IN VIVO EXPANSION OF GAMMA DELTA T CELLS BY A CD19-TARGETED BUTYROPHILIN HETERODIMER LEADS TO ELIMINATION OF PERIPHERAL B CELLS

Suresh De Silva*, George Fromm, Louis Gonzalez, Arpita Patel, Kyung Yoon, Zachery Opheim, Robert Farner, Dean Chamberlain, Taylor Schreiber. Shattuck Labs, Durham, NC, USA

Background: A primary mechanism of cancer immunotherapy resistance involves downregulation of specific antigens or major histocompatibility complex based antigen presentation, which renders tumor cells invisible to alpha-beta T cells, but not gamma-delta T cells. Recently, a two-step model of gamma-delta T cell activation has emerged, wherein one butyrophilin (BTN, ie. BTN2A1) directly binds the gamma-delta TCR but is only activated if certain molecular patterns (eg. phosphoantigens) facilitate recruitment of a second BTN (ie. BTN3A1) into a complex to form a BTN2A1/3A1 heterodimer. The BTN2A1/3A1 complex specifically activates the predominant gamma-delta T cell population in the peripheral blood, comprising the Vg9d2 T cell receptor (TCR), but does not activate the primary gamma-delta T cell population in mucosal tissues, comprising the Vg4 TCR. The unique mechanism of action and specificity of gamma-delta TCR/BTN interactions suggests that therapeutic proteins comprising specific BTN heterodimers could be used to target specific gamma-delta T cell populations, with a lower risk of off-target activation common with CD3-directed T cell engagers.

Methods: Human BTN2A1/3A1-Fc-CD19scFv and mouse BTN1/6-Fc-CD19scFv heterodimeric fusion proteins were purified and binding to CD19 or the respective gamma-delta TCRs was assessed by ELISA, Octet and flow cytometry using gd T-cells isolated from human peripheral blood and mouse blood, comprising the Vg9d2 T cell receptor (TCR), but is only activated if certain molecular patterns (eg. phosphoantigens) facilitate recruitment of a second BTN (ie. BTN3A1) into a complex to form a BTN2A1/3A1 heterodimer. The BTN2A1/3A1 complex specifically activates the predominant gamma-delta T cell population in the peripheral blood, comprising the Vg9d2 T cell receptor (TCR), but does not activate the primary gamma-delta T cell population in mucosal tissues, comprising the Vg4 TCR. The unique mechanism of action and specificity of gamma-delta TCR/BTN interactions suggests that therapeutic proteins comprising specific BTN heterodimers could be used to target specific gamma-delta T cell populations, with a lower risk of off-target activation common with CD3-directed T cell engagers.

Results: The CD19-targeting scFv domains of the BTN heterodimer fusion proteins bound to human and mouse CD19 with low nanomolar affinity. The BTN2A1/3A1-Fc-CD19scFv compound specifically bound to the Vg9d2 TCR on human gd T cells while the mouse BTN1/6-Fc-CD19scFv bound to Vg7d4 TCR on mouse gd T cells. Both compounds were able to activate gd T cells in a co-culture assay resulting in degranulation and increased surface expression of CD107a and also increased apoptosis of CD19+ tumor cells. Intraperitoneal administration of the mouse BTN1/6-Fc-CD19scFv led to anti-tumor effects in A20 tumor bearing BALB/c mice. Phenotyping from BTN1/6-Fc-CD19scFv treated mice revealed profound and rapid expansion of the endogenous gamma-delta T cells in the circulation and tumor, with concomitant depletion of peripheral CD19+ B-cells, confirming the mechanism of action of the heterodimer as a gamma-delta T cell specific engager.

Conclusions: These results provide proof of mechanism for in vivo manipulation of gamma-delta T cells using antigen-targeted butyrophilin heterodimeric fusion proteins for the treatment of cancer.