0279

BUTYROPHILINS: NOVEL IMMUNE CHECKPOINT TARGETS FOR HIV

Sai Vemula[1], Morgan A. Monslow[1], Guoxin Wu[1], Tassilo Hornung, Laura J. Garvin-Queen[1], Luca Micci[1], Min Xu[1], Lei Ba, Shuo Quan[1], Yvonne Meng[1], Steve Carroll[1], Hussam Shaheen[1], Dan Gorman[1], Daria J. Hazuda[1], and Bonnie J. Howell[1]*

[1]Merck & Co., Inc., Kenilworth, NJ, USA
[2]Caris Life Sciences, Irving, TX, USA

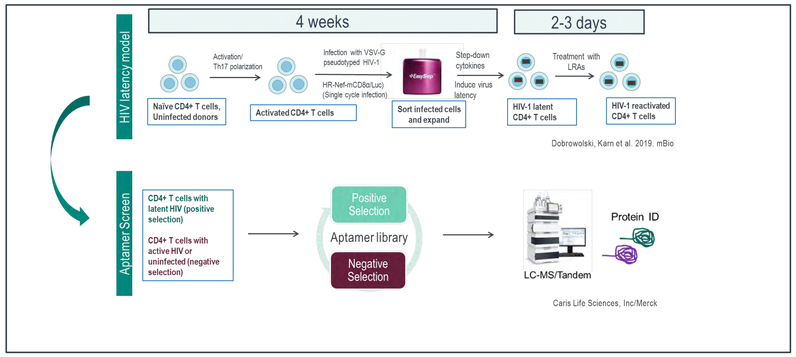
Background: Overexpression of immune-checkpoint receptors (IRs) has been associated with T-cell exhaustion and overall dysfunction in HIV. CD4 T-cells expressing IRs (e.g, PD-1, CTLA-4, LAG-3) enrich for integrated provirus and likely contribute to viral persistence during ART suppression. We identified a class of IRs, buytrophilins (BTNs), with high homology to B7 family members (e.g., PD-L1 and PD-L2) and the capability to modulate T-cell activation and HIV expression. We postulate BTNs can be exploited to induce both latent virus reactivation and/or T-cell function and serve as novel immunomodulatory targets for HIV cure research.

Methods: An aptamer screen was performed to identify proteins enriched on primary CD4 T-cells infected with HIV. Target enrichment was confirmed by flow cytometry as well as immuno-pulldown in CD4 T-cells from ART-suppressed donors using qPCR and ELISA. Functional assays were performed using recombinant BTN proteins or antibodies to demonstrate the impact of target modulation on HIV latency reversal and T-cell activation. Viral reactivation in a human latency model was measured by GFP or luciferase reporter virus and T-cell activation was evaluated concomitantly via IFNg release in the culture supernatant.

Results: BTN immune checkpoint receptors were identified as cell surface proteins overexpressed on in vitro infected HIV+ CD4 T-cells relative to uninfected cells as determined by aptamer screen and flow cytometry. Antibody-pulldowns in CD4 T-cells from ART-suppressed participants demonstrated BTN3A-expressing cells enrich for HIV integrase RNA (4 of 8 participants), LTR DNA (4 of 4), and p24 protein (5 of 8). Recombinant BTN-Fc fusion proteins inhibited activation of human CD4 T-cells following anti-CD3 antibody stimulation, verifying pathway function. In contrast, BTN3A-specific antibodies enhanced T-cell activation and reactivated HIV in response to anti-CD3 antibody; an activity blocked by recombinant BTN3A-Fc proteins. Novel antibodies were generated against three BTN3A protein isoforms using yeast display and characterized for modulation of HIV latency and T-cell activation. Work is ongoing to evaluate Gag-specific CD8 T cell response +/- BTN antibodies.

Conclusions: This data collectively implicates BTN3A family members as putative immune targets for HIV transcriptional regulation or T-cell activation. This novel finding warrants further investigation to determine if therapeutically modulating BTNs can impact the latent viral reservoir.

Methods



Results

Butyrophilins are novel immunomodulatory receptors with increased expression on HIV infected cells

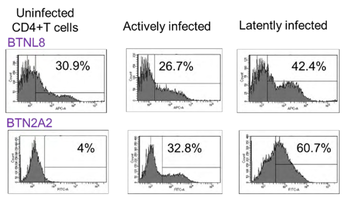


Figure 1: **Expression of butyrophilins (BTN) on HIV-1 infected CD4+ T cells**. Uninfected, active HIV-1 infected, and latent HIV-1 infected cells were stained with anti-BTNL8 (top) or anti-BTN2A2 (bottom) antibodies and protein expression was quantified by flow cytometry.

