TCR-Nck Modulators: Pioneering Oral Modulation of T Cell Receptor Activation Holding the Promise of Treating Autoimmune Diseases

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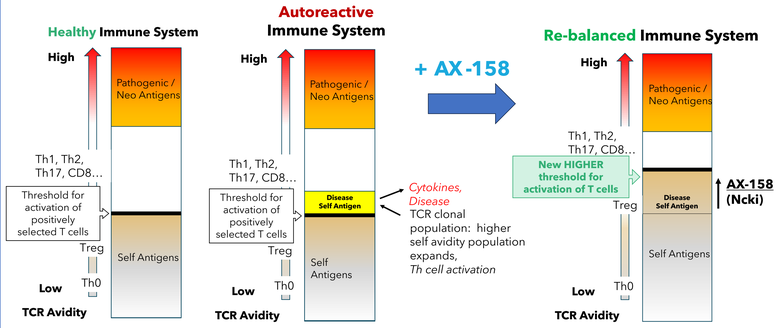
Abstract

Background/Purpose

Loss of T-cell tolerance to self-antigens underlies the development of all autoimmune diseases, and despite progress, there remains a significant unmet need for patients. The most effective modern therapeutic approaches focus on the suppression of key cytokines that are important for disease progression . In contrast, modulation rather than suppression of T cell receptor (TCR) activation offers the potential to normalize the patient's immune response to self, while preserving protection against pathogens. AX-158 is a Nck modulator currently in a Phase 2a trial for psoriasis.

Emergence of autoreactive clonal populations of T cells that drive autoimmune disease and relevance of Nck modulation to re-balance immune function



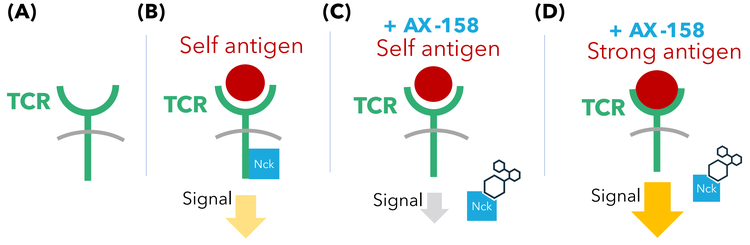
Mechanism: TCR signal modulation, not inhibition

(A) Nck is not associated with resting state T cell receptor (TCR)

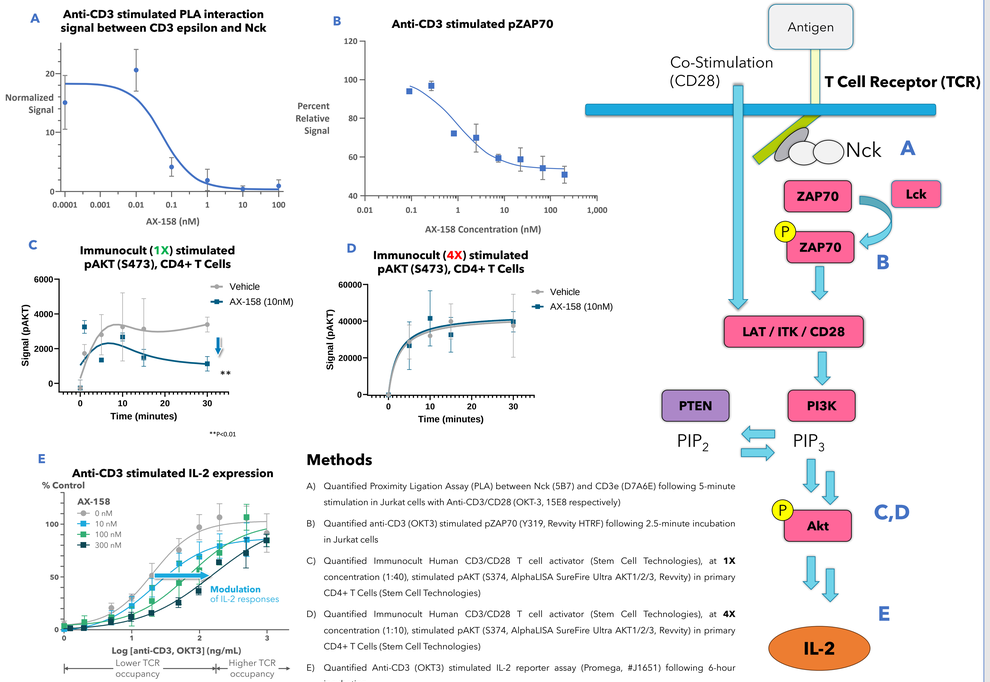
(B) Upon antigen binding, Nck is recruited to the activated TCR and positively modulates signaling

(C) AX-158 binds to Nck, preventing recruitment to the activated TCR, signal is negatively modulated

(D) Strongly avid antigens result in strongly activating TCR signals, Th activation is independent of Nck



AX-158 activity consistent through the TCR signal cascade



Methods

A) Quantified Proximity Ligation Assay (PLA) between Nck (5B7) and CD3e (D7A6E) following 5-minute stimulation in Jurkat cells with Anti-CD3/CD28 (OKT-3, 15E8 respectively)

