P560

Efzofitimod (ATYR1923) Treatment Reduces Pro-inflammatory Serum Biomarkers in Pulmonary Sarcoidosis Patients

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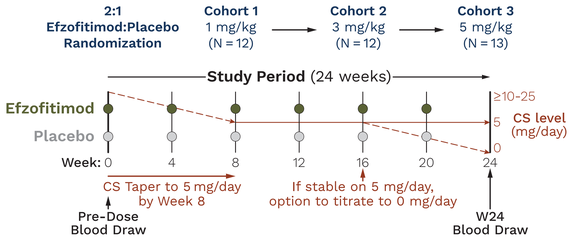
Introduction

- Pulmonary sarcoidosis is a form of interstitial lung disease characterized by the development of lung granulomas comprised of immune cells (T cells, monocytes, macrophages) that secrete pro-inflammatory chemokines and cytokines.[1]
- Left untreated, these granulomas promote aberrant inflammation both systemically and locally leading to fibrosis.
- Immunosuppressant agents, most notably oral corticosteroids (CS), are standard of care but are associated with significant unwanted side effects.[2]
- Efzofitimod (ATYR1923) is a novel immunomodulator shown preclinically to reduce inflammation and fibrosis in lung disease models.[3,4]
- We recently completed a randomized, placebo-controlled Ph1b/2a study of intravenous efzofitimod in pulmonary sarcoidosis patients who underwent a protocol-mandated CS taper during the study (NCT03824392).[5]
- In addition to assessing the safety and preliminary clinical efficacy of efzofitimod compared to placebo, the effect of efzofitimod on inflammatory serum biomarkers in the context of CS reduction was evaluated.

Materials and Methods

Phase 1a/2b Study Design



Materials

- **Human serum:** Serum samples were received frozen from a central laboratory. Samples were thawed at room temperature, aliquoted, and tested in duplicate at recommended kit dilutions. Samples found to be outside the range of the assay were diluted further and reanalyzed.
- **Biomarker Panels:** Biomarker panels were run using Meso Scale Diagnostics (MSD) V-PLEX Human Biomarker 40-Plex Kit (#K15209D).
- **Sarcoidosis Biomarkers:** Sarcoidosis biomarkers were run using IL-2Rα Single-Plex Kit (MSD #K151XGK), ACE protein quantitation kit (R&D Systems #DACE00), and KL-6 kit (Sekisui Medical Company #RSCKL6). ACE enzymatic activity was analyzed at ARUP laboratories (Salt Lake City, UT).
- **In-house Immunoassays:** HARS and NRP2 immunoassays utilized the MSD platform. NRP2 antibodies and recombinant protein were sourced from R&D Systems. HARS antibodies were produced at Abcellera (Vancouver, Canada) and TSRI (San Diego, CA).
- **Plate Readers:** MSD platform immunoassays were read on an MSD QuickPlex SQ 120 and other assays were analyzed on a Molecular Devices SpectraMax M2.

Methods

- Biomarker results were analyzed using GraphPad Prism v7.02 and sample values were calculated using an interpolated standard curve, multiplied by dilution factors and reported as an average of the replicate values in the units utilized by the assay calibrators (pg/mL, ng/mL, U/mL, or U/L).
- Patients who received less than 4 drug doses or missed the D1 or W24 blood draws were excluded from analysis post-hoc. Patients taking ACE inhibitors were excluded from the ACE enzymatic activity assay post-hoc.

	Placebo	Efzofitimod		
		1 mg/kg	3 mg/kg	5 mg/kg
Randomized and Dosed (n)	12	8	8	9
Evaluable for Biomarkers (n)	7	6	5	8
Evaluable for ACE-Enzymatic Activity* (n)	5	5	4	6

* ACE-enzymatic activity samples were also excluded due to sample stability requirements at the external testing facility

- Fold change between D1 and W24 for each individual patient was calculated as:

$$\text{Fold Change} = \frac{\text{W24 Value}}{\text{D1 Value}}$$

- Biomarkers were evaluated for Baseline levels in pulmonary sarcoidosis patients and compared against normal healthy control serum to identify elevated biomarkers while on daily CS.
- A result within normal limits for each biomarker was defined from normal healthy serum (n = 67) as: *sample value < normal limit*, where:

normal limit = normal median + 1.5 × [normal interquartile range (Q3-Q1)]

