TYK2 promotes IL-23 induced type 3 immunity and disease progression in SpA

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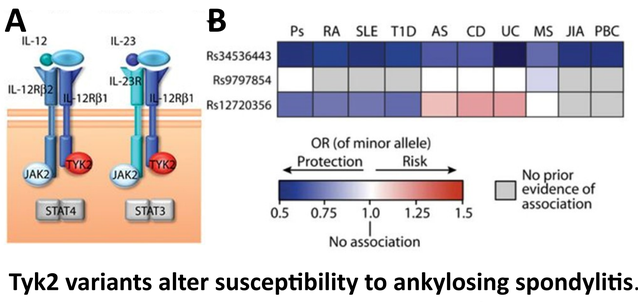
Introduction

Th17 cells play an important role in the pathogenesis of ankylosing spondylitis (AS), a debilitating arthritis of the axial skeleton[1]. TYK2, a member of the Janus Kinase (JAK) family of signaling molecules, associates with a number of receptors, including the type 1 interferon, IL-10/IL-22, and IL-12/IL-23 families of cytokine receptors. TYK2 plays a crucial role in Th17 cell function through mediating IL-23 intracellular signaling[2], making TYK2 an attractive target for the treatment of AS.



Tyk2 variants alter susceptibility to ankylosing spondylitis. A) Tyk2 mediates signaling through the IL12/IL23 family of cytokine receptors. B) AS Tyk2 risk variants shared with multiple autoimmune diseases. Adapted from Dendrou, 2016.

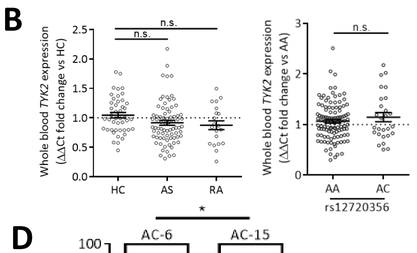
TYK2 was the first JAK to be associated with AS in genome-wide association studies[3] and is associated with psoriatic arthritis (PsA)[4]. The majority of AS/PsA-associated SNPs impart non-synonymous mutations[5], suggesting these variants impart a change in TYK2 function and not expression. Recent work suggest altered function of TYK2 is the most likely cause of TYK2's association across multiple autoimmune diseases[6].

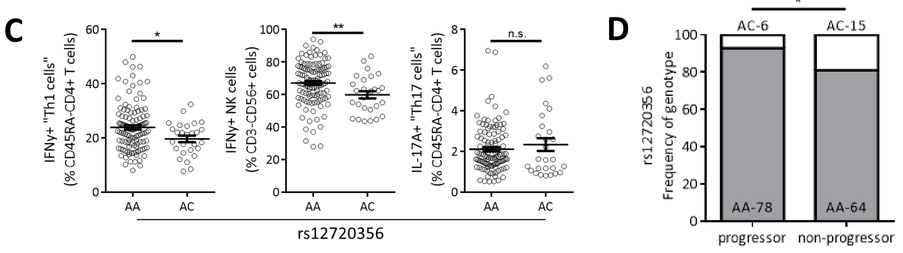
Nimbus Therapeutics have developed a selective and potent catalytic inhibitor of TYK2, NDI-031407. **Here we examine the role of TYK2 in murine models of local and systemic IL-23 inflammation, type 3 immune cells in vitro and correlate immune cell phenotype and AS disease progression with TYK2 risk SNPs.**

AS-associated TYK2 LoF SNP correlates with AS progression

A

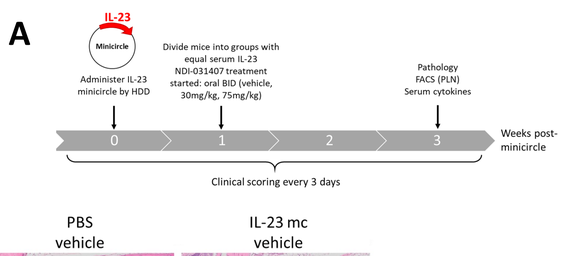
	Cohort 1			Cohort 2 (AS-only)	
	HC	AS	RA	Non-Progressors	Progressors
Sex (M/F)	29/18	49/27	6/15	55/24	71/14
Age (yr±SD)	37±12	42±16	54±11	38±13	51±11
HLA-B27+		79%		80%	74%
BASDAI		3.7±2.6		4.6±2.7	5.05±2.4
Mean CRP (mg/L±SD)		7.6±8.7		11.9±23	20.5±24

