Universal Protection of Allogeneic T Cells from Natural Killer Cells Via CD300a Agonism

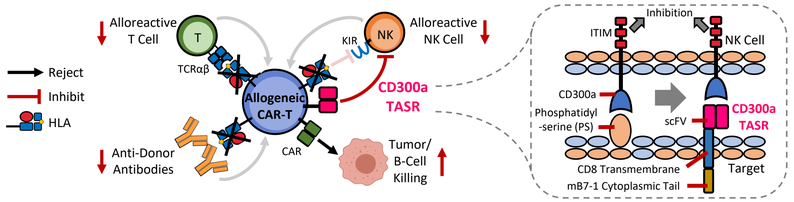
G. Grant Welstead[1,2], Shuqi Zhang[1], Faith Thomas[1], Justin Fang[1,2], Dustin Whitney[1,2], Andriana Lebid[2], Nicholas W. Asby[2], Hunter R. Hoffman[2], Michael Miller[2], Jill Carton[2], Jonathan Kurtz[1,2] Chad Cowan[1,2].

[1]Clade Therapeutics, [2]Century Therapeutics

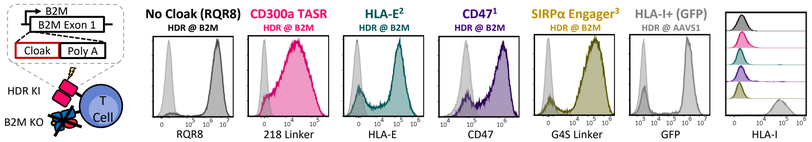
Poster # 4821

Overview

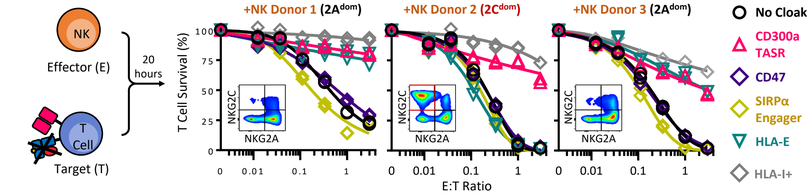
Immunogenicity limits the persistence of allogeneic cell therapies and transplants. While ablation of human leukocyte antigen (HLA) removes most T cell and humoral alloreactivity, no solution has enabled universal protection against the resulting natural killer (NK) cell response. To address this challenge, we engineered Trans Antigen Signaling Receptors (TASRs) to agonize CD300a as a novel and universal inhibitor of NK alloreactivity. CD300a TASR outperformed leading alternatives, including CD47[1] and HLA-E[2], against a large human cohort (45/45) and enhanced Chimeric Antigen Receptor (CAR) T cell mediated function under allogeneic pressure, broadening the population effectively treated by next-generation allogeneic cell therapies.



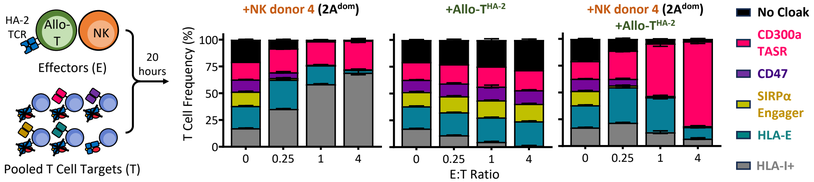
CD300a TASR outperforms HLA-E, CD47, and SIRPα engager in T Cells



Expression of CD300a TASR and alternative strategies from the B2M locus of human primary T cells. Phenotype by flow cytometry of T cells expressing the indicated transgenes at the B2M locus under control of B2M promoter and with concomitant ablation of B2M/HLA-I expression via non-viral CRISPR-mediated homology directed repair (HDR). CD47 is bicstronic with RQR8 tag using 2A peptide. Gated single-cell lymphocytes. dotted grey histogram indicates staining with unedited T cell.

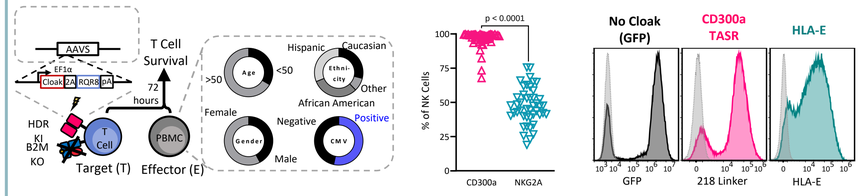


CD300a TASR enhances persistence of B2M KO T cells against NK cells from all donors while CD47, SIRPa Engager, and HLA-E does not. Survival of T cells expressing the indicated transgenes challenged with human primary NK cells from 3 donors. Inset shows phenotype of NK cells by flow cytometry at time of co-culture. N = 2 technical replicate curves per condition.

