A CD33 Antigen Targeted Gamma Delta T-cell Engager in Combination with Zoledronate Promotes Vγ9Vδ2⁺ T cell Proliferation and Cytotoxicity Against Acute Myeloid Leukemia

#6722

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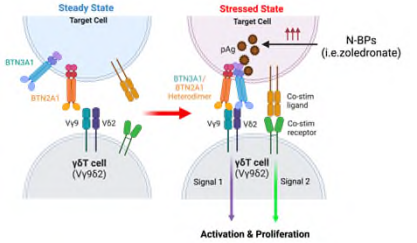
1. Introduction

- Vγ9Vδ2⁺ T cell (GDT) targeted immunotherapy is of interest to harness its MHC-independent cytotoxic potential to promote anti-tumor immunity.
- To date, clinical trials in cancer patients have failed to demonstrate monotherapy activity using a variety of GDT activating agents, including aminobisphosphonates such as zoledronic acid (ZA), Vδ2⁺ targeted T cell engagers and BTN3A1 targeted antibodies.
- It remains unclear whether GDT (1-5% of total T cells) are present in sufficient numbers, whether the agents in question are providing adequate GDT activation, and whether GDT are being directed to tumor cell targets.
- GDTs are activated by phosphoantigen-sensing butyrophilins on cell surface of target cells and APCs. Using a BTN-based GDT engager, the current study is designed to dissect the role of phosphoantigens, butyrophilins and antigen presenting cells (APCs) in the proliferation and activation of GDT.

2. GDT Activation by Phospho-Antigen Sensing Butyrophilins

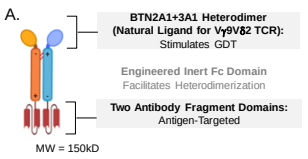


- GDT respond to transformed cells by sensing elevated phosphorylated non-peptide metabolites, or phosphoantigens (pAg) produced via the mevalonate pathway of cholesterol synthesis that becomes dysregulated in certain tumor cells.
- B7-related membrane protein BTN3A1, or CD277, expressed on target cells is responsible for direct pAg sensing through its cytoplasmic B30.2 domain. pAg binding to BTN3A1 initiates a conformational change in its extracellular domain, which facilitates interaction with BTN2A1 that can in turn engage with Vγ9Vδ2 TCR, leading to proliferation and activation of effector functions.
- Aminobisphosphonates (N-BPs), such as zoledronate, can induce phosphoantigen accumulation in target cells or antigen-presenting cells (APCs), leading to Vγ9Vδ2⁺ T cell activation.

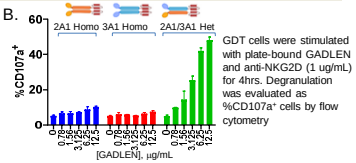
Figure adapted from Herrmann et al., 2020 and created with Biorender.com.

3. GADLEN: Butyrophilin-Based Gamma Delta T Cell Engager

Design of GADLEN

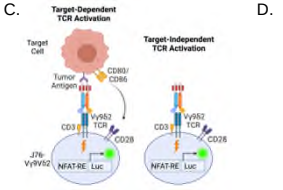


BTN2A1/3A1 Heterodimer Promotes GDT Degranulation in the Presence of Co-stimulation

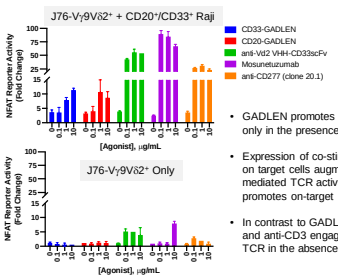


GDT cells were stimulated with plate-bound GADLEN and anti-NKG2D (1 ug/mL) for 4hrs. Degranulation was evaluated as %CD107a⁺ cells by flow cytometry

GADLEN Activates GDT Upon Binding to Antigen Expressed on Target Cells

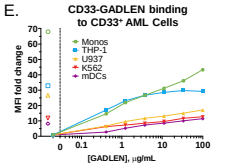


J76-Vγ9Vδ2⁺ cells containing Luciferase reporter driven by NFAT response elements were incubated with GDT agonists with and without target cells (1:1 ratio, 5hrs) to evaluate target-dependent or target-independent TCR activation, respectively. Raji cells transduced with human CD33 was used as target cells to interrogate TCR activation by CD33- and CD20-targeting GADLEN.

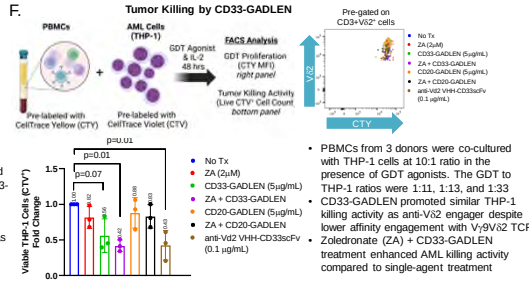


- GADLEN promotes TCR activation only in the presence of target cells.
- Expression of co-stimulatory ligands on target cells augments GADLEN-mediated TCR activation and promotes on-target GDT activation
- In contrast to GADLEN, anti-Vδ2 and anti-CD3 engagers both activate TCR in the absence of target cells

