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ATYR1923 Modulates the Inflammatory Response in Experimental Models of Interstitial Lung Disease

C. Burkart, M. Seikkula, L. Eide, S. Paz, D. Chu, C. Polizzi, D. King, S. Rosengren, K. Ogilvie

aTyr Pharma, San Diego, CA

Abstract

Rationale: ATYR1923 is a novel immunomodulatory therapeutic protein that consists of the histidyl-tRNA synthetase (HARS) N-terminal immunomodulatory (iMod) domain fused to human IgG1 Fc which extends the circulating half-life of the molecule resulting in a longer pharmacological duration of action. We have previously shown that secreted forms of the HARS iMod domain reduce bleomycin-induced lung fibrosis in rodents and reduce activation of human T cells *in vitro*. Based on this knowledge, we hypothesized that ATYR1923 might also modulate inflammatory and fibrotic processes in other rodent models of interstitial lung disease (ILD).

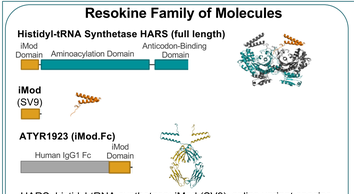
Methods: ATYR1923 was evaluated in the following murine models of ILD: Sclerodermatous chronic graft-versus-host disease (scl cGvHD), *Saccharopolyspora rectivirgula*-induced chronic hypersensitivity pneumonitis (CHP), *Propionibacterium acnes*-induced pulmonary fibrosis (sarcoidosis) and SKG mice [rheumatoid arthritis-associated interstitial lung disease, (RA-ILD)]. ATYR1923 was given intravenously once a week at 0.4 - 3 mg/kg. At study termination, lung tissue was collected for protein and histopathological analysis. Lung homogenates were analyzed for cytokines and chemokines implicated in lung fibrosis using a multiplex immunoassay platform (Luminex). Lung-derived single cell suspensions were immunophenotyped by flow cytometry.

Results: In the scl cGvHD model, low-dose ATYR1923 (0.4 mg/kg) significantly decreased both skin and lung fibrosis as determined by histopathological and biochemical analyses. Likewise, ATYR1923 reduced lung protein levels of several fibrosis-related cytokines or chemokines (e.g. IFN-γ, MCP-1/CCL2, IL-6, CXCL10) in the highly inflammatory experimental CHP and sarcoidosis models. In addition, flow cytometric analysis of cells isolated from lungs of SKG mice showed significantly lower numbers of lymphocytes in ATYR1923 treated animals.

Conclusions: ATYR1923 has pharmacological activity in a murine model of scl cGvHD when dosed during the active inflammatory phase of the model. Furthermore, protein and cellular analyses indicate that ATYR1923 has potent immunomodulatory activity in other animal models of ILD and that these effects were most prominent in models that are highly inflammatory or T cell driven. These data are compatible with our hypothesis that ATYR1923 modulates inflammatory responses that may lead to subsequent downstream inhibition of fibrosis, as observed in the scl GvHD model. In a recently completed Phase I study in healthy volunteers, ATYR1923 was well-tolerated at all doses tested, supporting further evaluation of this potential therapy in patients with inflammatory ILD.

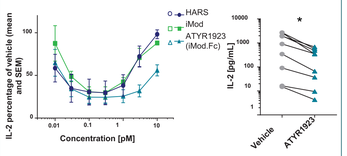
Introduction

Resokine Family of Molecules



HARS, histidyl-tRNA synthetase; iMod (SV9), splice variant species 9; ATYR1923 (iMod.Fc), a Resokine N-terminal domain (iMod) fused to human Fc

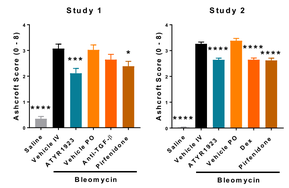
iMod-Containing Proteins Decrease Activation of Human T Cells[1]



Paired Student's t-test.

- IL-2 measured 24 hours after stimulation with anti-CD3 and anti-CD28 antibodies of human T cells isolated from healthy donor peripheral blood mononuclear cells
- Similar findings with other cytokines (TNFα, IL13, CCL20, IL10) and granzyme B as well as surface activation markers (CD69, CD40L, ICOS,4-1BB, OX40).