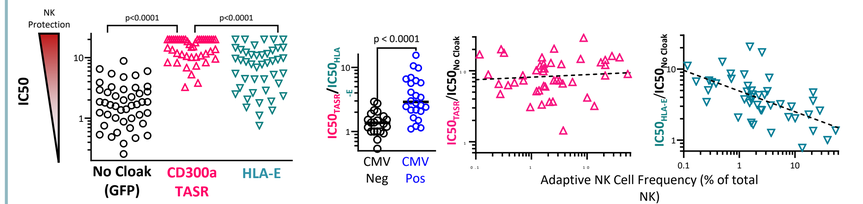


B2M KO with CD300a TASR outcompete alternative strategies and protects against both alloreactive T and NK cells. Relative frequency of the indicated T cell member within a pool of 6 T cell members challenged with human primary NK cells and/or an alloreactive T cell clone (Allo-T). Each T cell member can be discerned by its transgene expression using flow cytometry. Allo-T contains a TCRαβ reactive to minor histocompatibility antigen 2 (HA-2) presented by HLA-A2. Effectors are serotyped HLA-A2- while targets are HLA-A2+. N = 3 technical replicates per condition.

CD300a TASR is a universal NK cell inhibitor



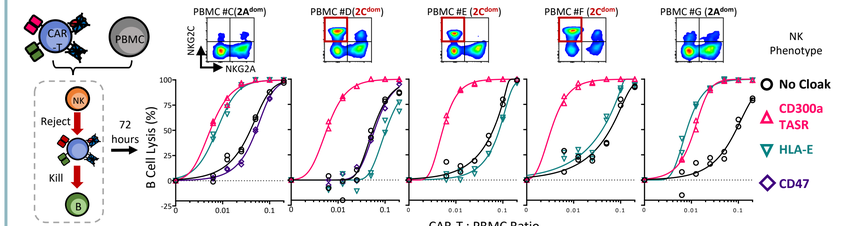
Demographic and phenotypic correlates CD300a TASR and HLA-E function using long-term PBMC challenge with a large human cohort. (left panel) study design. Peripheral blood mononuclear cells (PBMCs) from 45 donors were selected for demographic diversity as shown. (middle panel) Expression of indicated marker on NK cells from PBMCs by flow cytometry. Gating set by FMO control. N = 45 donors. (right panel) phenotype by flow cytometry of the three engineered T cell targets, gated on live single lymphocytes. Label indicates cloaking transgene. grey dotted histograms indicate negative control T cells stained with the same markers



CD300a TASR enhances T cell survival against PBMCs from all donors and is more effective than HLA-E. T cell survival expressing the indicated cloaking ligand against PBMCs from one donor. IC50 value represents E:T ratio corresponding to 50% T cell survival. Limitation of quantification (LoQ) set to be twice the highest E:T ratio used. N = 45 donors.

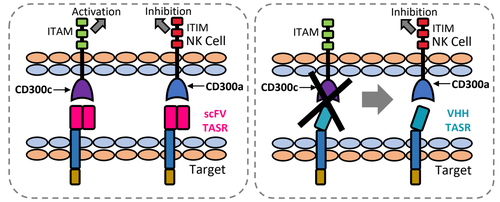
CD300a TASR is more effective than HLA-E in CMV+ patients who contain high percentages of NKG2C+ adaptive NK cells[4]. (left panel) Association of CD300a TASR vs. HLA-E function with CMV serostatus of the challenging PBMC donor, with one indicating similar function. (middle, right panel) Relationship between the adaptive NK cell frequency of the PBMC donor (phenotype NKG2A-NKG2C+CD57+CD56loCD16hi) and functional potency of CD300a TASR (left) and HLA-E (right), normalized to non-cloaked control. Dotted line represents linear fit of log-log transformed data. N = 45 PBMC donors

CD300a TASR enhances CAR-T function under allogeneic pressure



CD300a TASR enhances the functional potency of CAR-T cells under physiological allogeneic pressure in an *ex-vivo* model of systemic lupus erythematosus. Model of B cell killing by dosing anti-CD19 CAR-T cells expressing the indicated cloaking transgene directly into PBMCs under NK cell pressure. (bottom row) B cell killing potency for each of the four CAR-T cell members against the indicated PBMC donors. N = 2 technical replicate curves per condition. (top row) phenotype of NK cells from the respective PBMC donor, gated CD3-CD56+CD14-CD20-.

