Impacts of TCR-Nck Interaction Inhibitors on T Cell Activation Driven By Mixed Lymphocyte Reactions

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) using allogeneic donors is associated with a high risk of graft-versus-host disease (GvHD). GvHD is a systemic disorder that usually involves the skin, gastrointestinal track, liver, kidneys, eyes and also the hematopoietic system leading to severe morbidity and mortality. The major cause of GvHD is the activation of T cells of the donor by mismatched HLA class I and II haplotypes present in the tissues of the acceptor. The T cell antigen receptor (TCR) of the donor's T cells binds mismatched HLA and becomes activated leading to T cell activation and differentiation into effector T cells that promote tissue attack. TCR triggering involves the recruitment of the adapter protein Nck among other cytoplasmic effector proteins. The TCR-Nck interaction is an amplifier of TCR-peptide/MHC (TCR-pMHC) interactions.

Here we show that an oral, clinical-stage second generation Nck inhibitor (AX-158) potently modulates the activation of human T cells in Mixed Lymphocyte Reactions (MLR) in vitro, suggesting that disruption of the TCR-Nck interaction is modulatory for T cell responses driven by allogeneic stimuli. Specifically, AX-158 lowered cytokine production upon MLR (Interferon gamma, TNF alpha, IL-2). MLR driven proliferative responses were not significantly impacted, suggesting that Nck inhibition leads to less activated T Cell populations to drive potential benefit. By modulating T Cell activation in response to allogeneic stimuli while sparing responses to strong foreign antigens, Nck inhibitors offer the promise of potentially treating GVHD without strong immunosuppression.

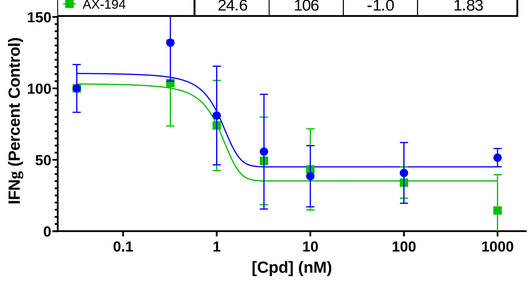
STUDY DESIGN

- ❑ Human PBMCs purified from healthy donors.
- ❑ Cells were tested for responsiveness and mixed together in pairs in equal volumes.
- ❑ Cells were pre-treated with compounds for 30 minutes.
- ❑ Cells were incubated for 18 hours at 37 ˚C.
- ❑ Cells were spun down and superintendents collected.
- ❑ Supernatants were analyzed for various cytokines using a Luminex 200 and quantified with a standard curve for each analyte.
- ❑ Results shown are typically four replicates and representative of multiple experiments.

RESULTS

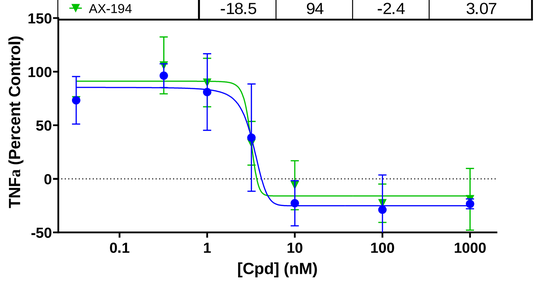
Artax Compounds Reduce IFNg Release in a Dose-dependent Manner

	Bottom	Top	HillSlope	IC50 (nM)
AX-158	43.7	110	-2.7	1.20
AX-194	24.6	106	-1.0	1.83

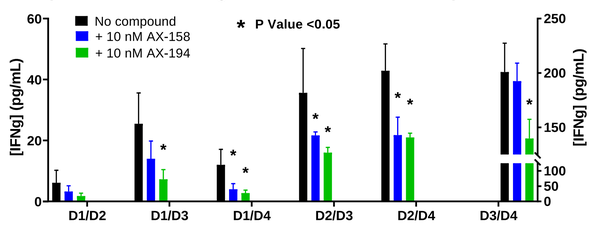


Artax Compounds Reduce TNFa Release in a Dose-dependent Manner

	Bottom	Top	HillSlope	IC50 (nM)
AX-158	-26.8	85	-3.0	3.52
AX-194	-18.5	94	-2.4	3.07