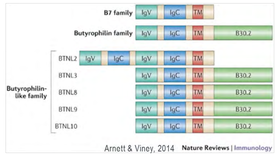


Figure 2: **BTNs are novel class of immunomodulatory receptors**

- Share considerable structural homology with B7 family members (e.g, CD80, CD86, PD-L1, PD-L2); have similar extracellular IgV and IgC domains)
- Primarily expressed on macrophages, DCs, B cells, NK cells, T cells
- Regulate γδ and αβ T cell function
- Expression levels of BTNs elevated on stressed cells and in several human cancers.

Results Continued

Peripheral blood CD4+ T cells from ART-suppressed HIV+ participants expressing BTN3 enrich for HIV DNA

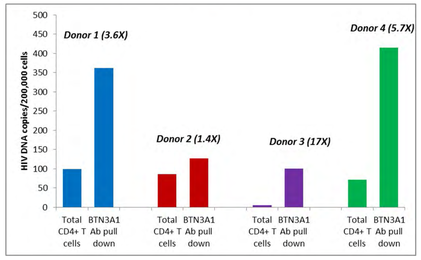


Figure 3: **Enrichment in HIV DNA levels following pull down with antibody beads.** HIV DNA levels in HIV infected CD4+ T cells from four ART-suppressed participants were quantified after BTN3A antibody pull down. Data is presented as HIV DNA copies/200,000 cells. Black bars = total CD4+ T cells; grey bars = BTN3A antibody pulldown cells.

Butyrophilins modulate T cell activation

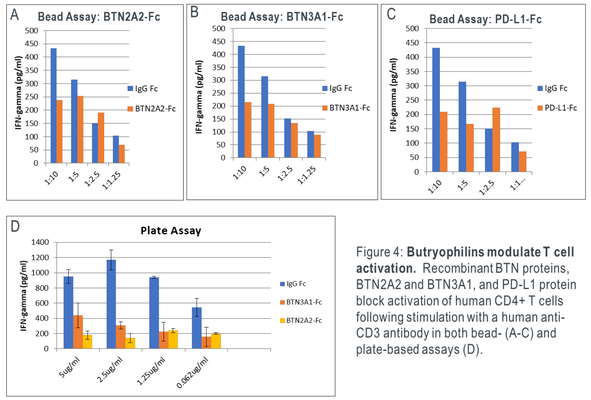


Figure 4: **Butryophilins modulate T cell activation.** Recombinant BTN proteins, BTN2A2 and BTN3A1, and PD-L1 protein block activation of human CD4+ T cells following stimulation with a human anti-CD3 antibody in both bead- (A-C) and plate-based assays (D).

Anti-BNT3A antibody enhances T cell activation and HIV reactivation

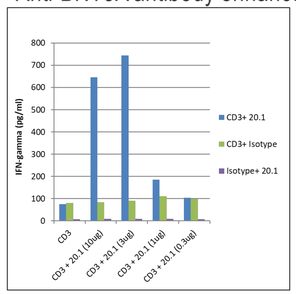


Figure 5: **Anti-BTN3A antibody (clone 20.1) enhances T cell activation mediated by anti-CD3 antibody**. Plates were coated with anti-CD3 antibody and varying concentrations of anti-BTN3A antibody (20.1). As controls, plates were coated with anti-CD3 antibody and varying concentrations of isotype control, anti-CD3 antibody alone, or isotype control alone.

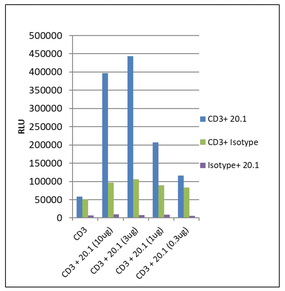


Figure 6: **Anti-BTN3A antibody (clone 20.1) enhances virus reactivation mediated by anti-CD3 antibody in HIV latent primary CD4+ T cells.** Plates were coated with anti-CD3 antibody and varying concentrations of anti-BTN3A antibody (20.1). As controls, plates were coated with anti-CD3 antibody and varying concentrations of isotype control, anti-CD3 antibody alone, or isotype control alone. Luminescence is measured by RLU.

