Robust immune evasion technology enables durable persistence of allogeneic Engineered Regulatory T cells (EngTregs)

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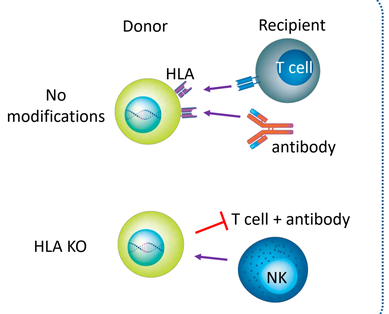
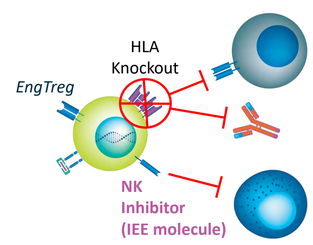
Immune Evasion Engineering (IEE) for allogeneic cell therapies

Problem: Host immune responses reject allogeneic cells



Solution: GentiBio's immune evasion engineering strategy



Combine HLA KO with proprietary NK inhibitor to evade host antibody, T cell, and NK cell responses

Problem

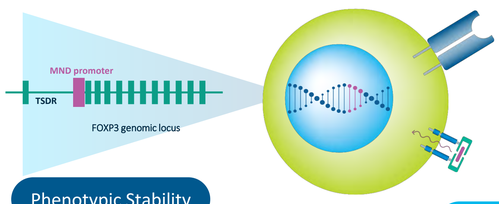
- Rejection of allogeneic cell therapies limits durability of the cell product
- Recipient T cells and antibodies mediate rejection by targeting donor human leukocyte antigen (HLA)
  - Can form memory responses that prevent re-dosing
- HLA knockout (KO) largely evades T and antibody-mediated responses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

Solution

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

GentiBio's Engineered Regulatory T cells (EngTregs)

EngTregs address key shortcomings of sorted Tregs



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

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.

Cytokine Support

Tregs require IL2 but do not produce it

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

Pipeline

GNTI-122: Islet antigen-specific clinical stage asset to treat T1D
- Lead autologous product
- Actively recruiting patients (POLARIS)

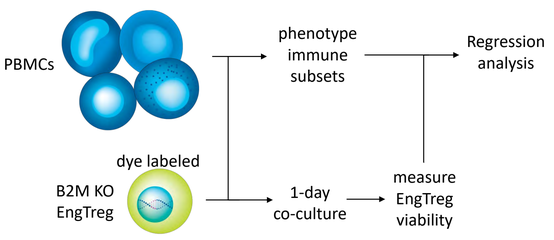
GNTI-148: allogeneic islet antigen-specific asset **with IEE** to treat T1D

GNTI-932: allogeneic asset **with IEE** and CAR targeting a gut-specific antigen to treat IBD

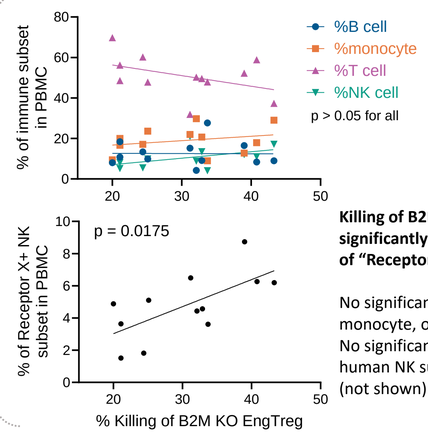
GNTI-823: Allogeneic asset to treat acute inflammatory diseases

GNTI-350: CAR-based allogeneic asset **with IEE** to treat B cell mediated autoimmune diseases

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.

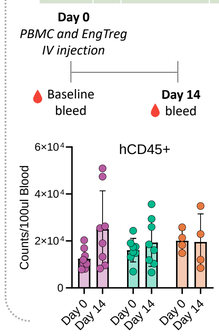


Killing of B2M KO EngTreg was only significantly related to the frequency of "Receptor X+" NK cells in PBMCs.

