A Novel NK Inhibitor Provides “Best-in-Class" Protection of HLA Knockout Allogeneic Human Engineered Regulatory T Cells to Effectively Evade Immune Rejection

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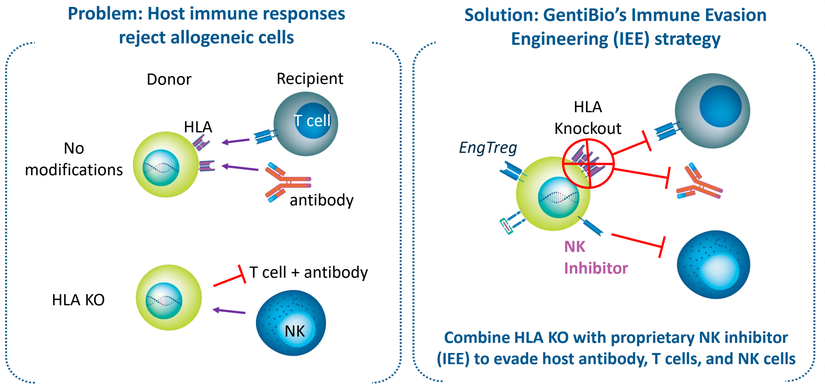
Immune evasion for allogeneic cell therapies



Problem

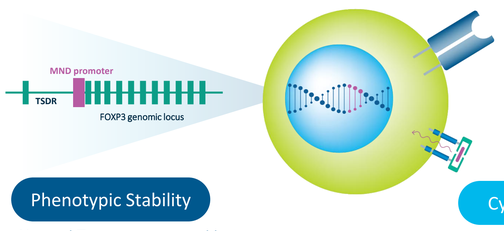
- Rejection of allogeneic cell therapies by the recipient immune responses limits durability of the cell product
- Recipient T cells and antibodies mediate rejection of allogeneic cells mainly by targeting donor human leukocyte antigen (HLA) and can form memory responses that prevent re-dosing
- HLA knockout (KO) can largely evade T and antibody-mediated responses, but HLA class I KO cells are susceptible to NK killing

Solution

- HLA KO combined with an effective NK inhibitor can mitigate T cell, antibody, and NK mediated rejection
- Such approach also enables re-dosing
- GentiBio has developed a proprietary and robust NK inhibitor, referred herein as IEE, to enable HLA KO allogeneic EngTreg products

GentiBio’s Engineered Regulatory T cells (EngTregs)

EngTregs address key shortcomings of sorted Treg approach (see Poster 1769 for platform overview)



Phenotypic Stability

Natural Tregs are rare and heterogenous

- Tregs engineered from more abundant bulk T cells are scalable and achieve better phenotypes by stabilizing Treg differentiation factors (e.g. FOXP3) from either autologous or allogeneic sources.

Tissue Specificity

Modular engineering enables tissue specificity

- Modular TCRs or CARs for optimal efficacy and safety
- Antigen specificity confers tissue localization, engages bystander suppression and drives infectious tolerance
- Genti approach to TCR & CAR screening and qualification identifies TCRs & CARs that are optimal for EngTregs

Cytokine Support

Tregs require IL2 but do not produce it

- Engineered Tregs incorporate Treg-selective and titratable IL2 signaling through a chemically-induced signaling complex (CISC)
- CISC enhances manufacturing and transforms Tregs into potent, long-lived drugs

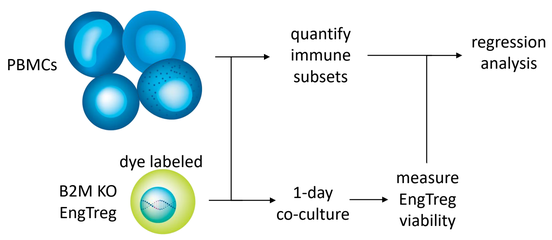
GNTI-122: Islet antigen-specific asset to treat T1D
- Lead autologous asset
- Entering clinic in 2025

