Rapid Serial Killing of Target Cells by Vγ9Vδ2 T Cells in Cynomolgus Macaques and Humanized Mice Treated with a CD20-Directed Heterodimeric Butyrophilin 2A1/3A1 Fusion Protein

#1815

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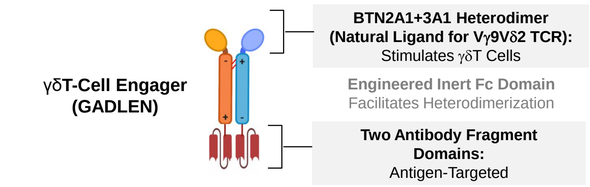
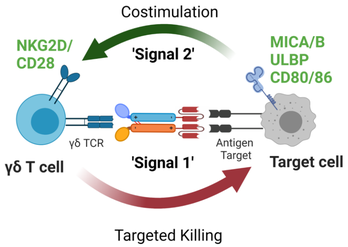
Introduction

γδT cell targeted immunotherapy is of interest to harness its MHC-independent cytotoxic potential to promote anti-tumor immunity. However, it remains unclear whether γδT cells (1-5% of total T cells) are present at sufficient numbers to be therapeutically harnessed. While CD3-directed T cell engagers provide clinical benefit in a variety of tumors and can activate γδT cells, this class of immunotherapy has a narrow therapeutic window due to toxicity. If the smaller fraction of γδT cells could be targeted, such an approach may be preferred to prevent systemic activation of T cells.

The Vγ9Vδ2 T cell receptor is activated by a butyrophilin 2A1 and 3A1 (2A1/3A1) heterodimer as "signal 1", together with costimulatory signaling through CD28 or NKG2D as "signal 2" (Lai et al., *J. Immunol* 2022). Here we evaluated the ability of a γδT engager (GADLEN) comprising the extracellular domains of 2A1/3A1 adjoined via an Fc linker to an antibody fragment targeting the CD20 antigen, to mediate target-cell depletion *in vivo* at physiologically relevant frequencies of Vγ9Vδ2 T cells.

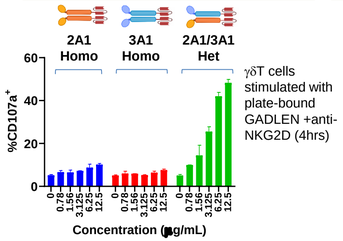


GADLEN MOA

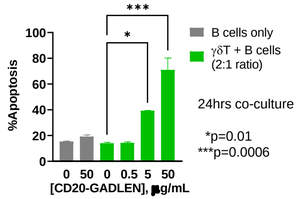


- Activates γδT cells upon binding to antigen expressed on target cell
- Butyrophilin heterodimer engages the TCR with low affinity similar to αβTCR and MHC/antigen
- Costimulation is required for optimal signal strength and to prevent activation-induced cell death
- TCR activation through GADLEN leads to sustained killing and preservation of γδT cell viability

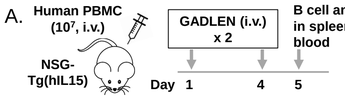
BTN2A1/3A1 Heterodimer Mediates γδT Cell Degranulation



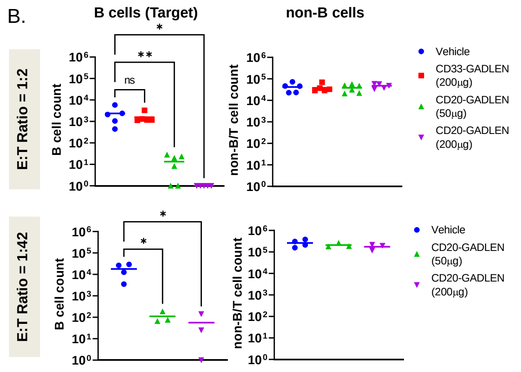
CD20-GADLEN Promotes Apoptosis in Target Cells in the Presence of γδT Cells

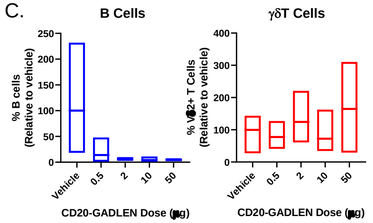


Efficient Target Cell Depletion by CD20-GADLEN at a Wide-Range of Effector to Target (E:T) Ratios in PBMC-Humanized Mice



PBMC Donor	%Vδ2+T Cells	% B Cells	E:T Ratio	%B Cell Depletion (mean ± SD)
1	2.85	5.96	1:2	97.1 ± 1.3
2	2.73	8.48	1:3	95.1 ± 2.6
3	1.22	6.13	1:5	99.3 ± 0.7
4	0.36	9.42	1:26	94.7 ± 2.1
5	0.43	20.0	1:42	97.6 ± 0.7
6	0.34	20.4	1:60	96.4 ± 1.8



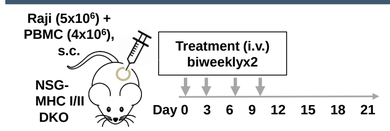


A) B cell depletion by CD20-GADLEN was evaluated in PBMC-humanized mice as indicated. Human PBMCs containing varying Effector (Vδ2+T cells) to Target (B cells) ratios were evaluated as indicated in the table. Near complete B cell depletion upon CD20-GADLEN (50ug) treatment at all donor E:T ratios.