Novel CD300a VHH binders lead to TASRs with improved protection of iPSC-derived T cells from alloreactivity

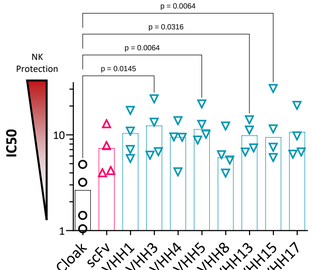


A VHH binder campaign[7] to improve specificity for CD300a. The original scFv binder for CD300a TASR was found to cross-react with CD300c, which raises the possibility of eliciting activating signals via CD300c ITAMS (left). A VHH binder campaign was initiated to select for CD300a and against CD300c affinity for new VHH-format TASRs that will only induce inhibitory signals (middle). 16 VHHs with <10uM KD for CD300a and KD above the detection limit for CD300c were identified (right)

CD300a TASR screens

Binder	Monomer (%)[1]	Jurkat binding[2]	VHH surface expression[3]	CD300a-Fc capture[4]
Untransduced	n/a	n/a	3.04	3.30
scFv	97.9	0.0	3.08	5.01
VHH_01*	97.9	0.0	5.04	4.13
VHH_02	1.0	25.0	4.55	4.09
VHH_03*	99.0	0.0	4.97	4.11
VHH_04*	56.9	4.0	4.24	3.87
VHH_05*	99.0	31.0	4.76	4.27
VHH_06	1.0	25.0	4.76	4.09
VHH_07	1.0	56.0	4.59	4.12
VHH_08*	99.0	10.0	5.24	3.74
VHH_09	1.0	36.0	4.46	4.07
VHH_00	1.0	66.0	4.22	3.85
VHH_11	1.0	35.0	4.30	4.06
VHH_12	1.0	45.0	4.24	3.88
VHH_13*	99.0	1.0	4.76	4.15
VHH_14	1.0	48.0	4.10	3.76
VHH_15*	99.0	29.0	4.38	3.90
VHH_16	1.0	15.0	4.88	3.52
VHH_17*	99.0	0.0	4.95	4.36

[1]Analytical size exclusion chromatography of soluble (Fc') fragments
[2]Flow cytometry to assess non-specific binding of soluble (Fc') fragments to CD300a⁻ Jurkat cells
[3]Flow cytometry to assess VHH surface expression of full-length TASR on transduced primary T cells
[4]Flow cytometry to assess capture of soluble CD300a by primary T cells transduced to express full-length TASRs
*TASRs selected for functional screening in iT cells. These 8 VHH-format TASRs conferred allo-evasion of primary T cells from 2 allogeneic PBMC donors similar to scFv-format TASR (not shown).



CD300a TASR VHH formats support survival of iPSC-derived T (iT) cells from allogeneic PBMCs. *B2M* was disrupted via CRISPR knock-out in a T cell-derived iPSCs (TiPSC) line. The line was then differentiated to iT cells and transduced with LV to express the indicated CD300a-targeting TASR formats. The iT lines were then cultured across a titration of allogeneic PBMCs (n=4) and IC50 values were calculated. ANOVA with Freidman's multiple comparison post-test indicates difference from uncloaked (*B2M* ko with no TASR) cell survival.

Summary and Conclusions

- Surface expression of an engineered CD300a agonist (TASR) at physiological levels universally protects HLA-deficient allogeneic T cells from NK-mediated rejection.
- Relative to HLA-E, CD300a TASR expands the addressable population to include CMV seropositive patients, which comprise 50-90% of the human population[5]
- CD300a TASR enhances CAR-T efficacy under allogeneic immune pressure
- CD300a TASR VHH candidates show comparable protection and donor universality to scFv with no binding to CD300c

References

1. Hu, X. et al. Hypoimmune anti-CD19 chimeric antigen receptor T cells provide lasting tumor control in fully immunocompetent allogeneic humanized mice. Nat. Commun. 14, 2020 (2023).
2. Jo, S. et al. Endowing universal CAR T-cell with immune-evasive properties using TALEN-gene editing. Nat. Commun. 13, 3453 (2022).
3. Gravina, A. et al. Synthetic immune checkpoint engagers protect HLA-deficient iPSCs and derivatives from innate immune cell cytotoxicity. Cell Stem Cell 30, 1538-1548.e4 (2023).
4. Gumá, M. et al. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood 104, 3664–3671 (2004).
5. Zuhair, M. et al. Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. Rev. Méd. Virol. 29, e2034 (2019).
6. Zhang et al. Universal Protection of Allogeneic T-Cell Therapies from Natural Killer Cells via CD300a Agonism. Blood Adv. 2024 Oct 5:bloodadvances.2024013436. doi: 10.1182/bloodadvances.2024013436. Online ahead of print.
7. Coding Bio, UK