GNTI-823: Allogeneic asset to treat acute inflammatory diseases
- Posters 1047 and 1528

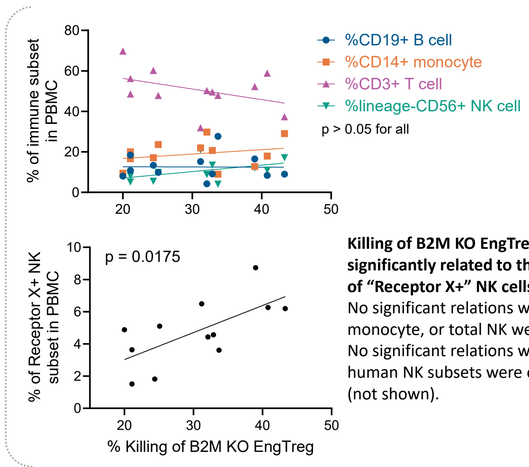
GNTI-932: CAR-based allogeneic asset with IEE targeting a gut-specific antigen to treat IBD
- Poster 2031

GNTI-350: CAR-based allogeneic asset with IEE to treat B cell mediated autoimmune diseases
- Session: CAR-T Innovations in Autoimmune and Infectious Disease

1. Rejection of B2M KO EngTreg is significantly related to the frequency of “Receptor X”+ NK cells in PBMCs

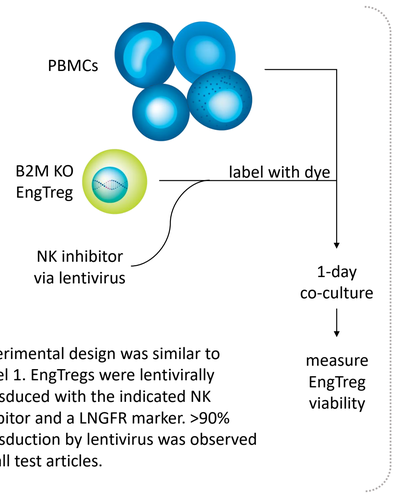


Twelve PBMC donors were characterized for frequencies of major immune and NK subsets, and co-cultured with B2M KO (HLA class I deficient) EngTreg at 30:1 PBMC:EngTreg ratio. Killing of dye-labeled EngTreg was quantified by subtracting baseline cell death without PBMCs. Percent killing was assessed for relation with frequency of various immune subsets in PBMCs using simple linear regression.

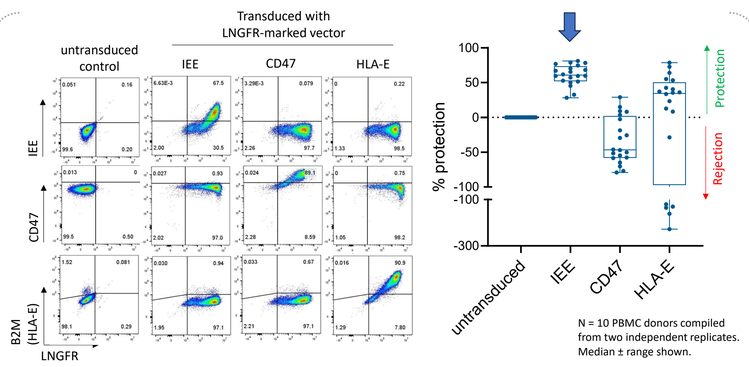


Killing of B2M KO EngTreg was only significantly related to the frequency of “Receptor X+” NK cells in PBMCs. No significant relations with % B, T, monocyte, or total NK were observed. No significant relations with other human NK subsets were observed (not shown).

