Reduced Frequency of RORγt Tregs in Axial Spondyloarthritis Secukinumab Responders

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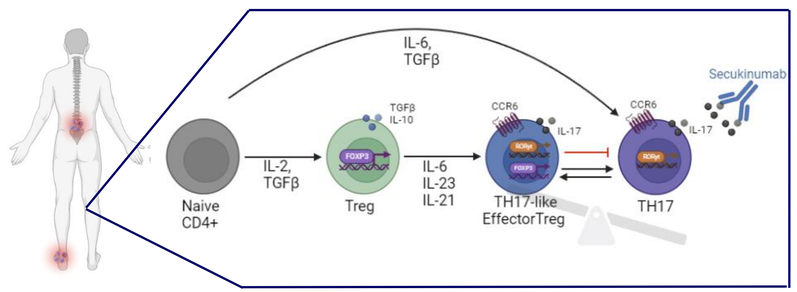
Introduction and Background



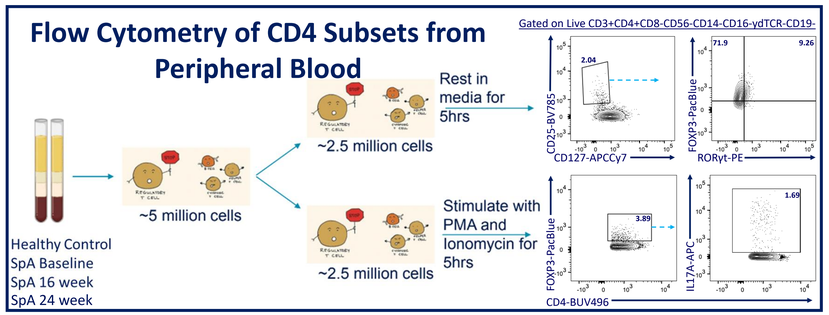
Axial Spondyloarthritis (axSpA) is an inflammatory disease that predominantly affects the spine and the sacroiliac joint. Although the spine is the primary site, other peripheral joints often also become inflamed. Although the pathobiology of axSpA is not completely known, biologics that target the cytokine IL-17A, such as secukinumab, have demonstrated improved clinical outcome for majority of patients. T-helper 17 (Th17) cells produce IL-17A and are regulated by Regulatory T cells (Tregs). Tregs gain an effector phenotype (RORγt+ Treg) to suppress Th17 proliferation and cytokine production.

Objective/Hypothesis

Objective: The Predicting Clinical and Immune Secukinumab Effects (PreCISE) study aims to address biomarkers that may predispose treatment outcomes.

Hypothesis: We hypothesize a decrease in the frequencies of IL-17A-producing cell subsets in secukinumab responders and increase of Type 3 regulatory cells.

Flow Cytometry of CD4 Subsets from Peripheral Blood



Patient Cohort Characteristics

Characteristic	Flow Cytometry Analysis			Pilot NanoString Study	
	Healthy Control (n=13)	Responder (n=13)	Non-Responder (n=8)	Responder (n=8)	Non-Responder (n=7)
Age, Years	42.7	43.14	46	43.57	43.57
Sex, Men	9 (69.2%)	8 (61.5%)	5 (62.5%)	7 (87.5%)	6 (85.7%)
HLA-B27, Positive	N/A	10 (76.9%), 2 unk	5 (62.5%), 2 unk	7 (87.5%)	6 (85.7%)
BASDAI, Baseline -> Followup	N/A	5.66 -> 3.46	5.87 -> 5.66	5.63 -> 2.98	6.38 -> 6.45
BASDAS, Baseline -> Followup	N/A	3.41 -> 2.52	3.07 -> 3.32	3.77->2.17	3.38 -> 3.67
Psoriasis, Yes	N/A	2 (15.4%)	2 (25%)	1 (12.5%)	3 (42.9%)
Uveitis, Yes	N/A	1 (7.69%)	1 (12.5%)	1 (12.5%)	1 (14.3%)

Fig 1. Patient Characteristics. Sample characteristics are described for healthy controls, baseline axSpA patients and 24-week secukinumab treated axSpA patients.