No significant relations with % B, T, monocyte, or total NK were observed. No significant relations with other human NK subsets were observed (not shown).

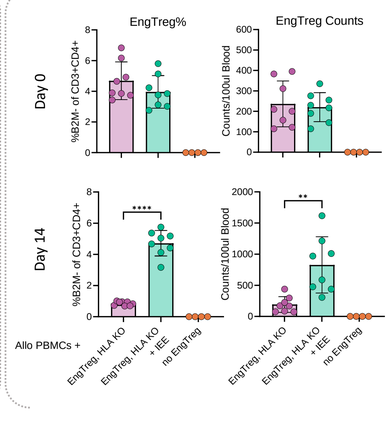
4. IEE protected HLA KO EngTreg in vivo using a PBMC humanized NSG-hIL15 mouse model

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	

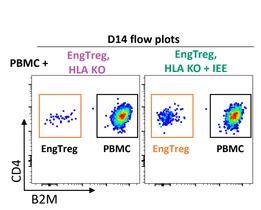


PBMC and allogeneic HLA KO EngTreg, with or without IEE expression, were transferred to NSG-hIL15 mice, which supports human NK function in vivo[ref.5]. Peripheral blood was collected at baseline and on day 14.

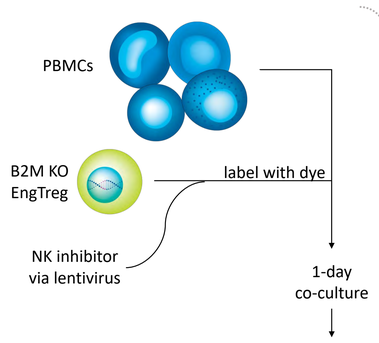
Levels of humanization were comparable between different treatment conditions.



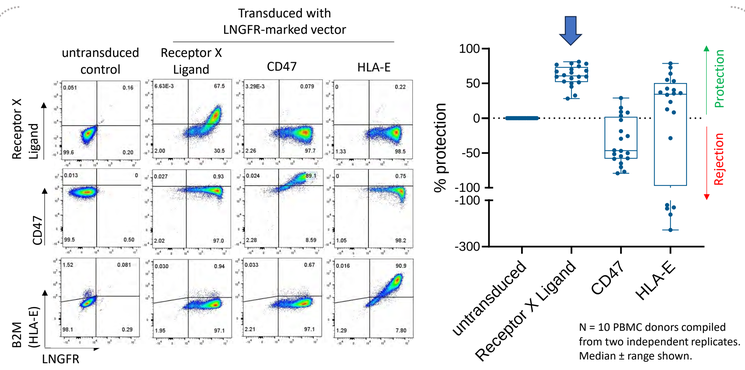
HLA KO EngTregs with IEE persisted at significantly higher levels than HLA KO EngTregs by day 14, despite starting at comparable levels at baseline.
HLA KO EngTregs are distinguished from PBMC in this system based on surface B2M expression.



2. Expression of a prototypic Receptor X Ligand protected B2M KO EngTreg from PBMC killing and provided consistently better protection than field standard NK inhibitors



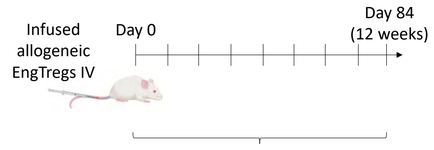
Experimental design was similar to Panel 1. EngTreg were lentivirally transduced with the indicated NK inhibitor marked by LNGFR. >90% transduction by lentivirus was observed for all test articles.