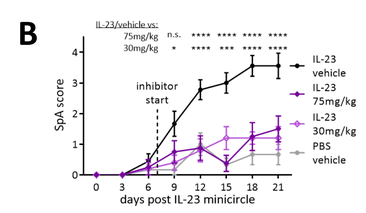


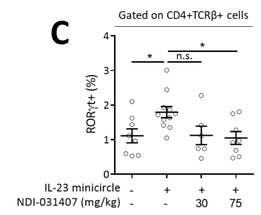


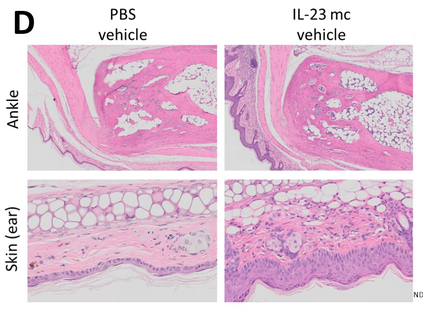
AS-associated TYK2 SNPs do not alter TYK2 expression, but associate with altered Th1 frequency and AS disease progression. A) Clinical features of two AS patient cohorts from the Toronto Western Hospital SpA clinic. All patients fulfilled mNY criteria for AS and the imaging arm for axSpA. B) Cohort 1 examined for TYK2 gene expression in whole blood by qPCR. Data analyzed by patient type (left) or by rs12720356 genotype (right). C) Cohort 1 examined for Th1 and Th17 frequency by flow cytometry in PMA/ionomycin stimulated PBMC. AS patients had elevated Th17 and reduced Th1 frequency compared to healthy controls (not shown). All subjects (AS/RA/HC) pooled and data stratified by rs12720356. D) Cohort 2 genotyped for rs12720356. Data in B and D analyzed by One-way ANOVA or Mann-Whitney test. Data in D analyzed by Fisher's exact test.

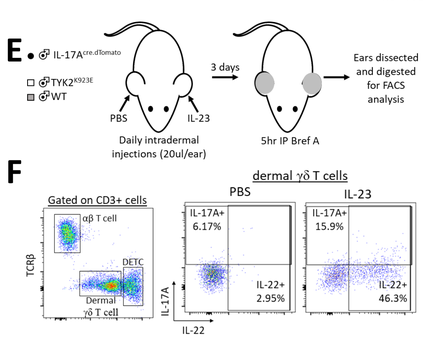
Genetic and pharmacologic inhibition of TYK2 protects against IL-23 induced inflammation





