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Preclinical Characterization of ATYR1923 (iMod.Fc), an Immune-Modulatory Therapeutic With Potentially Broad Application in Interstitial Lung Diseases

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Abstract

INTRODUCTION: During the evolution of complex organisms, aminoacyl-tRNA synthetase genes evolved to incorporate new sequences and generate multiple splice variants, which lose their tRNA synthetase activity and take on novel functions (Lo et al. *Science.* 2014;345(6194):328-32). Histidyl-tRNA synthetase (HARS) and its splice variants are secreted and exhibit extracellular activity, which we have termed the Resokine pathway. Based on the overexpression in the lung of a splice variant encoding the N-terminal domain of Resokine, we hypothesized that it modulates the activity of immune cells in interstitial lung diseases (ILDs) and consequently ameliorates disease.

RATIONALE: In previous work, we showed that administration of Resokine proteins containing the N-terminal immunomodulatory (iMod) domain reduced bleomycin-induced lung fibrosis in mice, demonstrating the functional significance of the Resokine pathway in the lung. Based on these observations, we sought to engineer and characterize a clinical candidate with appropriate pharmaceutical properties for clinical study in ILD. Specifically, we sought to extend the duration of action of the iMod by fusion to the fragment crystallizable region (Fc) of human immunoglobulin G1 (IgG1 Fc).

METHODS: ATYR1923 (iMod.Fc), a Resokine N-terminal domain fused to human Fc, was expressed in *Escherichia coli* and purified to homogeneity, confirming low endotoxin (limulus amebocyte lysate [LAL] assay) and pathogen-associated molecular pattern signals by a novel cell-based method. A rat model of bleomycin-induced lung fibrosis was employed to explore the effects ATYR1923 *in vivo*, including whole body plethysmography and histological disease scoring on day 22. Pharmacokinetic studies and Good Laboratory Practice (GLP)-compliant 1- and 3-month toxicology studies were conducted in rats and nonhuman primates (NHPs).

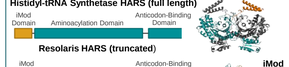
RESULTS: ATYR1923 exhibited the therapeutic potential of the iMod domain while having a long *in vivo* half-life. ATYR1923 had a terminal half-life of ~3 days in rats and ~4.5 days in NHPs, in contrast to the isolated iMod domain that had a terminal half-life of ~20 minutes in rats. In rat bleomycin-induced lung fibrosis, ATYR1923 at 0.1–3 mg/kg weekly beginning on day 9 exerted therapeutic activity as revealed by reversal of bleomycin-induced changes in respiratory parameters and decreased histological fibrosis (Ashcroft score) and immune infiltration. One- and 3-month GLP-compliant studies found no adverse test article–related findings. The no-observed-adverse-effect level was 60 mg/kg in both species.

CONCLUSIONS: ATYR1923 has been engineered to have a long duration of action and is efficacious in bleomycin-induced lung fibrosis preclinical models when administered weekly. Based on the preclinical data, clinical testing is planned.

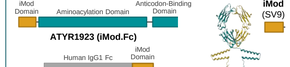
Introduction

Resokine Family of Molecules

Histidyl-tRNA Synthetase HARS (full length)

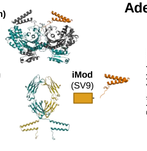


Resolaris HARS (truncated)



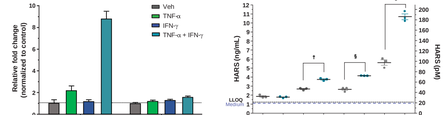
ATYR1923 (iMod.Fc)

iMod (SV9)



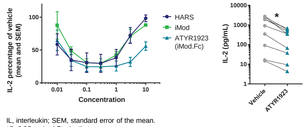
HARS, histidyl-tRNA synthetase; IgG1 Fc, fragment crystallization region of human immunoglobulin G1; ATYR1923 (iMod.Fc), a Resokine N-terminal domain fused to human Fc; SV9, splice variant species 9.

SV9 Transcript and HARS Release From A549 Lung Adenocarcinoma Cells Are Upregulated by Inflammatory Cytokines



FL, full length; IFN, interferon; LLOQ, lower limit of quantification; TNF, tumor necrosis factor; Veh, vehicle.
*P<0.05; †P<0.01; ‡P<0.001; §P<0.0001.

