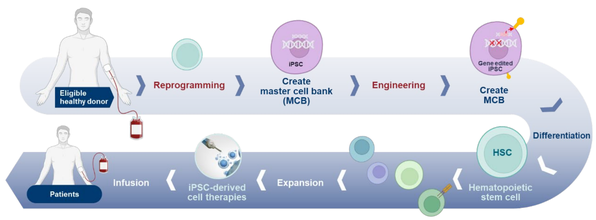
The IL-2/IL15-mediated signaling pathway is critical for T cell potency and persistence. The SOCS family regulates this pathway by inhibiting JAK/STAT signaling. Our work revealed that iPSC-derived γδT (iγδT) cells deficient in two SOCS family members (**CISH and Gene X**) exhibited synergistic enhancement in persistence and cytotoxic capabilities. Cytokine deficiency- and activation-induced cell death are key factors that diminish effector cell persistence post-infusion. To prolong cell survival, we identified a mediator of the apoptotic pathway (**Gene Y**) and found that deficiency of this proapoptotic protein is necessary for extending the survival of iγδT cell following IL-15 withdrawal. Additionally, we demonstrated that **FAS** deficiency protects functionally enhanced iγδT cells from FASL-induced apoptosis, thereby enhancing their durable killing ability. Apart from functional enhancements, elimination of **B2M and CIITA** combined with **HLA-E** overexpression enabled evasion of host immune surveillance.

Building on the foundation of functional enhancing gene edits, we further incorporated **chimeric antigen receptor (CAR)** and a proprietary **signal converter**. This signal converter transforms soluble factors generated during tumor-effector engagement into signaling that drives iγδT proliferation and activation. These engineered CAR-iγδTs exhibited robust durable killing of tumors without any supporting cytokines *in vitro.*

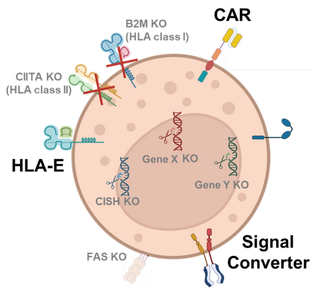
Together, our combination of gene edits reduced iγδT cell's threshold for IL2/IL15-mediated survival, promoting enhanced persistence and functionality in circulation prior- and post- tumor engagement. Meanwhile, our signal converter and CAR heightened efficacy and expansion during tumor engagement. In fact, in humanized mouse model mimicking high tumor burden without lymphodepletion, engineered CAR-iγδTs, in the presence of PBMC, eradicated the engrafted tumor.

This comprehensive strategy has enabled us to generate iPSC-derived γδT cells with extended persistence and enhanced anti-tumor efficacy without relying on exogenous cytokine support, highlighting its potential in allogeneic cell therapy.

iPSC-derived γδT Cell Therapy



Representative production journey depicting the generation of IPSC-derived γδT cells, starting from the donor specimen acquisition to therapeutic administration. Note that each batch of product manufacturing will be sourced directly from the MCB of the selected gene-edited iPSC clone.



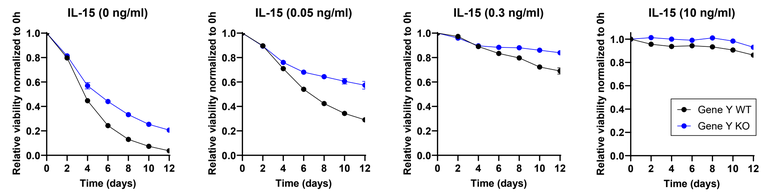
To enhance the persistence and efficacy of iγδT cells, we employed a gene editing strategy that includes:

1. **Immunoevasion:** Disabling HLA class I/II expression and overexpressing HLA-E to evade host immune responses.
2. **Efficacy:** Knocking out CISH and Gene X to enhance efficacy and persistence.
3. **Cell Survival:** Knocking out FAS and Gene Y to extend cell survival.
4. **Signal Conversion:** Introducing a novel signal converter to enhance cell proliferation, activity and durability in tumor environment.