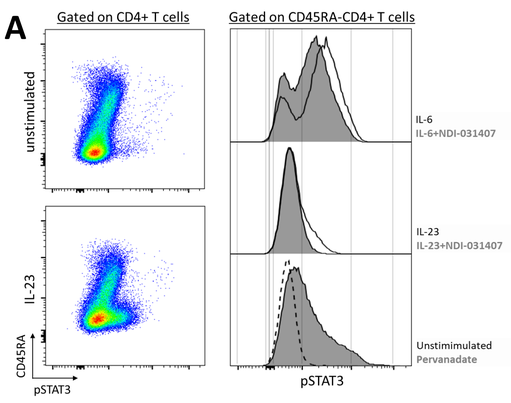


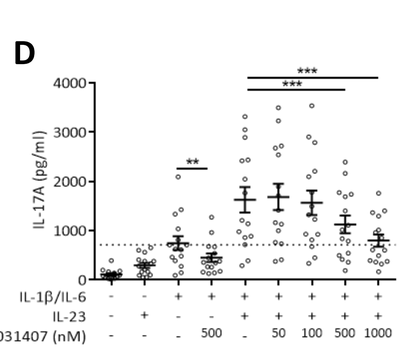


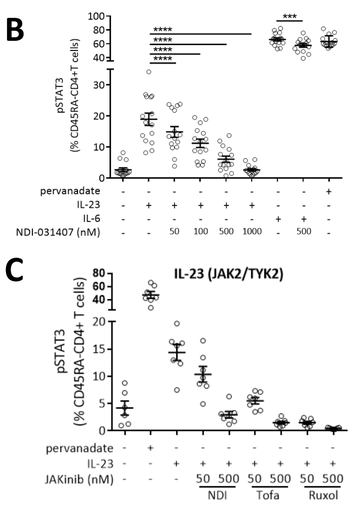


TYK2 plays a pro-inflammatory model in IL-23 induced inflammation in vivo. The IL-23 minicircle was used to induce systemic IL-23 overexpression in male B10.RIII mice. A) Schematic of IL-23 minicircle experiment. Briefly, 6µg minicircle administered by hydrodynamic delivery and NDI-031407 treatment begun one week post minicircle treatment. B) Composite SpA score (blepharitis, dermatitis, arthritis) assessed every 3 days. C) RORγt+CD4+TCRβ+ "Th17" cell frequency in popliteal lymph nodes assessed by flow cytometry at endpoint. D) H&E sections and scoring of skin and ankle enthesis. To induce local inflammation (dermatitis), 400ng IL-23 was injected intradermally into the ear for 3 days. E) Schematic of the dermatitis experiment. 300µg Bref A administered IP prior to mouse sacrifice to allow for cytokine analysis without restimulation. TYK2 inhibited pharmaceutically with 100mg/kg NDI-031407 delivered oral BID starting 1 day before first IL-23 injection. TYK2 inhibited genetically using mice with kinase-dead TYK2 (TYK2^{K923E}). F) Representative flow cytometry gating showing dermal γδ T cell identification and cytokine production. G) Dermal γδ T cell IL-17/IL-22 with NDI-031407 treatment or in TYK2^{K923E} mice. Data assessed by Mann-Whitney test, one-way ANOVA or Wilcoxon test where appropriate.