2. Expression of a Receptor X ligand (IEE) protected B2M KO EngTreg from PBMC killing and consistently conferred higher protection than field standard NK inhibitors

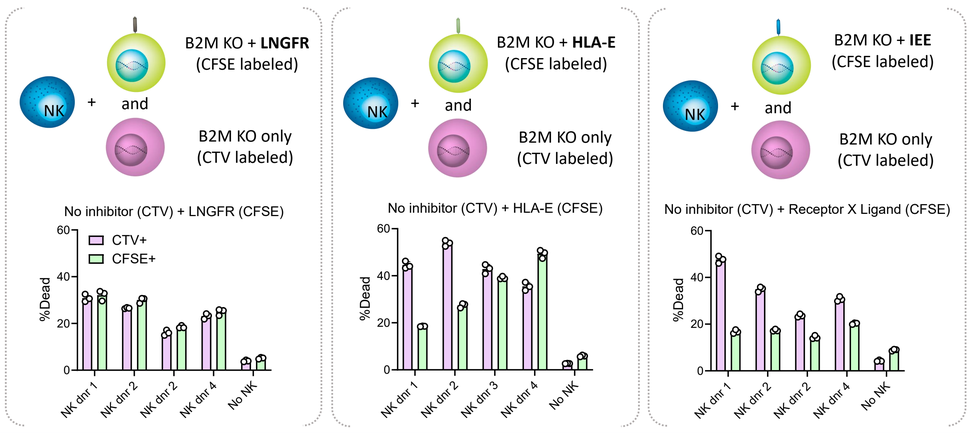


Experimental design was similar to Panel 1. EngTregs were lentivirally transduced with the indicated NK inhibitor and a LNGFR marker. >90% transduction by lentivirus was observed for all test articles.



(left) Representative phenotype of B2M KO EngTreg transduced with indicated NK inhibitor, or untransduced as a control. (right) **Expression of IEE protected B2M KO EngTreg consistently better than the field-standard NK inhibitors HLA-E[ref.1] and CD47[ref.2].** “% protection” was calculated by first subtracting baseline cell death without PBMC from co-culture conditions, then normalizing to the killing of untransduced target cells. Note that higher % NKG2C+ NK cells were observed in PBMCs where HLA-E aggravated rejection (not shown).

3. IEE protected B2M KO EngTreg in a cell-intrinsic manner in a three-way co-culture system



Purified NK cells from 4 donors were co-cultured overnight with two independent B2M KO EngTreg target cells at a 10:1:1 ratio of NK:CFSE+ target:CTV+ target. Viability of each dye-labeled population was measured by flow cytometry.

IEE protected expressing cells (CFSE+) without interfering with NK elimination of bystander targets (CTV+).

Similar to Panel 2, IEE protected B2M KO targets more consistently than HLA-E.

4. IEE functionally integrates into GentiBio’s existing lead asset for Type I Diabetes without impacting EngTreg activity

- GNTI-122 is GentiBio’s lead autologous asset for T1D that targets a pancreatic islet antigen presented by HLA-DRB1*04:01 (DR4)
- As a proof-of-concept, an hypoimmune version was generated with HLA (B2M and CIITA) KO and IEE expression via lentivirus
- Expression of IEE protected HLA KO cells from PBMC killing (middle), and did not interfere with EngTreg responses as measured by LAP and GARP upregulation when stimulated with DR4+ antigen-presenting cells loaded with cognate peptide (right)

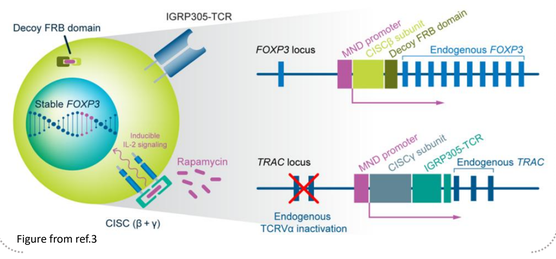
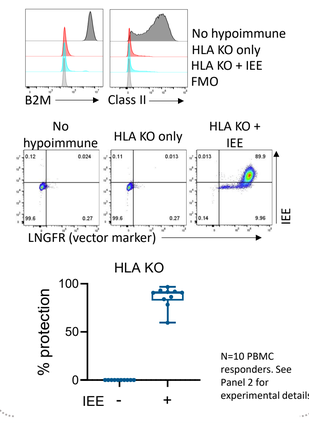
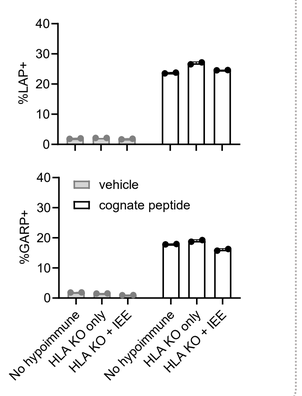