	IFNγ	IL-6	IP-10	MCP-1	TNFα	IL-2Rα	SAA	ACE Enzyme	ACE Protein
Normal Limit	3.67	1.27	350.8	306.5	2.125	2450	7777.5	67	202
Units	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	ng/mL	U/L	ng/mL

Results

Baseline Levels of Inflammatory and Sarcoidosis Biomarkers are Elevated in Pulmonary Sarcoidosis Patients Despite Immunosuppressant Therapy

ACE	bFGF	CRP	Eotaxin	Eotaxin3	Fit-1	GM-CSF	HARS	ICAM-1	IFNγ	IL-1α
IL-1β	IL-2	IL-2Rα	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12p40	IL-12p70
IL-13	IL-15	IL-16	IL-17A	IP-10	KL-6	MCP-1	MCP-4	MDC	MIP-1α	MIP-1β
NRP2	PIGF	SAA	TARC	Tie-2	TNFα	TNFβ	VCAM-1	VEGF-A	VEGF-C	VEGF-D

Detected *Elevated* *Elevated and/or Disease/MOA Biomarkers*

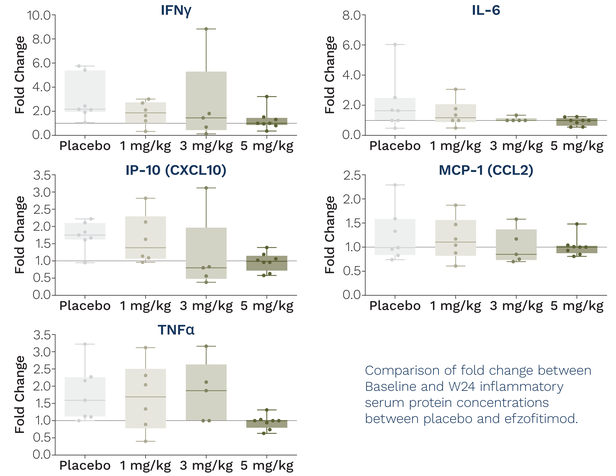
Data from samples drawn on D1 prior to study drug dosing (Baseline). Out of 44 tested biomarkers, 32 were detected (yellow, red, and green). Elevated biomarkers (> 2-fold median or > 1 SD from the average of normal control samples (red & green)) reflect analytes that were elevated in pulmonary sarcoidosis patients on D1 (pre-dose).

Results

Dose-Dependent Control of Inflammatory Biomarkers

- Across treatment groups, biomarker levels increased after protocol-mandated CS taper, with the greatest elevations generally observed for the placebo group.
- Following efzofitimod treatment, there was a dose-responsive decrease in IFNγ and IL-6.
- Following CS taper, inflammatory markers in the 5 mg/kg treatment group showed overall maintenance or improvement.

Biomarker	Placebo		Efzofitimod					
			1 mg/kg		3 mg/kg		5 mg/kg	
Timepoint	D1	W24	D1	W24	D1	W24	D1	W24
IFNγ								
Median (pg/mL)	13.3	28.3	22.1	45.2	30.2	54.3	5.9	9.7
Median Increase (%)	—	38	—	118	—	57	—	0
Within Normal Limits (%)	43	0	0	0	0	0	38	38
IL-6								
Median (pg/mL)	1.53	2.58	1.27	2.49	1.27	1.27	1.27	1.30
Median Increase (%)	—	69	—	29	—	0	—	0
Within Normal Limits (%)	43	29	67	33	100	80	63	50
IP-10								
Median (pg/mL)	281.2	521.3	513.1	879.0	524.0	420.0	335.7	350.4
Median Increase (%)	—	61	—	46	—	-21	—	0
Within Normal Limits (%)	57	29	33	17	40	40	63	50
MCP-1								
Median (pg/mL)	190.8	239.2	296.7	288.6	163.2	150.8	212.7	224.9
Median Increase (%)	—	-3	—	11	—	-12	—	0
Within Normal Limits (%)	71	57	67	67	100	80	88	88
TNFα								
Median (pg/mL)	1.38	3.13	2.18	3.05	1.38	2.58	1.49	1.39
Median Increase (%)	—	127	—	47	—	87	—	0
Within Normal Limits (%)	86	43	50	33	100	40	88	100