Compound Effects on IFNg are Consistent Across Multiple Donor Pairs



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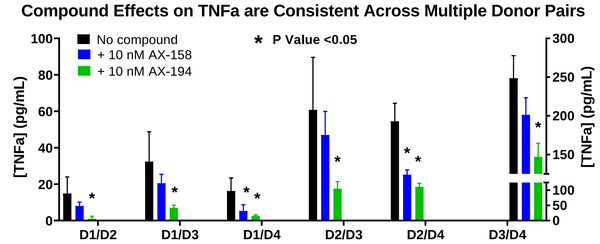
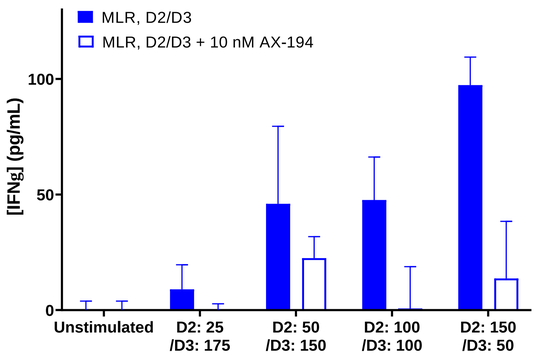
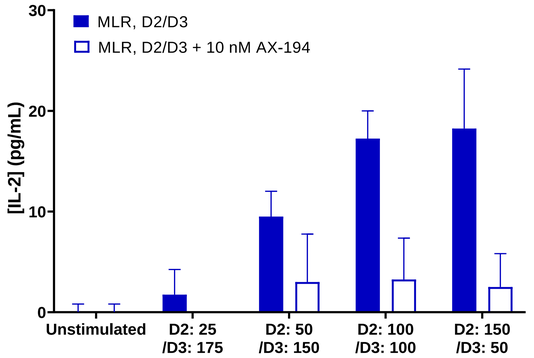


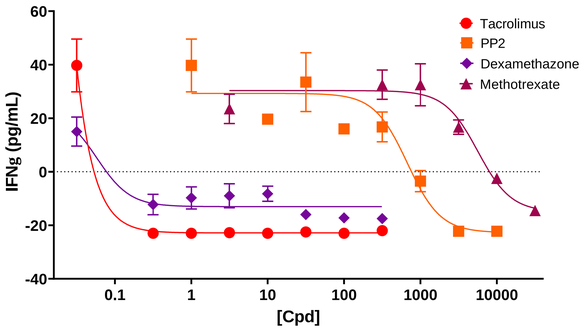
Effects on IFNg Release are Dependent on Donor Volumes



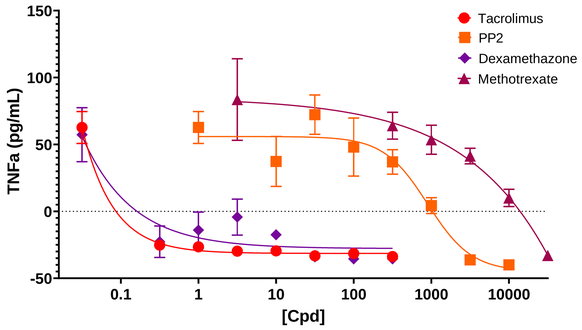
Effects on IL-2 Release are Dependent on Donor Volumes



Immuno-suppressive Control Compounds Completely Prevents IFNg Release



Immuno-suppressive Control Compounds Completely Prevents TNFa Release



PRINICPLE FINDINGS

- ❑ **Artax compounds exhibit a dose dependant inhibition of MLR-dependant cytokine production with an IC50 of ~1-5 nM.**
- ❑ **Potency was found to be consistent across multiple donor pairs.**
- ❑ **Adjusting the relative amounts of the two donor's cells produced a cell volume-dependant effect, demonstrating that the compounds are impacting the MLR response.**
- ❑ **Immune suppressive control compounds had a much stronger effect and eliminated the baseline levels of the cytokine release. This was consistent with immuno-suppressive side effects.**
- ❑ **Artax Nck inhibitors did not share this pattern of effects in these studies.**

CONCLUSIONS

Modulation of T cell stimulated reactions to allogenic stimulation offers the potential to modulate GvHD.

The modulation is rapid, robust and consistent. The compounds are selective and potent.

The current data, combined with other internal data suggest that these compounds should not exhibit strong immunosuppression.

Nck inhibitors have the potential to be an effective treatment for GvHD.

Other in-house data demonstrate direct impact on the TCR complex via the SH3.1 domain of Nck. This is a promising mechanism for modulation of several T cell mediated pathologies.

CONTACT INFORMATION

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