1203 ANTIGEN TARGETED BUTYROPHILIN HETERODIMER-BASED BISPECIFIC ENGAGERS INDUCE Vγ9δ2$^+$ T CELL-MEDIATED ANTI-TUMOR ACTIVITY

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Background Decreased antigen expression and antigen presentation via major histocompatibility complexes (MHCs) evades αβ T cell recognition. γδ T cells recognize stressed cells in an MHC-independent manner, and consequently, may be exploited to overcome immunotherapy resistance. The butyrophilin (BTN) 2A1/3A1 heterodimer specifically activates Vγ9δ2$^+$ T cells, the predominant subtype in peripheral blood. BTN2A1 directly binds to the Vγ9 chain of the γδ T cell receptor (TCR), but only activates the γδ T cell if phosphoantigen-sensing BTN3A1 forms a heterodimer complex with BTN2A1. To mimic BTN-mediated activation of γδ T cells, we generated bispecific γδ T-cell engagers (GADLEN) containing heterodimeric BTN2A1 and BTN3A1 extracellular domains fused via inert Fc linkers to scFv domains targeting a tumor antigen. We previously reported that in the presence of costimulatory signals from either a cytotoxicity receptor (NKG2D) or T-cell co-stimulatory receptor (CD28), GADLEN compounds activated Vγ9δ2$^+$ T cells to facilitate antigen-dependent tumor cell killing. The specificity of γδ TCR/BTN interactions and dependence upon a secondary co-stimulatory signal suggests that GADLENs could be used to redirect Vγ9δ2$^+$ T cells against hematologic and solid tumors, with a lower risk of off-target activation common with other bispecific engagers. Here, we report the functional characterization of CD20- and B7H3-targeting GADLEN compounds for targeting heme malignancies and solid tumors, respectively.

Methods Specificity of CD20- and B7H3-targeting GADLENs were evaluated using ELISA and cell-based assays by flow cytometry. The functionality of the compounds to activate Vγ9δ2$^+$ T cells and mediate killing of tumor cells was assessed *in vitro* in tumor co-cultures using flow cytometry and live cell imaging, as well as *in vivo* in murine xenograft models.

Results CD20- and B7H3-targeting GADLENs bound to human cells expressing CD20 or B7H3 and to Vg9d2$^+$ T cells with low nanomolar affinity. GADLEN compounds activated Vγ9δ2$^+$ T cells in *in vitro* co-culture assays resulting in degranulation and apoptosis of CD20+ or B7H3+ tumor cells, respectively. Importantly, GADLEN treatments induced the secretion of pro-inflammatory cytokines suggesting the potential of both direct and indirect tumor killing mechanisms via additional immune cell subset activation and recruitment. Introduction of CD20-GADLEN into NSG-hIL15 mice engrafted with human PBMCs efficiently depleted human CD20+ B cells in the blood and spleen. Similarly, coadministration of GADLEN with Vγ9δ2$^+$ T cells reduced tumor growth in tumor xenografts.

Conclusions These results provide proof of concept for *in vivo* manipulation of γδ T cells using antigen targeted GADLENs for the treatment of hematologic and solid tumor malignancies.

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