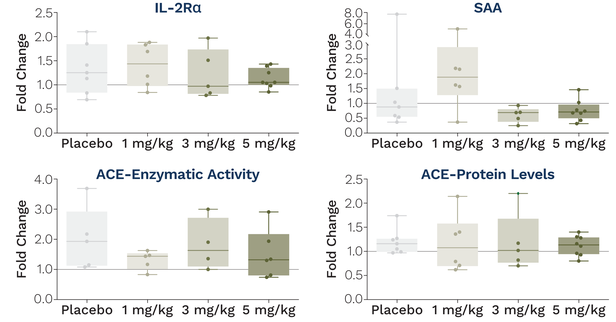


Comparison of fold change between Baseline and W24 inflammatory serum protein concentrations between placebo and efzofitimod.

Dose-Dependent Control of Sarcoidosis Biomarkers

- Compared to placebo, there was an overall greater control of SAA following 3 mg/kg efzofitimod.
- Compared to placebo, 5 mg/kg efzofitimod demonstrated a decreased median fold change for ACE enzymatic activity, IL-2Rα, and SAA.
- Compared to placebo, the 5 mg/kg group maintained the percentage of patients within normal limits for SAA and showed improvement for ACE enzymatic activity.

Biomarker	Placebo		Efzofitimod					
			1 mg/kg		3 mg/kg		5 mg/kg	
Timepoint	D1	W24	D1	W24	D1	W24	D1	W24
IL-2Rα								
Median (pg/mL)	2315	2957	2575	3180	1570	2243	1544	1704
Median Increase (%)	—	20	—	28	—	-2	—	6
Within Normal Limits (%)	57	29	50	17	80	80	100	100
SAA								
Median (ng/mL)	14271	8745	23855	47373	18031	9976	6784	4487
Median Increase (%)	—	-23	—	86	—	-31	—	-41
Within Normal Limits (%)	14	43	17	17	0	20	63	63
ACE Enzyme								
Median (U/L)	33	40	48	56	32	61	34	45
Median Increase (%)	—	42	—	29	—	47	—	32
Within Normal Limits (%)	100	80	100	80	100	75	83	100
ACE Protein								
Median (ng/mL)	128.3	138.9	120.4	132.6	129.5	154.5	117.5	139.4
Median Increase (%)	—	10	—	7	—	2	—	13
Within Normal Limits (%)	100	100	100	83	100	100	100	100



Comparison of fold change between Baseline and W24 sarcoidosis-associated disease serum protein concentrations between placebo and efzofitimod.

Abbreviations: ACE=angiotensin-converting enzyme; D=day; IFNγ=interferon gamma; IL-2Rα=interleukin-2 receptor alpha; IL-6=interleukin-6; IP-10/CXCL10=interferon gamma-induced protein 10; MCP-1/CCL2=monocyte chemoattractant protein-1; NPR2=neuropilin 2; SAA=serum amyloid A; SD=standard deviation; TNFα=tumor necrosis factor alpha; W=week.

References: 1) Baughman et al. 2011; *Am J Respir Crit Care Med* 183: 573-581. 2) Baughman et al. *Eur Respir J* 2012;58:20040709. 3) Paz et al. Keystone Symposia Conference 2019;B7. 4) Nangle et al. *Am J Respir Crit Care Med* 2017;195:A7068. 5) Sporn et al, ATS 2022, 7232, P559.

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Conclusions

- Efzofitimod demonstrated dose-dependent control of inflammatory and sarcoidosis disease biomarkers over 24 weeks in the context of a CS taper.
- The affected cytokines and chemokines are key drivers of sarcoidosis and other interstitial lung diseases, and are consistent with results from preclinical animal models and from a Phase 2 study in hospitalized COVID-19 pneumonia patients (NCT04412668).[3,4]
- These results are the first demonstration of efzofitimod's anti-inflammatory mechanism in the target patient population. The statistical analyses were exploratory and not adjusted for multiplicity to control for false positive results; therefore, these findings will need to be confirmed in a larger study.

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