Results Continued

Functional blockade of anti-BTN3A antibody mediated virus reactivation

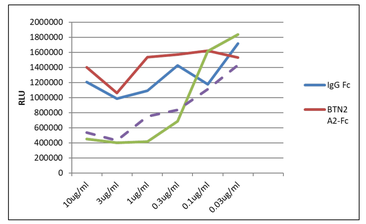


Figure 7: **Recombinant BTN3A proteins block anti-BTN3A antibody (clone 20.1)-mediated virus reactivation in HIV latent primary CD4+ T cells.** Plates were coated with anti-CD3 and anti-BTN3A antibody (20.1). BTN3A1-Fc or BTN3A2-Fc protein was added to block anti-BTN3A antibody-mediated virus reactivation. BTN2A2-Fc and IgG-Fc proteins were used as negative controls.

Anti-BNT3A antibodies enhance cytokine production in HIV+ and HIV- donor PBMCs in the presence of HIV gag/pol peptides

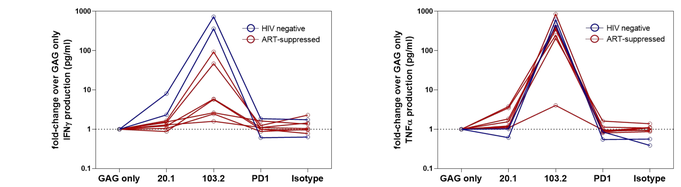


Figure 8: **Treatment of peripheral blood mononuclear cells (PBMC) with anti-BTN3A antibody (clone 103.2) increases IFNγ (left) and TNFα (right) cytokines production in HIV-positive (virally suppressed) and negative donors in the presence of HIV-gag pool-peptide stimulation.** PBMC were pre-incubated with either no antibody, anti-BTN3A (20.1 or 103.2), anti-PD-1 (in-house MK3475), or isotype control antibody 30 minutes prior to adding HIV-gag pool-peptide stimulation. After 6 hours of culture, cytokine production was assessed in the supernatant. Data are normalized as fold-change relative to HIV-gag alone stimulated wells.

Novel BTN3A1 antibodies display either single or dual functions

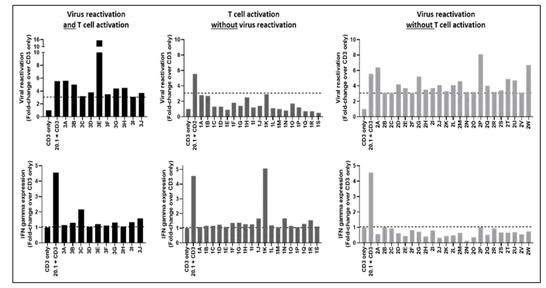


Figure 8 **BTN3A1 antibody campaign screening data**. Each column depicts antibodies that fall within one of the three functional categories: Virus reactivation and T cell activation, T cell activation without virus reactivation, and virus reactivation without T cell activation. Viral reactivation (top row) is expressed as fold change over CD3 control and dotted line represents the 95th percentile cut point. IFN gamma expression (bottom row), indicative of T cell activation, is expressed as fold change over CD3 control and dotted line represents the 95th percentile cut point.

Summary

- ADAPT aptamer screen reveals several immunomodulatory targets expressed on HIV+ CD4+ T cells
- Flow cytometric analysis of HIV infected CD4+ T cells revealed high level of expression of butyrophilin (BTN) family members
- Treatment with recombinant BTN proteins reduce T cell activation mediated by anti-CD3 antibody
- Anti-BTN3 antibody reactivates HIV in a primary T cell model of latency
- Pulldown of blood CD4+ T cells from HIV+ ART suppressed participants using anti-BTN3 antibody shows enrichment for HIV-1 DNA containing cells
- Anti-BNT3A antibodies 103.2 and 20.1 enhance cytokine production in HIV+ and HIV- donor PBMCs in the presence of HIV gag/pol peptides
- Novel BTN3A1 antibody screening campaign identified antibodies with either single or dual functions (T cell activation and/or HIV reactivation)

Presented at CROI; March 9 – 11, 2020, Boston, Massachusetts, USA

Copyright © 2020 Merck Sharp & Dohme Corp., a subsidiary of **Merck & Co., Inc.** All rights reserved.