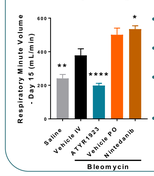
Weekly Dosing with ATYR1923 Ameliorates Fibrosis in Mouse Models of Bleomycin-Induced Lung Injury[2]



One-Way ANOVA with Dunn's multiple comparisons test (BLM + Vehicle IV)

- ATYR1923 administered therapeutically at 0.4 mg/kg (IV QW D8 and D15)
- Anti-TGF-β antibody 3 mg/kg (QOD D0 – 21), Pirfenidone 100 or 200 mg/kg (PO BID D8 – D21), Dexamethasone 0.25 mg/kg (PO QD D0 – D21)
- ATYR1923 drives efficacy as determined by Ashcroft score comparable to or greater than pirfenidone, anti–TGF-β antibody and dexamethasone in two separate studies

Early Intervention With ATYR1923 Improves Respiratory Function in a Rat Bleomycin Model



- ATYR1923 administered therapeutically starting day 2 post BLM at 1 mg/kg once weekly (IV QW).
- Nintedanib dosed at 50 mg/kg starting day 1 post BLM (PO QD)
- Administered ATYR1923 improved respiratory function on day 15 post BLM insult back to baseline (Saline - No BLM)
- No significant effect of ATYR1923 on inflammation or Ashcroft score at termination on day 22

One-Way ANOVA with Dunn's multiple comparisons test (BLM + Vehicle IV)

iMod Domain
- Encoded by a splice variant that is enriched in human lung
- Inhibits human T cell activation
- Exogenous administration reduces fibrosis in mouse bleomycin-induced lung fibrosis model
- Small protein readily cleared

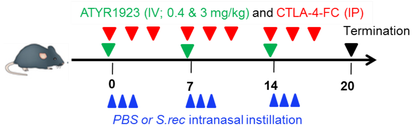
Fc domain
- Prolongs *in vivo* half-life

ATYR1923 Therapeutic Rationale
- Retains ability of the isolated domain to inhibit human T cell activation
- T cells are pathogenic in a variety of interstitial lung diseases (ILDs)
- Administration of ATYR1923 is therapeutic in rodent bleomycin-induced lung fibrosis models

Purpose:
Explore activity of ATYR1923 in additional animal models of ILD

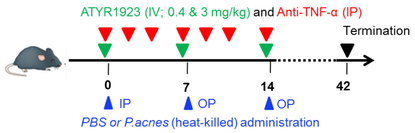
Methods: Four Experimental Models of ILD

S. rectivirgula



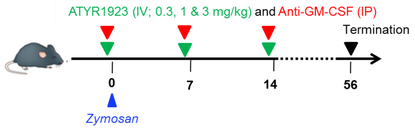
- Repeated challenges with *S. rectivirgula* extract is the most common model of experimental chronic hypersensitivity pneumonitis (CHP).
- In humans a common form of CHP is farmers's lung, caused by exposure to aerolized *S. rectivirgula*.
- Test articles dosed until study termination.

P. acnes



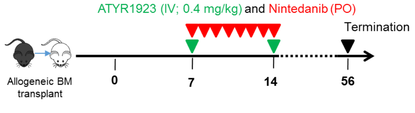
- *P. acnes* has been implicated as the etiological agent for sarcoidosis.
- Repeated *P. acnes* challenges induce chronic granulomatous inflammation.
- Test articles dosed until study termination.

SKG



- SKG mice are genetically prone to develop autoimmune arthritis.
- Following zymosan administration, SKG mice develop a disease resembling rheumatoid arthritis-associated interstitial lung disease (RA-ILD) with penetrance of joint (100%) and lung disease (20%)[3].
- Test articles dosed until study termination.

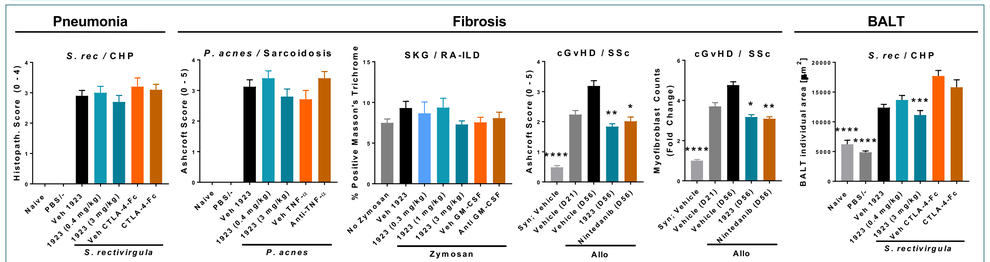
cGvHD



- Murine graft vs host disease (cGvHD) induces symptoms resembling the inflammatory stages of diffuse systemic sclerosis (SSc).
- B10.D2(H-2^{d})→Balb/c(H-2^{d}) minor histocompatibility antigen-mismatched transplant model used to induce cGvHD.
- Test articles dosed until study termination.