Abbreviations: B2M, Beta-2 microglobulin; **CIITA,** Class II major histocompatibility complex transactivator; **HLA-E**, major histocompatibility complex class I type E; **CISH**, cytokine inducible SH2 containing protein; **FAS**, Fas cell surface death receptor; **Gene X**, suppressor of cytokine signaling; **Gene Y,** mediator of cell death; **SC**, Signal converter **5KO: B2M/CIITA/CISH/GeneX/GeneY knockouts**

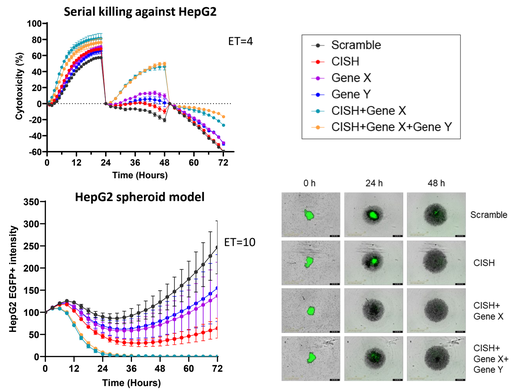
Functionally Enhanced iγδT with a Novel Combination of Genetic Editing

Knockout of a pro-apoptotic protein improves iγδT survival



Knockout of **Gene Y,** a pro-apoptotic protein, improved iPSC-derived γδT cell survival at physiological concentration of IL-15. Elevated survival was also observed in condition without any cytokine support.

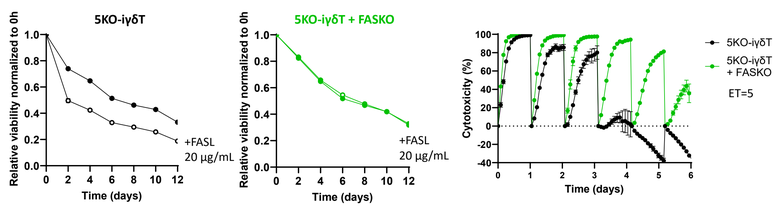
Novel combination of SOCS-family knockout enhances efficacy



Compared to single knockout of SOCS-family proteins, **CISH- and Gene X combined** knockout resulted in significant enhancement to iPSC-derived γδT cytotoxicity against HepG2 cells.

Both HepG2 serial killing and spheroid models were conducted in the absence of supporting cytokines.

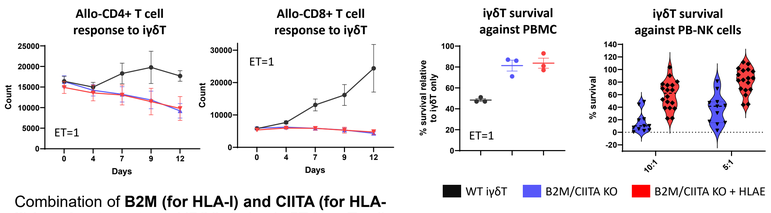
FAS KO prevents FASL-mediated apoptosis and promoted durable killing



FAS knockout prevented FASL-induced apoptosis in iPSC-derived γδT containing 5KO (B2M, CIITA, CISH, Gene X and Gene Y knockouts).

Combination of 5KO with **FAS knockout** promoted durable killing of HepG2 without exogenous IL-2/IL-15

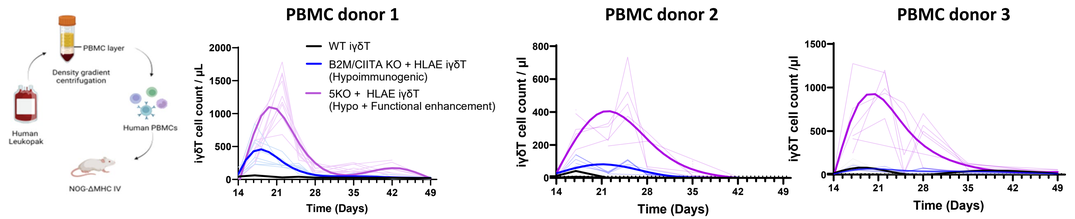
Hypoimmune modifications reduce allogeneic response against iγδT



Combination of **B2M (for HLA-I) and CIITA (for HLA-II) knockouts** protected iPSC-derived γδT from T cell-mediated allogeneic responses of multiple donors

B2M, CIITA with HLAE offered iPSC-derived γδT protection from PBMC and PB-NK cells