Immune Phenotyping Reveals a Decreased Frequency of RORγt+ Tregs in axSpA patients that correlates with Disease Severity

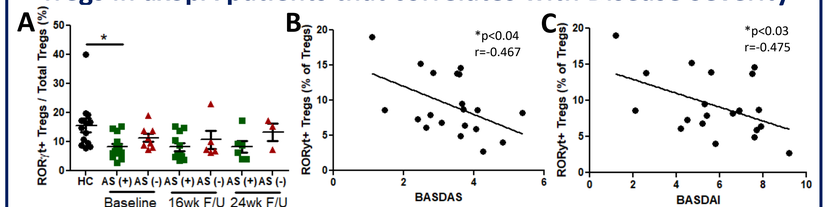


Fig 2. Immune Profiling of RORγt+ Tregs by Flow Cytometry. (A) Dot plot representing the frequency of RORγt+ Treg. Green squares represent responders to secukinumab and red triangles represent non responders. Scatterplot shows correlation of RORγt+ Treg frequency with **(D)** BASDAS and **(E)** BASDAI clinical scoring. Significance determined by Kruskal-Wallis test with Dunn's Multiple Comparison Test for dot plots or Spearman correlation test for scatterplot. p<0.05*

Immune Phenotyping Reveals that Secukinumab has a Significant Effect on IL17+ FOXP3+ CD4+ Frequency but this Subset does not correlate with Disease Severity

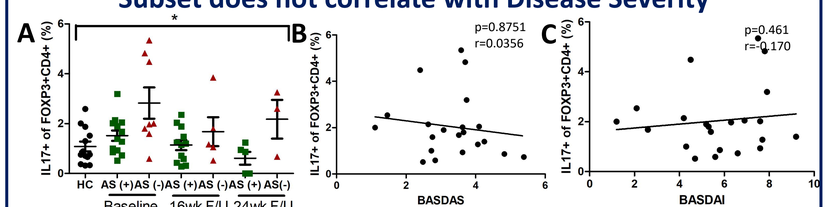


Fig 3. Immune Profiling of IL17+ FOXP3+ CD4 by Flow Cytometry. (A) Dot plot representing the frequency of IL17 producing FOXP3+ CD4s. Green squares represent responders to secukinumab and red triangles represent non responders. Scatterplot shows correlation of IL17+ FOXP3+CD4+ frequency with **(B)** BASDAS and **(C)** BASDAI clinical scoring. Significance determined by Kruskal-Wallis test with Dunn's Multiple Comparison Test for dot plots or Spearman correlation test for scatterplot. p<0.05*

Gene Expression Profiling of Mature CD4s Shows Decreased Type 1 Immunity in Responders compared to Non Responders after 24 week Secukinumab Treatment

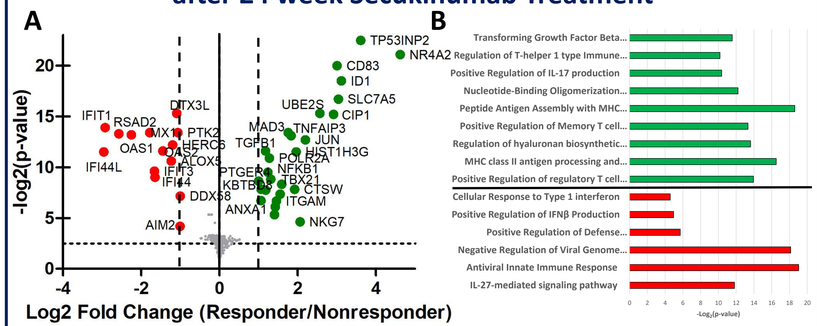
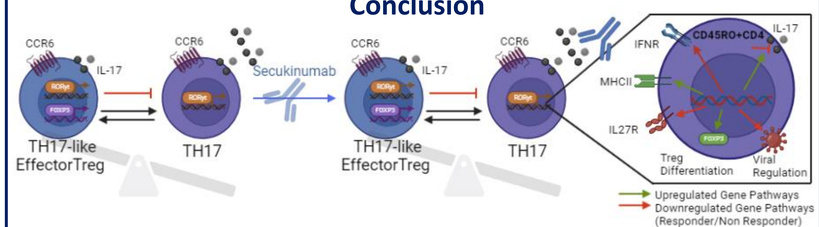


Fig 4. NanoString Analysis of FACS Sorted CD45RO+CD4+. (A) 24 week follow-up transcript levels were compared between secukinumab responders and non responders. Genes above Log2(Fold Change) of 1 are displayed. Benjamini-Hochberg determined significance and displayed above the dotted line. **(B)** Upregulated genes (green) and downregulated genes (red) were listed in pathway analysis using GO Enrichment.

Conclusion



Patients that respond to secukinumab have a reduced frequency of RORγt Tregs that does not restore to healthy control frequency after treatment. Mature CD4s in responders have upregulated MHC class 2 antigen presentation and T regulatory pathways after secukinumab compared to non responders. Responders have downregulated viral response and type 1 immunity pathways compared to secukinumab non responders.

References

1) Aschenbrenner et al., *Nature Immunology 2018;* doi: 10.1038/s41590-018-0200-5
2) Crome and Levings, *Clin Exp Immunol. 2010;* doi:10.1111/j.1365-2249.2009.04037
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