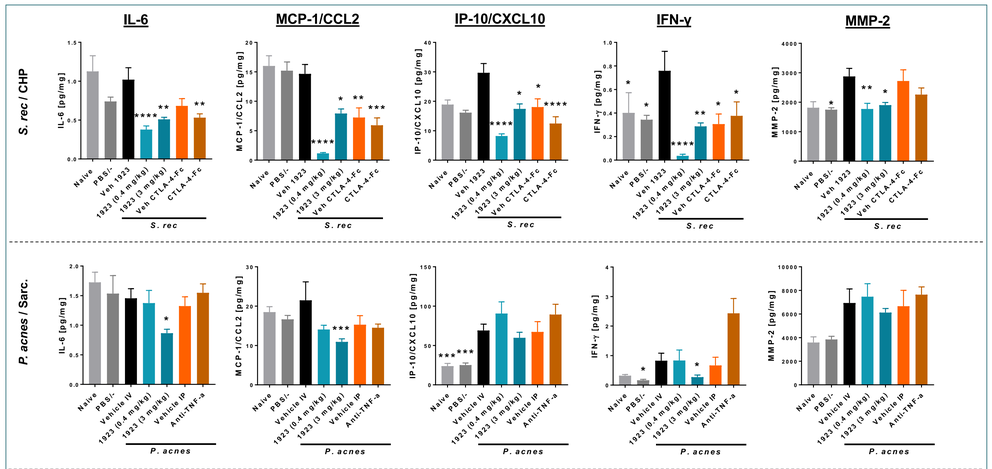
All data are shown as mean ± SEM. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

Results: ATYR1923 Modulates Histopathological Endpoints in Models of SSc and CHP



Lung tissue sections from study mice scored by a board-certified pathologist (Pneumonia, Ashcroft score) or quantified using the HALO image analysis platform (Masson's Trichrome, Myofibroblast counts, bronchus-associated lymphoid tissue (BALT)).
S. rectivirgula, *P. acnes*, SKG: One-Way ANOVA with Dunn's multiple comparisons test; cGvHD: Kruskal-Wallis test with Dunn's multiple comparisons test.

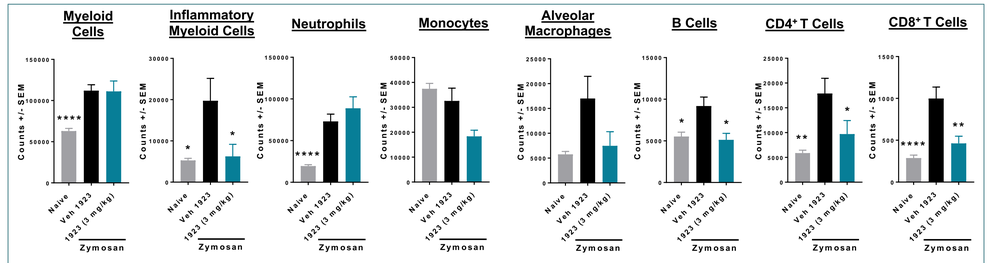
ATYR1923 Downregulates Fibrosis-Associated Cytokines and Enzymes in Granuloma-Forming Models of ILD



Lung homogenates were subjected to bead-based multiplex ELISAs (Luminex platform).
- 8-plex cytokine/chemokine panel: IL-6, IL-10, IL-17, IFN-γ, IP-10/CXCL10, KC/CXCL1, MCP-1/CCL2, TNF-α
- 5-plex matrix metalloproteases panel: MMP-2, MMP-3, MMP-8, proMMP-9, MMP-12

A subset of the proteins tested is shown. *S. rectivirgula*, *P. acnes*: One-Way ANOVA with Dunn's multiple comparisons test – Vehicle 1923; cGvHD: Kruskal-Wallis test with Dunn's multiple comparisons test – Vehicle 1923.