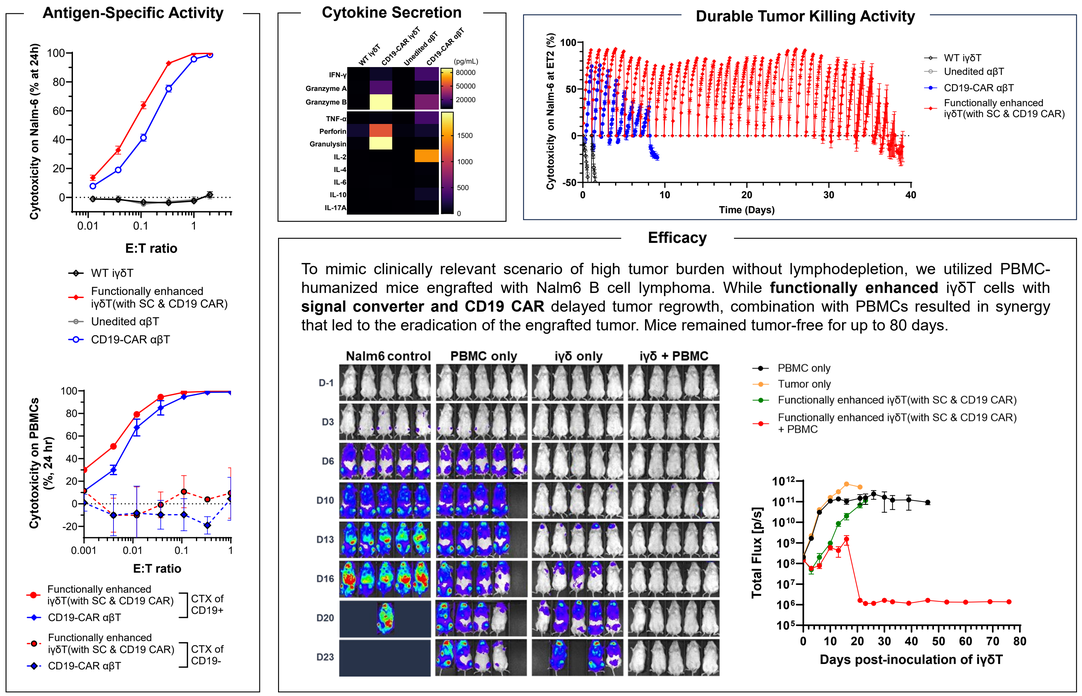
Combination of Hypoimmunogenic and Functionally Enhanced iγδT Exhibited Robust Proliferative Advantage in Humanized Mice



Utilizing PBMC engrafted humanized mice, we confirmed that **hypoimmune modifications** increased iPSC-derived γδT survival in the context of human immunity. **Functionally enhanced** gene knockouts further enhanced iPSC-derived γδT proliferation, which was consistent across multiple PBMC donors.

Functionally Enhanced iγδT with Signal Converter and CAR Demonstrated Improved Tumor Specific Cytotoxicity and Persistence Without Exogenous Cytokine Support

Utilizing **hypoimmune, functionally enhanced iγδT cells as a foundation, a signal converter (SC) and CD19-CAR were further incorporated**. These CD19-CAR iγδT demonstrated significant cytotoxicity at 24 hours and sustained long-term killing in tumor rechallenge studies, even in the absence of exogenous IL-2/IL-15. Correspondingly, increased secretion of tumor lysis enzymes further supported improved tumor-specific response.



Conclusion

BeiGene has developed a hypoimmunogenic, functionally enhanced iPSC-derived γδT cell therapy with specific editing of signal converter and CARs. By strategically modulating key signaling pathways involved in γδT cell efficacy and persistence, our approach led to enhanced efficacy while decreasing supporting cytokine requirement threshold, circumventing the potential disadvantages of constitutively active cytokine receptors.

- Engineered iγδT offer the opportunity to eliminate or mitigate apheresis, wait time, complexity, product variability, and failure of ex vivo engineering associated with the current approved autologous CAR T therapies.
- Engineered iγδT exhibit reduce reliance on IL-2/IL-15, enhancing persistence during pre- and post- tumor engagement circulation. While signal converter and CAR further facilitated durable anti-tumor killing. Together, this novel amalgamation holds significant promise for allogenic cell therapy