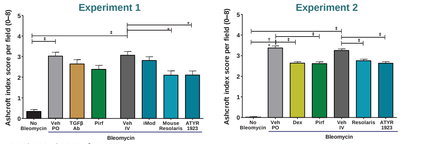
iMod-Containing Proteins Decrease Activation of Human T Cells



IL, interleukin; SEM, standard error of the mean.
*P<0.05, 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

Weekly Therapeutic Dosing of ATYR1923 Ameliorates Fibrosis in Mouse Bleomycin-Induced Lung Injury



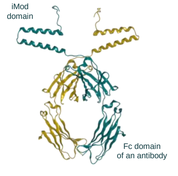
*P<0.05; †P<0.01; ‡P<0.001; §P<0.0001.

Dosing information

Oral vehicle: 1x PBS, BID D8-D21
Intravenous (IV) vehicle: 50 mM L-His, 140 mM NaCl, QD D8-D21
Transforming growth factor (TGF)-β antibody: clone 1D11, 3 mg/kg IP, QOD D0-D21
Pirfenidone (Pirf): 100 mg/kg (Exp 1) or 200 mg/kg (Exp 2) PO BID D8-D21
Dexamethasone (Dex): 0.25 mg/kg PO QD D0-D21
iMod: 2.5 mg/kg IV QD D8-D21
Resolaris™: 3 mg/kg IV QD D8-D21
ATYR1923: 0.4 mg/kg IV QW D8, D15

- ATYR1923 administered therapeutically at 0.4 mg/kg once weekly (QW) drives efficacy comparable to or greater than pirfenidone, anti–TGF-β antibody, and dexamethasone

ATYR1923



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 interstitial lung diseases (ILDs)
- Administration of ATYR1923 is therapeutic in rodent bleomycin-induced lung fibrosis models

Methods

Objectives

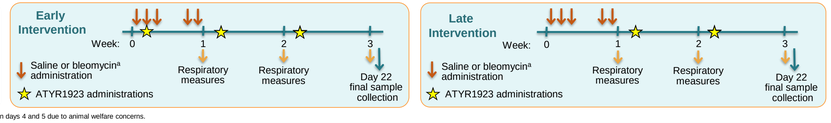
- Determine dose response when intervening on day 9
- Determine effects of intervention at day 2 vs day 9 (1 mg/kg)

Dosing Information

Nintedanib: 60 mg/kg PO QD (days 9–22)
Vehicle PO: QD (days 9–22)
Vehicle IV: QW (days 2, 9, and 16)
ATYR1923: 0.1–3 mg/kg (days 9 and 16)
ATYR1923ª: 1 mg/kg (days 2, 9, and 16)

ªOropharyngeal (OP) dosing was skipped on days 4 and 5 due to animal welfare concerns.

In-Life Protocol



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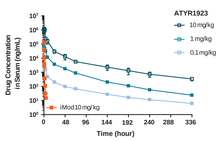
Results

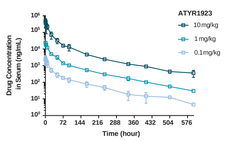
Fusion of iMod to Human Fc Sustains Exposure

Sprague Dawley Rats



Dose (mg/kg)	C_0 (ng/mL)	V_{ss} (L/kg)	CL (mL*h/kg)	$T_{1/2}$ (h)	AUC_{inf} (ng*h/mL)
0.1	3,070	0.353	0.0573	70	29,200
1	90,500	0.160	0.0309	60	542,000
10	1,550,000	0.188	0.0280	76	6,970,000

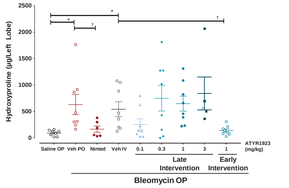
Cynomolgus Monkeys

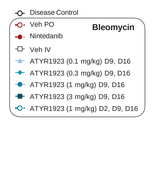


Dose (mg/kg)	C_0 (ng/mL)	V_{ss} (L/kg)	CL (mL*h/kg)	$T_{1/2}$ (h)	AUC_{inf} (ng*h/mL)
0.1	2,670	0.140	0.0260	94	67,300
1	24,400	0.133	0.0291	110	577,000
10	510,000	0.088	0.0229	127	7,880,000

AUC_{inf}, area under the curve extrapolated to infinity; C_0, initial plasma drug concentration; CL, total clearance; $T_{1/2}$, half-life; V_z, apparent volume of distribution during terminal phase.

Collagen Content Decreased by ATYR1923 and Nintedanib

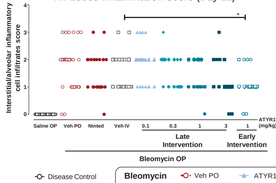




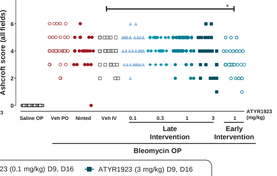
*P<0.05; Kruskal-Wallis analysis of variance (ANOVA) followed by Dunn's multiple comparisons test of intended comparisons.
†P<0.05; Mann-Whitney U test.