GADLEN Promotes On-Target Tumor Killing at Low E:T Ratio in the Absence of GDT Proliferation



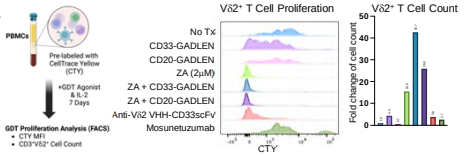
AML cell lines, monocytes and monocyte-derived dendritic cells (mDCs) were incubated with CD33-GADLEN, followed by detection with anti-human Fc by flow cytometry. MFI fold change from no GADLEN control is shown. CD33-GADLEN binding correlated with CD33 expression level, as indicated by anti-CD33 MFI in open symbols for each cell type



- PBMCs from 3 donors were co-cultured with THP-1 cells at 10:1 ratio in the presence of GDT agonists. The GDT to THP-1 ratios were 1:11, 1:13, and 1:33
- CD33-GADLEN promoted similar THP-1 killing activity as anti-Vδ2 engager despite lower affinity engagement with Vγ9Vδ2 TCR
- Zoledronate (ZA) + CD33-GADLEN treatment enhanced AML killing activity compared to single-agent treatment

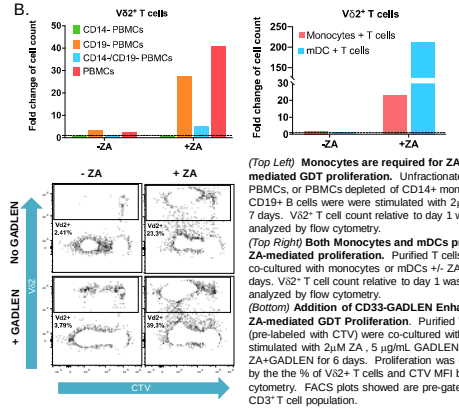
4. CD33-GADLEN in Combination with Zoledronate (ZA) Promotes Robust GDT Proliferation *in vitro* and *in vivo*

GADLEN Alone Promotes GDT proliferation, But Addition of ZA Further Augments GDT expansion



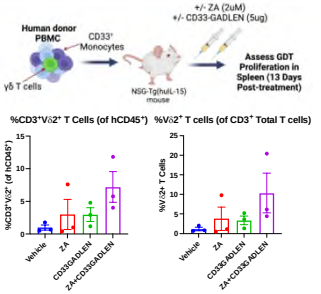
- Enhanced GDT proliferation by GADLEN+ZA combination was observed in 5 different donors (unfractionated PBMC or T+mDC co-cultures).
- Stimulation with anti-Vδ2 engager also resulted in robust proliferation, but the lower GDT count is likely due to activation-induced cell death resulting from high affinity engagement to TCR.

GDT Proliferation is Dependent on Monocytes or Monocyte-Derived Dendritic Cells (mDCs)



(Top Left) **Monocytes are required for ZA-mediated GDT proliferation.** Unfractionated PBMCs, or PBMCs depleted of CD14+ monocytes or CD19+ B cells were were stimulated with 2μM ZA for 7 days. Vδ2⁺ T cell count relative to day 1 was analyzed by flow cytometry.
(Top Right) **Both Monocytes and mDCs promote ZA-mediated proliferation.** Purified T cells were co-cultured with monocytes or mDCs +/- ZA for 8 days. Vδ2⁺ T cell count relative to day 1 was analyzed by flow cytometry.
(Bottom) **Addition of CD33-GADLEN Enhances ZA-mediated GDT Proliferation**. Purified T cells (pre-labeled with CTV) were co-cultured with mDCs, stimulated with 2μM ZA , 5 μg/mL GADLEN, or ZA+GADLEN for 6 days. Proliferation was evaluated by the the % of Vδ2+ T cells and CTV MFI by flow cytometry. FACS plots showed are pre-gated on CD3⁺ T cell population.

CD33-GADLEN in Combination with ZA Promotes GDT Proliferation Human PBMC-Engrafted NSG-Tg(hu-IL15) Mice



NSG-Tg(hu-IL15) mice engrafted with human PBMCs were treated with ZA (2μM) +/- CD33-GADLEN (10 μg). Spleens were harvested from mice 13 days after treatment for analysis of human GDT cell counts by flow cytometry (n=3 per treatment group). GDT cells were identified as human CD45⁺CD3⁺Vδ2⁺ population. Representative FACS plots of %GDT in spleen of PBMC-engrafted mice are shown in top panels.

5. Conclusions

- Although both GADLEN and ZA stimulation activate GDT via heterodimeric BTNs, the combination of the two agents significantly increase GDT proliferation. This suggests that ZA treatment provides additional co-stimulatory signal(s) in APCs to drive GDT proliferation.
- CD33-GADLEN is superior to CD20-GADLEN in promoting GDT proliferation. This is likely due to CD33-GADLEN mediating interaction between GDT and CD33⁺ monocytes/mDCs.
- Robust tumor killing activity and GDT expansion by CD33-GADLEN + low dose ZA demonstrates the potential benefits of this treatment combination in CD33⁺ AML settings.
- This study provides proof of concept for combining ZA and GDT engager for GDT therapies.