iPSC-derived CAR-γδT With Novel Combinatorial KO Demonstrated Extended Longevity And Profound Anti-tumor Efficacy Without Cytokine Support In Preclinical Studies

Abstract #4790

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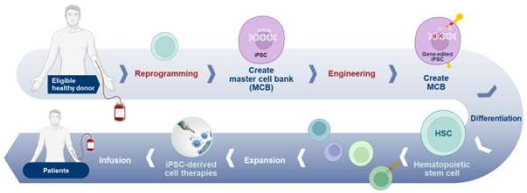
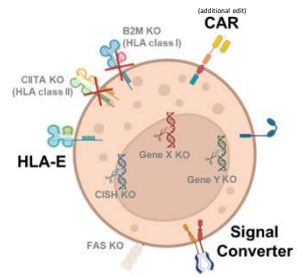
INTRODUCTION



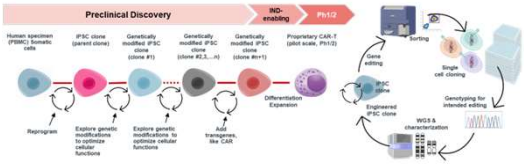
A representative product journey depicting the generation of iPSC-derived immune cells, including gamma delta T, starting from the donor specimen acquisition to therapeutic administration. Note that each batch of product manufacturing will be sourced directly from the MCB of the selected gene-edited iPSC clone.

METHODS



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.



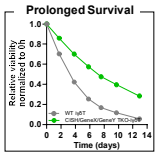
The GMP clone generation for iγδT cell therapy began with human PBMCs. After enrichment and reprogramming, iPSC clones were selected based on genome integrity tests, including residual gene expression, TCR sequencing, and morphological assessment. Qualified iPSC lines were cryopreserved and underwent multiple gene-editing rounds, with single cell sorting after each. Engineered iPSC clones were selected for cryopreservation into seed stocks based on cell health, on- and off-target editing, and genome integrity (via whole genome sequencing and oncogene mutation panels). Before differentiation, fully modified iPSCs will expand, mature into γδT cells, and after proliferation, iγδT cells be harvested as a pharmaceutical product.

ABBREVIATIONS

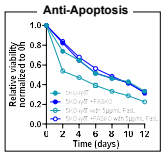
B2M, Beta-2 microglobulin; CIITA = Class II major histocompatibility complex transactivator; CISH, Cytokine inducible SH2 containing protein; FAS, Fas cell surface death receptor; Gene X, suppressor of cytokine signaling; Gene Y, a mediator of cell death; HLA-E, major histoco-mpatibility complex, class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

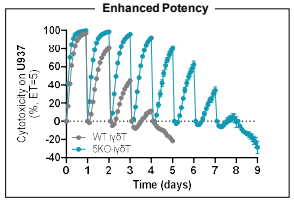
RESULTS – iγδT platform engineering

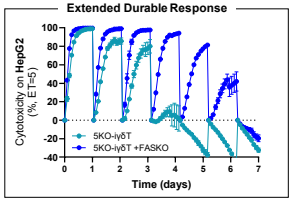
Combining gene knockouts to enhance key signaling pathways could improve cell survival without cytokine supplementation



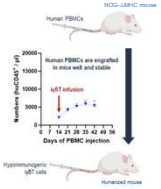
A particular gene knockout combination could enhance cell activity by preventing cytokine withdrawal- or FAS-FASL-induced cell death

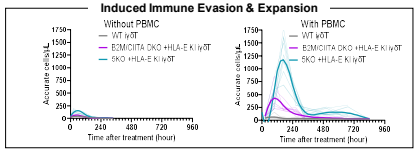


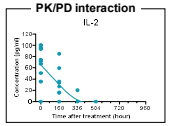




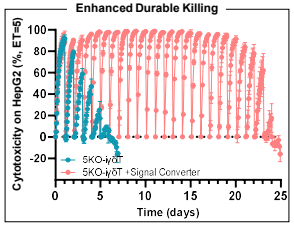
Hypoimmune modifications increased iγδT cell survival and persistence in the context of human immunity

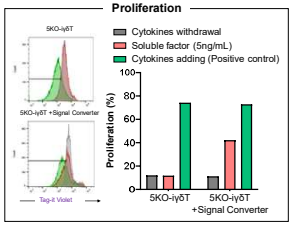




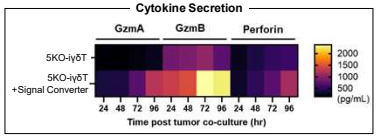


Combinatorial gene knockouts enhanced iγδT cell survival and multiplication *in vivo* due to IL-2 secretion from human PBMCs



