Supplemental Data:

Title: Dual Inhibition of IL-23 and IL-17 Offers Superior Efficacy in Mouse Models of Autoimmunity

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Supplemental Figure and Table Legends:

Supplemental Table 1: Biochemical analysis of ATI-1221 and ATI-1249

Kinetics for ATI-1221 and ATI-1249 (C-terminally PEGylated version of ATI-1221) were assessed against directly immobilized murine IL-23 using a Proteon instrument. Murine IL-23 at 2.5 to 5 µg/mL in acetate pH 5.5 was immobilized on a GLC chip to a surface density of ~ 1000-2000 RU. Concentration series of adnectins in PBS-T were injected and dissociation monitored for up to 3 hours at 37C. Kinetic parameters were evaluated using a 1:1 fitting model with local Rmax. Data are the averaged kinetic parameters from the number of experiments indicated in the table.

Supplemental Figure 1: ATI-1221 and ATI-1249 Do Not Inhibit IL-12-induced Th1 Differentiation and Secretion of IFN-γ

To differentiate Th1 cells, naive CD4+CD62L+ T cells were enriched from the pooled spleen and lymph nodes of C57/B6 mice (Charles River Laboratories) by magnetic bead selection (Miltenyi Biotec) and seeded on 96 well plates coated with anti-CD3 (145-2C11, generated at BMS) in presence of 10 ng/ml recombinant mouse IL-12 (R&D Systems), 1 µg/ml anti-CD28 antibody (3N7, generated at BMS) and 2 µg/ml of anti-IFN-γ (XMG1.2, eBioscience) neutralizing antibody. To test the activity of ATI-1221 and ATI-1249 for inhibiting IL-12-dependent responses, a range of each inhibitor as well as mouse anti-IL-12p40 antibody (R&D Systems) was added to the cultures during Th1 differentiation. After 4 days, the conditioned media from the cultures was harvested and assayed for IFN-γ concentrations by ELISA (R&D Systems). The IL-12-dependent response was measured by calculating the difference between the level of cytokine induced by media alone versus in the presence of IL-12 alone with no inhibitors added. Results are expressed as percent inhibition of IFN-γ and the IC_{50} curve was generated using GraphPad Prism software. IC_{50} curves shown were generated from one study and are representative of three

replicate studies. Each data point represents the percent inhibition calculated from the average IFN-γ concentration in duplicate wells.

Supplemental Table 2: Mouse IL-17RA-Fc Biacore Kinetics

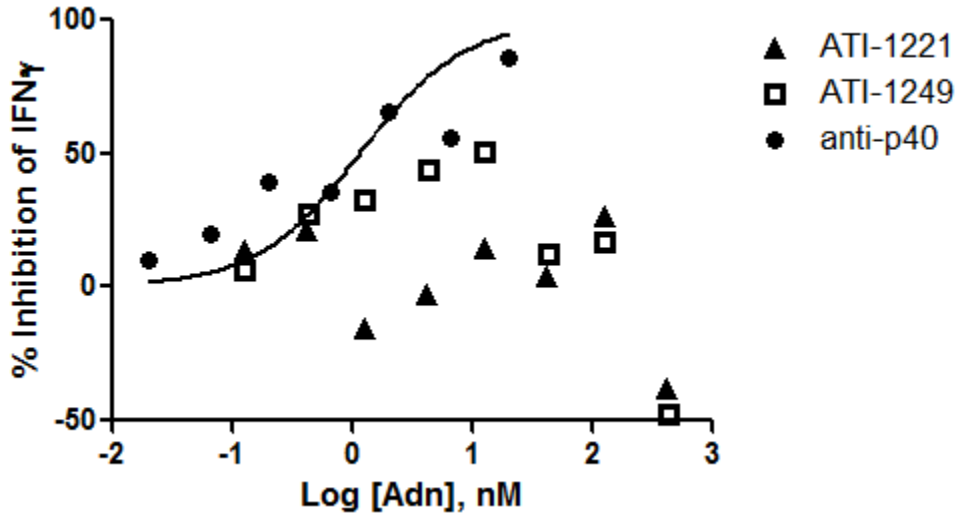
Kinetics analysis of mIL17RA-Fc was conducted by first amine coupling Protein A (Thermo Scientific, Rockford IL) using standard EDC-NHS chemistry to a Series S CM5 chip (GE Healthcare, Piscataway NJ), followed by capture of mIL17RA-Fc. A titration of 200 to 5.1nM murine IL17A (R&D Systems, Minneapolis MN) was injected across the IL-17RA-Fc surface in HBS-EP+ buffer (GE Healthcare, Piscataway NJ). The surface was regenerated between sample injections using 10mM glycine-HCl pH 2.0 (GE Healthcare, Piscataway NJ). Sensorgrams were double reference subtracted and globally fit to a 1:1 Langmuir model. Binding kinetics of the mouse anti-IL-17 antibody for murine IL-17A/A and IL-17A/F was determined using a Biacore T100 (GE Healthcare, Piscataway NJ). Previous Biacore studies indicated no reactivity of this antibody towards murine IL-17F/F. In brief, biotinylated IL-17A or IL-17A/F, expressed in house using HEK cells, was captured on a streptavidin CAPture chip (GE Healthcare, Piscataway NJ), followed by injection of a 200 to 2.5nM titration of anti-IL-17 antibody in HBS-EP+ buffer. Regeneration of the CAPture reagent surface was done according to the manufacturer's guidelines. Sensorgrams were double reference subtracted and locally fit to a 1:1 Langmuir model.

Supplemental Figures and Tables:

Supplemental Table 1:

Inhibitor	Replicates	ka (1/Ms)	kd (1/s)	KD (M)
ATI-1221	n=2	8.85E+04	2.17E-05	2.45E-10
ATI-1249	n=6	9.20E+04	2.15E-05	2.34E-10

Supplemental Figure 1:



Supplemental Table 2:

Inhibitor	KD (nM) IL-17A/A	KD (nM) IL-17A/F	KD (nM) IL-17F/F
mIL-17RA-Fc	0.022	ND	ND
Anti-IL-17 Ab	<0.07	<0.06	No Binding