ATYR1923 Reduces Immune Cells in Lungs of SKG Mice



Single cell suspensions from lung were analyzed by flow cytometry for different myeloid cell subsets as well as B and T cells. One-Way ANOVA with Dunn's multiple comparisons test – Vehicle 1923.

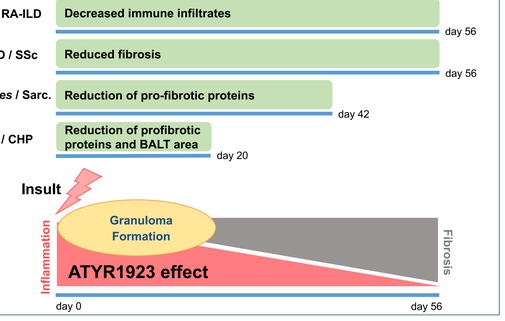
Summary

- ATYR1923 has anti-fibrotic activity in a murine model of sclerodermatous chronic GvHD.
  - Anti-fibrotic activity as determined by Ashcroft score and myofibroblast counts comparable to nintedanib.
  - Collagen covered area and hydroxyproline content in lungs of ATYR1923 treated animals were also reduced significantly[4].
  - No difference in lung cytokine levels detected at termination (day 56). This is likely due to resolution of the early cGvHD inflammation stage when several cytokines retrace to baseline, and hence no effect of ATYR1923's immunomodulatory activity is detected.
- ATYR1923 treatment led to reduction of several key inflammatory proteins in two granuloma-forming, highly inflammatory models of ILD (*S. rectivirgula* - CHP, *P. acnes* - Sarcoidosis).
  - ATYR1923 treatment at 3 mg/kg also lowered individual BALT area in the *S. rectivirgula* model.
- Significant reduction of infiltrating immune cells in a model of RA-ILD (SKG) upon ATYR1923 treatment.
  - T and B cells, which are implicated in RA-ILD pathogenesis, were significantly lower in lungs of ATYR1923 treated animals.
  - Low incidence of ILD in this model (~20%) possibly contributed to the lack of significant anti-fibrotic effects of ATYR1923 when comparing to its control group.

Conclusions

- Presented data support aTyr's hypothesis that ATYR1923 modulates inflammatory responses following lung injury, which may inhibit subsequent fibrotic processes.
- The robust anti-inflammatory effect of ATYR1923 across multiple experimental models of ILD can inform selection of additional indications for ATYR1923 therapy.

Stage-dependent anti-inflammatory and antifibrotic effect of ATYR1923 in experimental models of ILD



Clinical Program

- A Phase 1 study in healthy volunteers was successfully completed in 2018.
- A clinical Phase 1b/2a trial with ATYR1923 for treatment of pulmonary sarcoidosis was initiated in December 2018 (NCT03824392).
  - Randomized, double-blind, placebo-controlled, study will evaluate the safety, tolerability, immunogenicity, pharmacokinetic (PK), and preliminary efficacy of multiple ascending doses of IV ATYR1923 in patients with pulmonary sarcoidosis undergoing a protocol-guided oral corticosteroid (OCS) tapering regimen.

References

1) Identification of a T cell immunomodulatory domain in histydyl-tRNA synthetase. Mertsching et al., 2018 (aTyr Pharma); *poster presentation at American Academy of Immunology Annual Meeting, J Immunol 200 (1 Supplement) 112.3, 2018*
2) Preclinical characterization of ATYR1923 (iMod.Fc), an immune-modulatory therapeutic with potentially broad application in interstitial lung diseases. Ogilvie et al., 2018 (aTyr Pharma); *poster presentation at American Thoracic Society 2018 International Conference, Am J Resp Crit Care Med 197:A1064, 2018*
3) A novel model of rheumatoid arthritis-associated interstitial lung disease in SKG mice. Keith et al. 2012; *Experimental Lung Research; doi: 10.3109/01902148.2011.636139*
4) ATYR1923 Ameliorates Dermal and Pulmonary Fibrosis in a Murine Model of Sclerodermatous Chronic Graft vs. Host Disease. Ogilvie et al., 2018 (aTyr Pharma); *poster presentation at Scleroderma Foundation National Patient Education Conference 2018 (available at www.atyrpharma.com)*