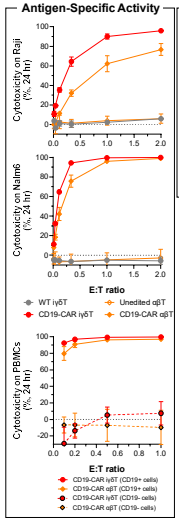


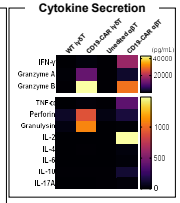
Modified iγδT cells expressing signal converter exhibited enhanced cytotoxicity, proliferation, and tumor-lytic enzyme secretion without exogenous cytokine support

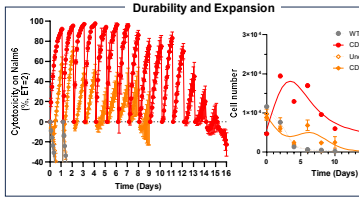


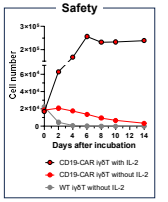
RESULTS – Proof-of-Concept of engineered iγδT with CAR

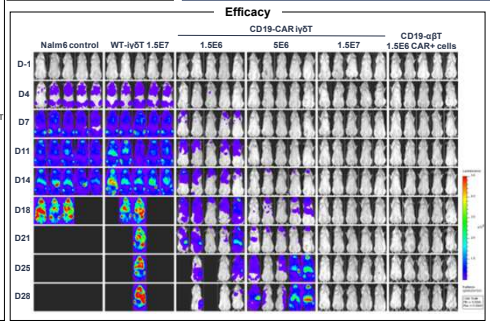
Utilizing this engineered iγδT platform, we incorporated CD19-CAR and demonstrated that these CD19-CAR iγδT (bulk) displayed significant cytotoxicity at 24 hours and sustained killing over the long term without any supporting cytokines in tumor rechallenge studies, along with increased secretion of tumor lysis enzymes. Furthermore, in the chosen B cell-malignancy model, CD19-CAR iγδT eradicated the engrafted tumor cells *in vivo* with increased proliferation.

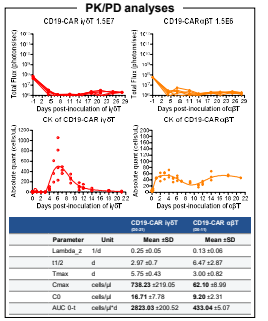






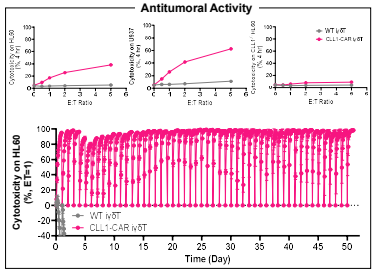


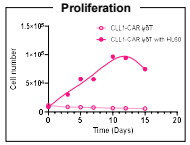


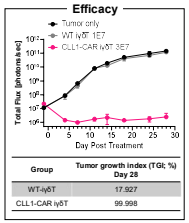


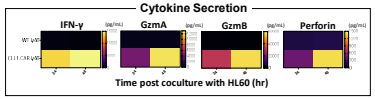
Parameter	Unit	CD19-CAR iγδT (n=7) Mean ±SD	CD19-CAR αβT (n=7) Mean ±SD
Lambda_z	1/d	0.25 ±0.05	0.13 ±0.06
t1/2	d	2.97 ±0.7	6.47 ±2.87
Tmax	d	5.75 ±0.43	3.00 ±0.82
Cmax	cells/µl	**738.23** ±215.05	**62.18** ±8.99
C0	cells/µl	**16.71** ±7.78	**9.20** ±2.31
AUC 0-t	cells/µl*d	**2823.03** ±200.52	**433.04** ±5.07

Moreover, serial killing experiments demonstrated that CLL1-CAR iγδT cells, without exogenous IL-2/IL-15, exhibit antigen-dependent (4hr) and long-lasting cytotoxicity. Additionally, these cells demonstrated increased tumor lysis enzyme and cytokine release, significantly inhibiting AML tumor growth.









Group	Tumor growth index (TGI; %) Day 28
WT-iγδT	17.927
CLL1-CAR iγδT	99.998

CONCLUSIONS

BeiGene is developing a hypoimmunogenic, functional enhanced iPSC-derived-γδT (iγδT) cell therapy with specific editing of signal converter and CARs. This approach has shown improved iγδT activity against target cells in both *in vitro* and *in vivo* preclinical studies.

Unique gene modifications, including combinatorial gene knockouts, hypoimmune editing, and signal converter engineering, improved iγδT cell survival and persistence both *in vitro* and *in vivo* and consequently enhanced anti-tumor activity and durable response.

These engineered iγδT cells can 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. Combining with the robust iPSC differentiation and expansion methodologies to derive iγδT, we believe that arriving to an inflection point of turning cell therapy into a massively produced medicine should not be far.

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