Figure from ref.3

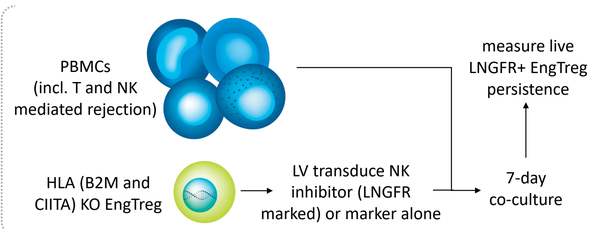
IEE was efficiently expressed and protected HLA KO cells



IEE did not interfere with autoantigen-dependent EngTreg responses

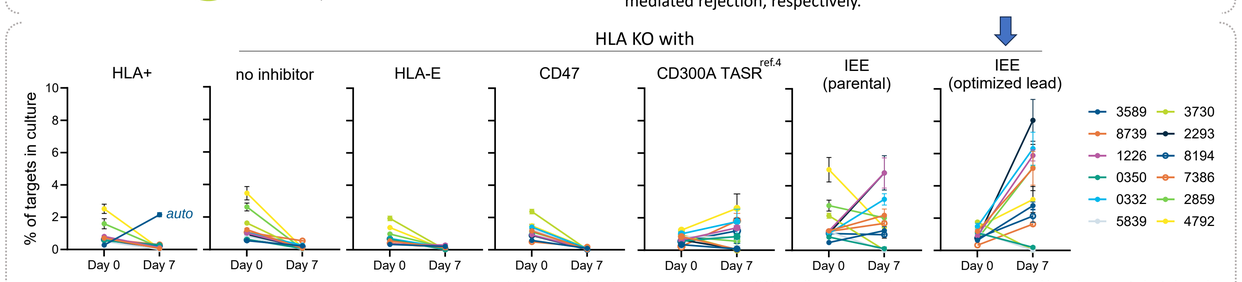


5. Optimized IEE outperforms field standard and emerging NK inhibitors in a rigorous 7-day in vitro rejection assay system



EngTreg with unedited HLA, HLA KO, or HLA KO and indicated NK inhibitor were co-cultured with the autologous PBMC and 11 allogeneic PBMCs at a ratio of 40:1 PBMC:EngTreg. Presence of EngTreg was measured by a LNGFR marker on day 0 and day 7 using flow cytometry.

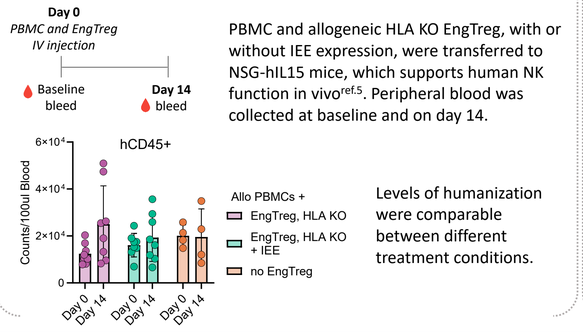
An optimized IEE enabled higher persistence than competing technologies in side-by-side comparisons after 7 days of culture. The lack of observable protection by HLA-E in this assay suggests a highly aggressive rejection system. Notably, HLA+ cells persisted only with the autologous PBMC and HLA KO cells without inhibitor were rejected by all PBMCs, consistent with the mechanisms of T cell and NK cell mediated rejection, respectively.