ATYR1923 Reduces Histological Inflammation and Fibrosis

All Lobes Inflammation Score (Day 22)

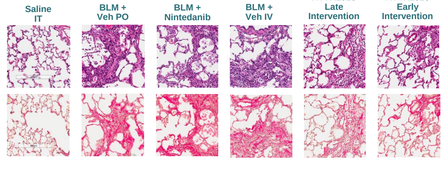


All Lobes Ashcroft Score (Day 22)

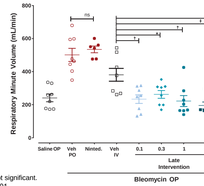


*P<0.05, 2-way repeated measures ANOVA followed by Dunnett's multiple comparisons test

Representative Images

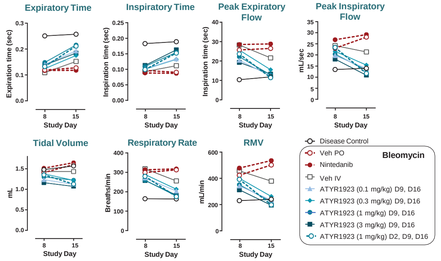


ATYR1923 Returns Respiratory Minute Volume to Normal (Day 15)



Ninted, nintedanib; ns, not significant.
*P<0.05; †P<0.01; ‡P<0.001.
Note: Four animals were euthanized before day 15 due to severe body weight loss.

ATYR1923 Ameliorates Bleomycin-Induced Respiratory Changes



D, day; RMV, respiratory minute volume.

Day 8 Respiratory Measures

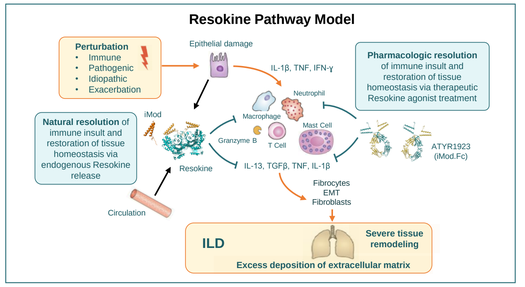
	Disease Control (Saline OP)	Bleomycin OP						
					ATYR1923 (mg/kg)/Treatment Days			
		Vehicle PO	Nintedanib	Vehicle IV	0.1 Days 9, 16	0.3 Days 9, 16	1 Days 9, 16	3 Days 9, 16	1 Days 2, 9, 16
Tidal volume (mL)	1.5 (0.06)	1.4 (0.05)	1.5 (0.05)	1.4 (0.05)	1.2 (0.05)	1.4 (0.09)	1.3 (0.11)	1.2 (0.07)	1.3 (0.11)
Respiratory rate (bpm)	164.4† (20.2)	298.9 (15.9)	313.0 (17.8)	318.2 (16.6)	270.8 (17.4)	288.6 (18.2)	259.5 (21.0)	257.9 (18.9)	279.3 (13.5)
Respiratory minute volume (mL/min)	230.5† (28.0)	423.0 (33.4)	477.5 (36.3)	456.3 (28.7)	342.4 (33.5)	400 (39.3)	351.2 (55.5)	312.6* (45.9)	391.9 (44.3)
Expiratory time (s)	0.25† (0.017)	0.12 (0.008)	0.12 (0.008)	0.11 (0.008)	0.14 (0.012)	0.12 (0.011)	0.15 (0.014)	0.13 (0.014)	0.13 (0.007)
Inspiratory time (s)	0.18† (0.016)	0.10 (0.004)	0.09 (0.005)	0.09 (0.005)	0.10 (0.005)	0.10 (0.007)	0.11 (0.008)	0.11 (0.006)	0.10 (0.005)
Peak expiratory flow (mL/s)	10.3† (1.5)	25.6 (2.5)	28.4 (1.9)	28.2 (1.8)	20.3 (2.1)	25.6 (3.5)	22.2 (4.0)	20.5 (2.7)	23.6 (3.1)
Peak inspiratory flow (mL/s)	13.4† (1.5)	22.0 (1.1)	26.7 (1.4)	24.1 (1.4)	19.6 (1.8)	21.6 (1.8)	203 (3.2)	18.1 (2.2)	23.3 (2.7)