NDI-031407 blocks IL-23 activation of human Th17s

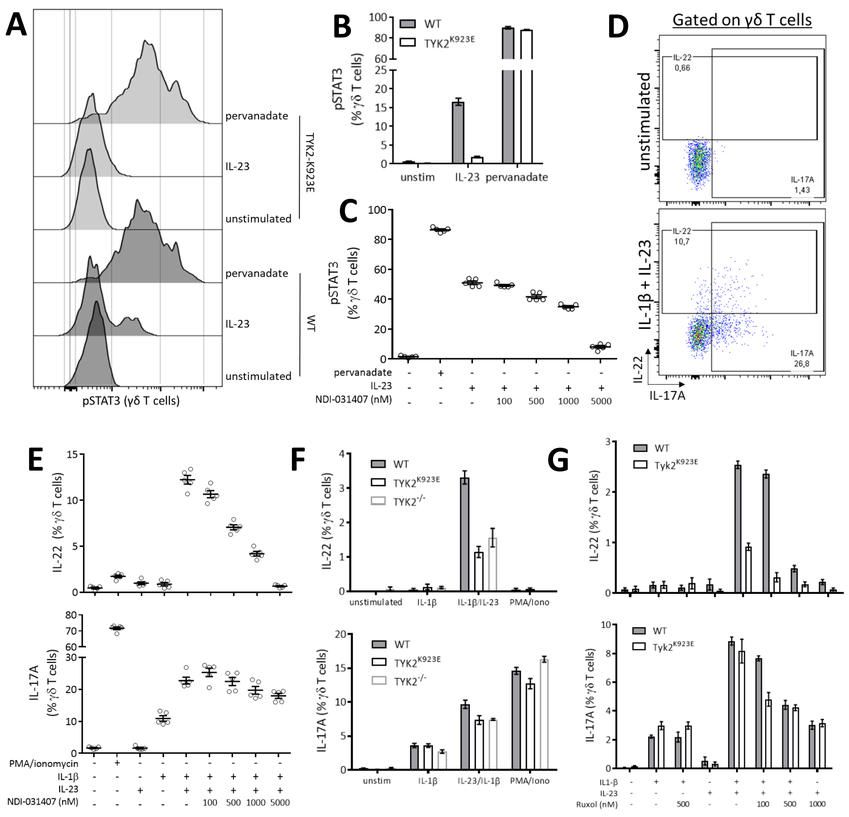






NDI-031407 is effective at blocking IL-23 signalling and cytokine release from human Th17 cells in vitro. For assessment of STAT phosphorylation by flow cytometry, PBMC activated for 3 days with anti-CD3/CD28 beads prior to 15 minute stimulation with 400ng/ml IL-23 or IL-6. A) Representative flow plots. B) STAT3 phosphorylation in cohort of healthy individuals with NDI-031407 treatment. C) IL-23 induced pSTAT3 in an independent cohort comparing NDI-031407 to non-selective JAK inhibitors. D) CD4+ T cells isolated by magnetic selection and skewed to Th17 cells for 4 days by anti-CD3/CD28 stimulation with the indicated cytokines. Data analyzed by one-way ANOVA with Dunnet's post-test or by Mann-Whitney test where appropriate.

TYK2 is essential for IL-23-induced IL-22, but not IL-17A



TYK2 does not play a universal role in IL-23 activation of murine γδ in vitro. γδ T cells identified by flow cytometry in whole lymph node extract. Lymphocytes stimulated for 15 minutes with 400ng/ml IL-23 for pSTAT3 detection by flow cytometry. A) representative γδ T cell pSTAT3 staining in WT or TYK2^{K923E} mice with the indicated stimulations. Lymphocytes treated under the indicated conditions from B) WT or TYK2^{K923E} γδ T cells or C) NDI-031407 treated WT cells. To examine down-stream effectors of IL-23 stimulation, whole lymph node extracts stimulated for 4.5 hours with 20ng IL-1β and 20ng IL-23. D) Representative gating strategy of IL-23 induced cytokines in γδ T cells. IL-17A/IL-22 production in E) WT cells pretreated with NDI-031407 or F) in WT, TYK2 kinase dead (K923E) or knock-out (KO) cells. G) WT and TYK2^{923E} cells pretreated with the JAK inhibitor, ruxolitinib, prior to stimulation with IL-1β/IL-23. All graphs from single experiment representative of 2-3 independent repeats per experiment.

Conclusion

Our results in AS patients support the notion that **TYK2 SNPs associated with AS (and PsA), likely exert their effect through altering TYK2 function**. It has been reported that TYK2 loss of function (LoF) SNPs are the primary variants associated with autoimmune disease[6]. Consistent with this, we find the LoF SNP rs12720356 to be associated with reduced frequency of IL-12 dependent cells (Th1 and NK) and to associate with protection against arthritic progression. Loss of TYK2 in humans[7] and mice[2] has no effect on Th17 frequencies, consistent with the lack of effect of rs1272056 on Th17 frequency.

We show that **TYK2 inhibition in SpA models has a protective effect** associated with inhibition of type 3 immunity. The protective effect may be mediated through blocking IL-22 via TYK2. Anti-IL-23 is beneficial for PsA and the SpA-linked diseases, psoriasis and IBD, but not AS. While this work does not explain why blocking IL-23 may be ineffective in AS, it does shed light on the dissociation between IL-23 and IL-17A. As has previously been reported, we have demonstrated that IL-23 alone cannot drive an IL-17A response. Co-stimulation, especially with IL-1β, is essential for IL-23 to induce effector cytokines. We show for the first time that **IL-17A and IL-22 are regulated by distinct IL-23 intracellular signaling pathways**, involving JAK2 independent of STAT3 and JAK2/TYK2/STAT3 respectively.

These data provide strong pharmacological support that TYK2 inhibition is a validate therapeutic target for SpA through inhibition of type 3 immunity.

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