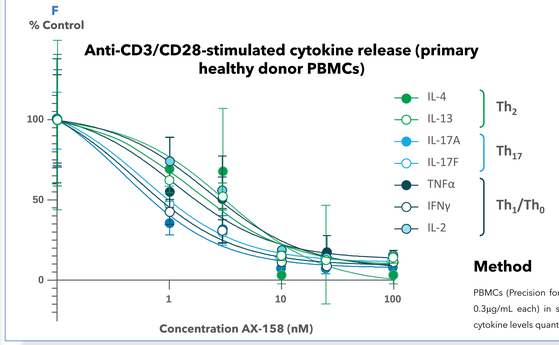
B) Quantified anti-CD3 (OKT3) stimulated pZAP70 (Y319, Revvity HTRF) following 2.5-minute incubation in Jurkat cells

C) Quantified Immunocult Human CD3/CD28 T cell activator (Stem Cell Technologies), at **1X** concentration (1:40), stimulated pAKT (S374, AlphaLISA SureFire Ultra AKT1/2/3, Revvity) in primary CD4+ T Cells (Stem Cell Technologies)

D) Quantified Immunocult Human CD3/CD28 T cell activator (Stem Cell Technologies), at **4X** concentration (1:10), stimulated pAKT (S374, AlphaLISA SureFire Ultra AKT1/2/3, Revvity) in primary CD4+ T Cells (Stem Cell Technologies)

E) Quantified Anti-CD3 (OKT3) stimulated IL-2 reporter assay (Promega, #J1651) following 6-hour incubation

AX-158 modulates TCR driven Th cell activation across subtypes



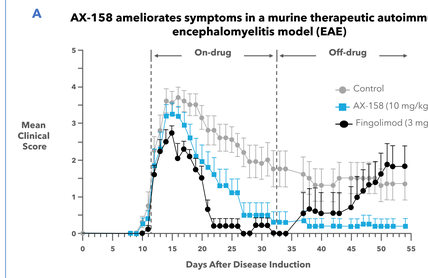
in vitro Results

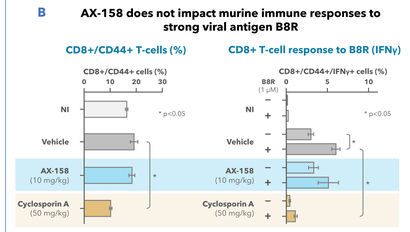
Cellular assays along the TCR cascade consistently demonstrate the activity of AX-158 in modulating TCR signaling. At the TCR, the PLA and pZAP assay observations illustrate the inhibition of Nck recruitment and lowering of pZAP70 kinetics (**A,B**). At later time points, the pAkt assay demonstrates the effect of AX-158 at weak, but not strong TCR activation (**C,D**). An IL-2 assay illustrates that AX-158 modulates but does not inhibit TCR stimulated cytokines (**E**). Finally, AX-158 demonstrates broad modulation of Anti-CD3/CD28 stimulated cytokines across Th cell subtypes in primary PBMCs (**F**).

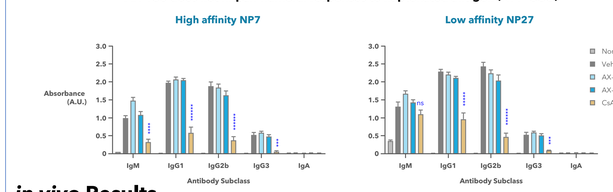
Method

PBMCs (Precision for Medicine) were stimulated with Anti-CD3/anti-CD28 (OKT3, 15E8 respectively, 0.3µg/mL each) in serum free RPMI 1640 media for 48 hours. Supernatants were collected and cytokine levels quantified (Procarta Plex, Thermofisher)

AX-158 Selectively impacts murine inflammation models







Methods

A) Female C57BL/6 mice were induced using MOG (MOG_{35-55}, Espikem) in CFA (Sigma-Aldrich). When mice reached a clinical score assessment of 2 (moderate hind limb weakness) they were randomized into groups and administered treatments for 22 days. Study was continued until day 54, monitoring and scoring clinical signs of disability. AX-158 was orally administered once daily (qd) at 1 and 10 mg/Kg (statistically significant at both 1 and 10 mg/Kg, only 10mg/Kg shown for clarity).

B) C57BL/6 mice were inoculated intraperitoneally with syngeneic bone marrow-derived dendritic cells pre-loaded with peptide B8R (immunodominant epitope common to several poxviruses). Treatments were administered for 7 days. AX-158 was orally administered b.i.d. at 10mg/Kg (high dose).

C) C57BL/6 mice were immunized with NP-CGG (nitrophenol chicken immunoglobulin) and randomized for treatment. Animals were treated up to 10mg/Kg qd with AX-158. ELISA was used to detect low affinity and high affinity immunoglobulins by varying the number of Nitrophenol units per bait protein (derivatized BSA).

in vivo Results

In the self-antigen driven murine experimental autoimmune encephalomyelitis (EAE) model, AX-158 conferred long-lasting protection in a therapeutic and in a self-antigen rechallenge setting (**A**). In contrast, AX-158 did not suppress the T cell response to a viral antigen (B8R of poxviruses) or the T-dependent humoral response to a model haptenated (NP-CGG) antigen (**B**,**C**).

Conclusion

These data suggest that selective TCR-Nck modulation represents a fundamental paradigm shift in the treatment of autoimmune diseases, one aligned with the restoration of self/non-self discrimination by the immune system.

Disclosures

Christopher VanDeusen, Shannon Dwyer, and D. Scott Batty Jr are employees of Artax Biopharma Inc. Andres Gagete is a consultant for Artax Biopharma Inc. Balbino Alarcon has a financial interest in Artax Biopharma and a sponsored research agreement with Artax Biopharma Inc. This work was sponsored by Artax Biopharma Inc.