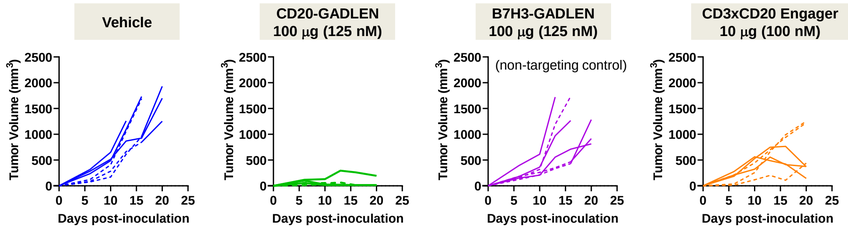
B) CD20-GADLEN, but not CD33-GADLEN (non-targeting control), mediated B cell depletion at both high and low E:T ratios in spleen and peripheral blood (data not shown). Non-B cell compartments remained unchanged after treatment. *p<0.05, **p<0.01

C) Increase in $CD3^+Vδ2^+$ T cells was not observed in animals treated with CD20-GADLEN at various dose levels, demonstrating the ability of γδT cells in promoting serial killing of target cells in the absence of proliferation or expansion.

Robust Anti-Tumor Activity by CD20-GADLEN at Low E:T Ratios *in vivo*



- Anti-tumor activity by CD20-GADLEN was evaluated in a Raji ($CD20^+$) lymphoma and PBMC xenograft model as indicated.
- CD20-GADLEN, but not B7H3-GADLEN (non-targeting control), significantly inhibited tumor growth compared to vehicle control at low input E:T ratios.
- These results further highlight the feasibility of targeting γδT cells to mediate depletion of target cells.



Dose-Range Finding and Safety Assessment of CD20-GADLEN in Non-Human Primates

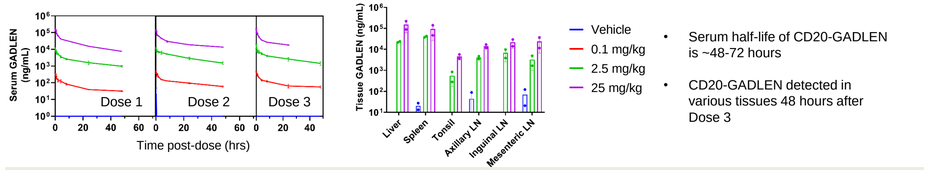
Study Design

Dose Schedule	Dose Level	No. of Animals
Day 1, 4, 7	0 (Vehicle)	1M/1F
	0.1 mg/kg	1M/1F
	2.5 mg/kg	2M/2F
	25 mg/kg	1M/1F

Safety Profile

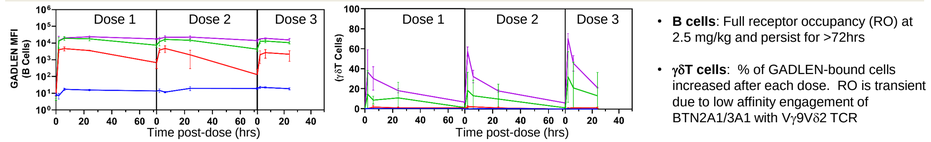
- All doses were well tolerated
- No clinically relevant changes observed with any hematological parameters
- Liver and kidney function were normal throughout the study as were coagulation, D-Dimer, serum proteins and electrolytes
- Minimal cytokine changes and no IL-6 elevation

CD20-GADLEN PK Profile and Distribution in Lymphoid Tissues



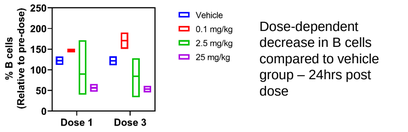
- Serum half-life of CD20-GADLEN is ~48-72 hours
- CD20-GADLEN detected in various tissues 48 hours after Dose 3

On-Target Binding of CD20-GADLEN on B and γδT Cells



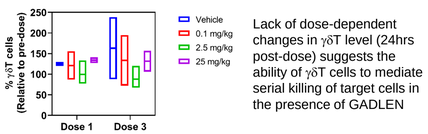
- **B cells**: Full receptor occupancy (RO) at 2.5 mg/kg and persist for >72hrs
- **γδT cells**: % of GADLEN-bound cells increased after each dose. RO is transient due to low affinity engagement of BTN2A1/3A1 with Vγ9Vδ2 TCR

Depletion of B Cells by CD20-GADLEN



Dose-dependent decrease in B cells compared to vehicle group – 24hrs post dose

No Significant Change in γδT Cell Compartment



Lack of dose-dependent changes in γδT level (24hrs post-dose) suggests the ability of γδT cells to mediate serial killing of target cells in the presence of GADLEN

Conclusions

- In both humanized mice and non-human primates, proliferation of γδT cells was not required to achieve B cell depletion.
- These observations, together with the speed at which B cell depletion occurred, suggest that the γδT cells eliminate the B cell pool by serial killing following treatment with GADLEN.
- Collectively, these results indicate that low frequencies of γδT cells can be harnessed to achieve similar target-cell killing potential as the broader $CD3^+$ T cell pool, but with potentially less toxicity.
- The CD20-GADLEN safely directed low frequencies of γδT cells to eliminate CD20 expressing cells in vivo, providing pre-clinical proof of concept for a differentiated T-cell engager for depletion of malignant or auto-antibody producing B cells.

Reference: Lai et al., 2022. Cutting Edge: Bispecific γδT cell engager containing heterodimeric BTN2A1 and BTN3A1 promotes targeted activation of Vg9Vd2+ T cells in the presence of co-stimulation by CD28 or NKG2D, *J Immunol* 209 (8): 1475-1480.