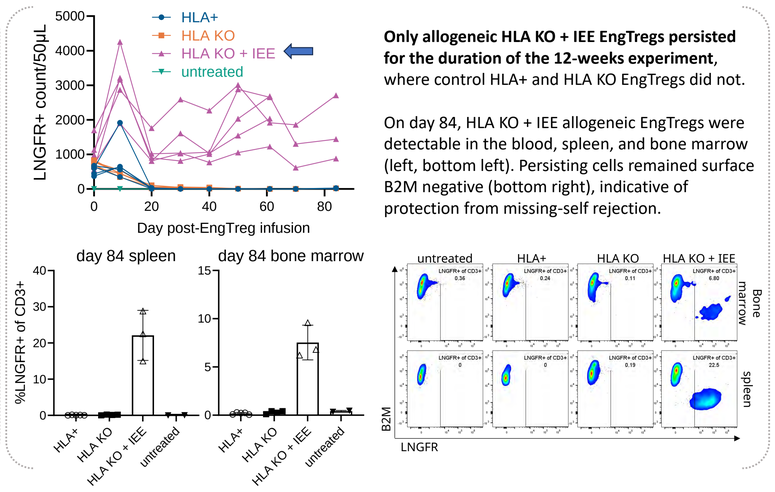
(left) Representative phenotype of B2M KO EngTreg test articles. (right) **Expression of Receptor X Ligand protected B2M KO EngTreg consistently better than the field-standard NK inhibitors HLA-E[ref.1] and CD47[ref.2].** "% protection" was calculated by first subtracting baseline cell death without 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).

5. IEE enabled months-long persistence of HLA KO EngTreg in vivo using a CD34+ HSC humanized NSG-hIL15 mouse model

n	Test article (LNGFR marked)	Host
5	Allogeneic EngTreg, HLA+	CD34+ HSC humanized NSG-hIL15 (~12-weeks post-HSC)
5	Allogeneic EngTreg, HLA KO	
5	Allogeneic EngTreg, HLA KO + IEE	
2	Untreated	



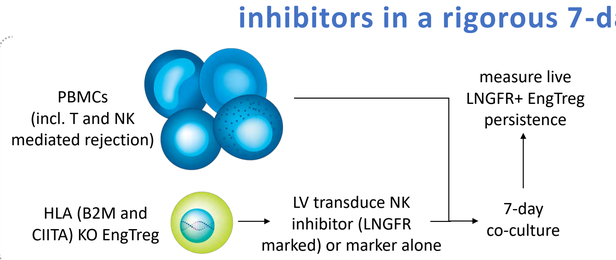
- Sampled peripheral blood ~every 10 days
- Supported EngTreg persistence with rapalog (activates CISC)
- Analyzed blood, bone marrow, and spleen at day 84 take-down



Only allogeneic HLA KO + IEE EngTregs persisted for the duration of the 12-weeks experiment, where control HLA+ and HLA KO EngTregs did not.

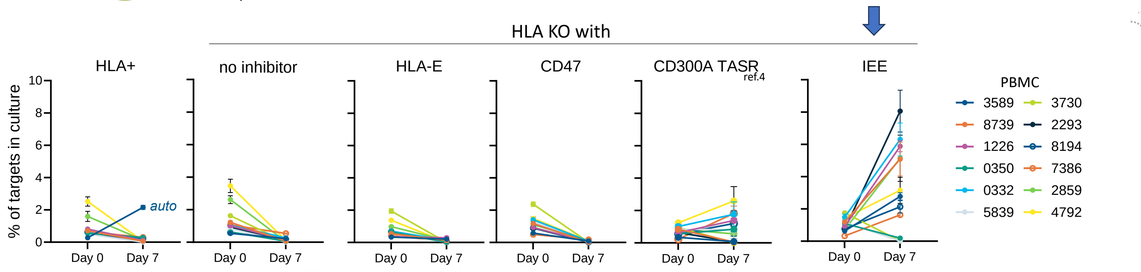
On day 84, HLA KO + IEE allogeneic EngTregs were detectable in the blood, spleen, and bone marrow (left, bottom left). Persisting cells remained surface B2M negative (bottom right), indicative of protection from missing-self rejection.