N = 12 PBMC donors. Mean ± SD of triplicates shown. Frequency of live, CD14- cells were quantified. Similar trend observed based on counts (not shown).

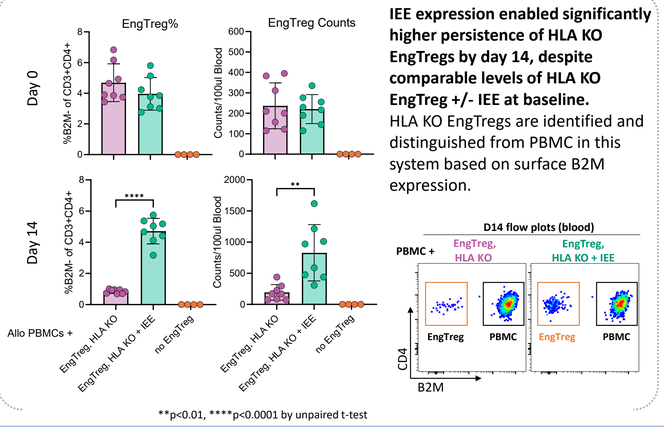
6. Optimized IEE protected HLA KO EngTreg in vivo using a humanized NSG-hIL15 mouse model

Group	n	Responder	EngTreg Targets	Host strain
1	8	2e7 PBMC	2e6 Allogeneic, HLA KO	NSG-hIL15
2	8	2e7 PBMC	2e6 Allogeneic, HLA KO + IEE	
3	4	2e7 PBMC	None	



PBMC and allogeneic HLA KO EngTreg, with or without IEE expression, were transferred to NSG-hIL15 mice, which supports human NK function in vivo[ref.5]. Peripheral blood was collected at baseline and on day 14.

Levels of humanization were comparable between different treatment conditions.



IEE expression enabled significantly higher persistence of HLA KO EngTregs by day 14, despite comparable levels of HLA KO EngTreg +/- IEE at baseline. HLA KO EngTregs are identified and distinguished from PBMC in this system based on surface B2M expression.

**p<0.01, ****p<0.0001 by unpaired t-test

CONCLUSIONS

- Killing of B2M KO EngTreg by PBMCs was significantly related to the frequency of “Receptor X” expressing NK cells, and not other immune subsets, suggesting Receptor X+ NK cells may be a key driver of HLA KO T cell rejection (Panel 1)
- B2M KO EngTreg expressing IEE, GentiBio’s proprietary Receptor X ligand, was protected from PBMC killing consistently better than CD47 or HLA-E (Panel 2)
- IEE protected B2M KO EngTreg in a cell-intrinsic manner and did not interfere with antigen-specific EngTreg activation (Panels 3 and 4)
- In an aggressive in vitro model of rejection, an optimized IEE enabled superior persistence of HLA KO EngTreg compared to CD47, HLA-E, and CD300A TASR (Panel 5)
- HLA KO EngTreg expressing IEE persisted significantly better in vivo than HLA KO EngTreg without an NK inhibitor in the presence of allogeneic PBMC (Panel 6)
- IEE technology can be readily incorporated in GentiBio’s gene editing platform to generate future off-the-shelf allogeneic EngTreg products with durable persistence

References:
1. Gornalusse, G, *et al.* HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat Biotechnol* 35, 765–772 (2017).
2. Deuse, T., *et al.* Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol* 37, 252–258 (2019).
3. Uenishi, G.I., *et al.* GNTI-122: an autologous antigen-specific engineered Treg cell therapy for type 1 diabetes. *JCI Insight* 9(6):e171844 (2024).
4. Zhang, S.-Q., *et al.* Universal protection of allogeneic T-cell therapies from natural killer cells via CD300a agonism. *Blood Adv* 9 (2): 254–264 (2025).
5. Aryee, K-E, *et al.* Enhanced development of functional human NK cells in NOD-scid-IL2rgnull mice expressing human IL15. *FASEB J* 36:e22476 (2022).

We make Tregs. *Better.*