Proposed
Martin E. Dahl, Tyson Chase, Stephanie Baguley, Eric Hare, Gerald Manorek, Kristine Storey, April Fraley, Gregory Gold, Robert Morse, Margaret Marino, Scott Lashbrook, Justin Choi, JingUei Verkade, Yu-yu Ren and Stephen Parmley, AnaHyBio, Inc., San Diego, CA

Abstract

B and T lymphocyte attenuator (BTLA) is an immune checkpoint molecule that contributes to the regulation of T cell, B cell and dendritic cell function. The role of BTLA as a regulator of autoimmune disease and cancer has been demonstrated via genetics and animal models. More recently, studies have described the finding of soluble checkpoint molecules, including BTLA, in serum or in tumors of diseased patients that correlates negatively with disease outcome, suggesting immune suppression.

We previously disclosed ANB032, a novel anti-BTLA monoclonal antibody that modulates BTLA signaling. In cynomolgus monkeys, we observed elevated levels of soluble BTLA (sBTLA) in the serum that correlated in an anti-inflammatory manner to ANB032 exposure and reduced BTLA expression on T and B cells. The circulating sBTLA in serum represents the intact extracellular domain (ECD) of BTLA.

ANB032 was highly efficacious in a human PBM-C-NOD-SCID (NSG) graft versus host disease (GvHD) model. ANB032 reduced TBLA expression on human T cells and increased human sBTLA in the serum. Flow cytometric analysis of human T cells demonstrated that ANB032 dose-dependently reduced pathogenic T cell activation and expansion.

We found that BTLA is a substrate of PR3 and has a PR3 specific cleavage site in its ECD, suggesting that BTLA is naturally shed by proteolytic cleavage. We propose a mechanism whereby enzymatic cleavage of BTLA is enhanced by monoclonal antibody targeting, which may play a role in the anti-inflammatory therapeutic effect of ANB032.

Introduction: ANB032 modulates trans-BTLA-HVEM interactions and induces the release of soluble BTLA in-vivo

BTLA was first identified as a negative immune regulator on T and B cells, naturally related to PD-1 and CTLA-4. BTLA transcripts vary substantially among different lymphoid and myeloid subtypes with highest expression in B cells. BTLA and its ligand HVEM are upregulated in some mature dendritic cells, macrophages and natural killer cells (Shah et al., 2004). Human BTLA can be upregulated in T cells following transduction with HVEM (HVEM+). In the past decade, HVEM has emerged as a major and complex co-stimulatory signaling molecule.

Engagement of BTLA and HVEM in trans results in bi-directional signaling, with contribution on the BTLA side and costimulation on the HVEM side. We have previously shown that ANB032 binds to BTLA on an epitope that does not disrupt HVEM interactions, stimulated antigen of BTLA that makes BTLA leading to co-inhibition on the BTLA side, while simultaneously disrupting HVEM signaling in trans to lead to crosstimulation.

Although the interactions of BTLA and HVEM have been characterized, recent evidence has identified the presence of soluble checkpoint molecules, including soluble BTLA (sBTLA) in numerous oncology settings where immune suppression is occurring.

Specifically, sBTLA is a predictor of negative outcome in CLL (Simo-Bahamonde, et al., 2021), prostate cancer (Wang et al., 2019), hepatocellular carcinoma (Ding et al., 2020), non-melanoma adenocarcinoma (Shah et al., 2019), renal cell carcinoma (Wang et al., 2019), and in a predictor of overall survival in patients undergoing immune checkpoint inhibitor therapy for solid malignancies (Quebbemann et al., 2019). Outside of oncology, in the setting of autoimmune disease, the role of sBTLA has not been reported, proteolytic cleavage and shedding of a related checkpoint molecule, TIM-3 has been reported. Proteinase 3 (PR3) is a neutrophil serine protease that is released into the extracellular space upon neutrophil activation. PR3 has previously been shown to cleave the checkpoint receptor T-cell immunoglobulin and mucin domain 3 (TIM-3), reducing the levels of TIM-3 on the surface of cells. The ability of PR3 to cleave BTLA activation. PR3 has previously been shown to cleave the checkpoint receptor T-cell immunoglobulin and mucin domain 3 (TIM-3), reducing the levels of TIM-3 on the surface of cells. The ability of PR3 to cleave BTLA has not been reported, proteolytic cleavage and shedding of a related checkpoint molecule, TIM-3 has been reported.

Variable cleavage via serine proteases on the surface of cells. The ability of PR3 to cleave BTLA has not been reported, proteolytic cleavage and shedding of a related checkpoint molecule, TIM-3 has been reported.

We propose a mechanism whereby enzymatic cleavage of BTLA is enhanced by monoclonal antibody targeting, which may play a role in the anti-inflammatory therapeutic effect of ANB032.

Figure 2: Serum concentrations of ANB032 and sBTLA in cynomolgus monkey were determined in a DRF study

sBTLA (ng/mL) 0 20 40 60 80 100 ANB032 (µg/mL) 0 1 3 10

Figure 3: Immunoprecipitation and sequencing of sBTLA from cynomolgus monkey were determined in a DRF study

Figure 4: ANB032 is highly efficacious in a humanized mouse GvHD model

Conclusion

• Studies have described soluble checkpoint molecules, including sBTLA, in serum or in tumors of diseased patients that correlates negatively with disease outcome, suggesting that soluble checkpoint molecules such as sBTLA are biomarkers of immune suppression, effects on BTLA suppression both.

• In cynomolgous monkeys treated with ANB032, we observed elevated levels of sBTLA in serum that correlated to ANB032 inhibition of BTLA expression on T and B cells. The discovered sBTLA represents the intact extracellular portion of BTLA.

• ANB032 was found to be highly efficacious in a xenogenic GvHD model in NSG mice. ANB032 led to anti-inflammatory regulation of pathogenic human T cells in this model, with reduced T cell activation, reduced T cell expansion, reduced T cell BTLA expression and induction of human sBTLA in the serum in a dose-dependent manner. This finding supports the hypothesis that sBTLA is a biomarker of ANB032 activity in-vivo.

• We report that BTLA is cleaved by PR3, similar to what has been published for TIM-3, suggesting that BTLA is naturally shed by proteolytic cleavage, likely upon ligation in trans-in-vivo. We propose a mechanism whereby enzymatic cleavage of BTLA is enhanced by monoclonal antibody targeting, which may play a role in the anti-inflammatory therapeutic effect of ANB032.

• The in-vivo efficacy of ANB032 was evaluated in a xenogenic humanized mouse model of acute GvHD, blood from a limited number of animals from each group was pooled via cardiac puncture. Circulating human T cells were analyzed by flow cytometry for the characteristic BTLA expression, enurethane human T cell numbers, and characteristic the activation marker CD25. ANB032 reduced BTLA expression on human T cells. ANB032 reduced expression of the activation marker CD25 at all doses. ANB032 was measured by ELISA and shown to increase in a dose-dependent manner. Based on robust efficacy observed in this model, the ANB032 effects on pathogenic human T cells, and the observation of sBTLA in the serum, our hypothesis is that BTLA is cleaved from the surface of T cells and that BTLA is a pharmacodynamic marker of ANB032 activity in-vivo.

Figure 5: ANB032 demonstrated inhibition of T cell expansion, reduced BTLA expression and induction of sBTLA in a humanized murine GvHD model.