3. Optimized Receptor X Ligand (IEE) outperforms field standard and emerging NK inhibitors in a rigorous 7-day in vitro rejection assay



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 Receptor X Ligand (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 replete cells persisted only with the autologous PBMC, demonstrating the presence of allogeneic rejection in the model.

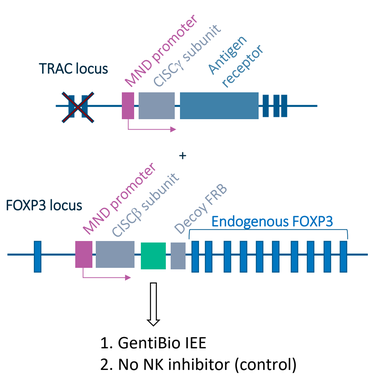


N = 12 PBMC donors. Mean ± SD of triplicates shown. Frequency of live, CD14- cells were quantified. Similar trend observed based on counts (not shown).

6. IEE readily integrates with GentiBio's AAV-based platform for producing next-generation off-the-shelf EngTreg therapies for autoimmune diseases

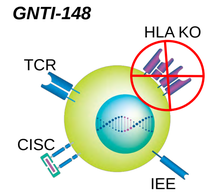
Cell engineering method

4 RNPs: *B2M, CIITA, TRAC, FOXP3*
2 AAVs: knock-in at *TRAC* and *FOXP3* (below)

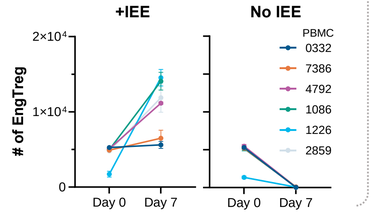


1. GentiBio IEE
2. No NK inhibitor (control)

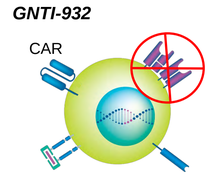
GNTI-148



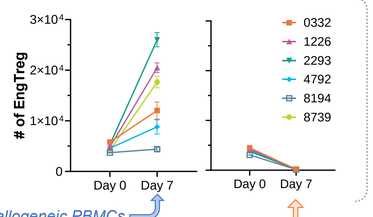
- Allogeneic EngTreg for T1D
- Targets pancreatic islet-specific antigen present by HLA-DRB1*04:01
- IEE functioned effectively in GNTI-148 (right) using the Panel 3 assay system



GNTI-932



- Allogeneic EngTreg for IBD
- Targets gut-specific antigen
- IEE functioned effectively in GNTI-932 (right) using the Panel 3 assay system



*EngTregs **with IEE persist** when cultured with allogeneic PBMCs*
*EngTregs **without IEE are rejected** by allogeneic PBMCs*

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, implicating Receptor X+ NK cells as key drivers of HLA KO T cell rejection (Panel 1)
- B2M KO EngTreg expressing a prototypic Receptor X Ligand was protected from PBMC killing consistently better than CD47 or HLA-E (Panel 2)
- In an aggressive in vitro model of rejection, an optimized Receptor X Ligand (IEE) enabled superior persistence of HLA KO EngTreg compared to CD47, HLA-E, and CD300A TASR (Panel 3)
- HLA KO EngTreg expressing IEE persisted significantly better in vivo than HLA KO EngTreg without an NK inhibitor using a PBMC humanized model (Panel 4)
- HLA KO + IEE enabled months-long persistence of allogeneic EngTreg in a CD34+ HSC humanized NSG-IL15 model, where control cells were quickly rejected (Panel 5)
- IEE readily incorporated into GentiBio's dual-AAV platform to generate off-the-shelf allogeneic EngTreg products with durable persistence (Panel 6)
- **GentiBio IEE approach could enable long-term persistence of other allogeneic therapies to deliver on lower cost per dose and greater accessibility than autologous products**

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* 2025; 9 (2): 254–264.
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.*