Day 15 Respiratory Measures

	Disease Control (Saline OP)	Bleomycin OP						
					ATYR1923 (mg/kg)/Treatment Days			
		Vehicle PO	Nintedanib	Vehicle IV	0.1 Days 9, 16	0.3 Days 9, 16	1 Days 9, 16	3 Days 9, 16	1 Days 2, 9, 16
Tidal volume (mL)	1.6 (0.06)	1.6 (0.04)	1.6 (0.04)	1.4 (0.05)	1.1* (0.06)	1.2 (0.05)	1.2 (0.08)	1.1† (0.08)	1.1* (0.04)
Respiratory rate (bpm)	163.1† (17.1)	312.5 (20.0)	317.3 (10.5)	255.5 (20.0)	204.2 (14.8)	213.5 (15.5)	178† (15.1)	183.1* (14.5)	175.1† (8.5)
Respiratory minute volume (mL/min)	241.1* (23.6)	501.2 (39.6)	534.6* (20.1)	378.4 (39.0)	233.5* (26.2)	262.2 (23.5)	222.1† (33.3)	195† (20.9)	198† (13.3)
Expiratory time (s)	0.26† (0.016)	0.13 (0.013)	0.12 (0.006)	0.16 (0.012)	0.19 (0.015)	0.18 (0.010)	0.22† (0.021)	0.18 (0.013)	0.21† (0.010)
Inspiratory time (s)	0.19† (0.016)	0.09 (0.004)	0.09 (0.002)	0.11 (0.010)	0.13 (0.010)	0.13 (0.009)	0.15† (0.011)	0.16† (0.014)	0.15* (0.008)
Peak expiratory flow (mL/s)	11.8* (1.1)	26.4 (2.8)	28.8 (1.2)	21.6 (2.1)	12.3* (1.1)	15.5 (1.5)	12.8* (2.0)	13.3 (2.3)	11.4* (0.5)
Peak inspiratory flow (mL/s)	14.0* (1.5)	27.9 (1.8)	29.0* (1.0)	21.3 (2.1)	13.8* (1.4)	15.4 (1.4)	13.1* (2.0)	10.8† (1.6)	11.7† (0.7)

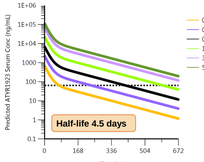
*P≤0.05; †P≤0.01; ‡P≤0.001.
Each respiratory endpoint was subject to 2-way repeated measures ANOVA followed by Dunnett's multiple comparisons test vs vehicle IV.

Resokine Pathway Model



- Pharmacokinetic predictions potentially enable once-monthly dosing in patients

Single-Dose Simulations: 0.3–3 mg/kg



ATYR1923 Single Ascending IV Infusion Dose Level (mg/kg)	Duration of Time Plasma Conc is >1 nM (64 ng/mL)* [Half-life 4.5 days]	Duration of Time Plasma Conc is >1 nM (64 ng/mL)* [Half-life 7 days]
0.03	3.1 days	4.8 days
0.1	9.8 days	15.1 days
0.3	16.9 days	26.2 days
1.0	24.8 days	38.3 days
3.0	>29.2 days	49.4 days
10.0	>33.3 days	>58 days

*1 nM is assumed to be the concentration above which therapeutic benefit is expected.

Favorable Good Laboratory Practice (GLP) Safety Profile

Nonhuman Primates

- **2 weekly** IV doses of 3 mg/kg
- No increase in ~30 serum immune markers
- **1- and 3-month** weekly IV dose at 0, 10, 30, and 60 mg/kg
- No adverse test article–related findings
- Systemic exposure increased with increasing dose and did not appear to change with repeated dosing
- Anti-drug antibodies (ADA) did not appear to have an impact on systemic exposure
- No-observed-adverse-effect level (NOAEL) = 60 mg/kg (C_{trough} = 228 nM)

Rodents

- **1- and 3-month** weekly IV dose at 0, 10, 30, and 60 mg/kg
- No adverse test article–related findings
- Systemic exposure increased with increasing dose and did not appear to change with repeated dosing
- ADA did not appear to have an impact on systemic exposure
- NOAEL = 60 mg/kg (C_{trough} = 75 nM)

Summary

- ATYR1923 had a terminal half-life of ~3 days in rats and ~4.5 days in NHPs
- ATYR1923 at 0.1–3 mg/kg weekly beginning on day 9
  - Reversed bleomycin-induced changes in respiratory parameters
- ATYR1923 at 1 mg/kg weekly beginning on day 2
  - Decreased histological fibrosis (Ashcroft score)
  - Decreased histological inflammation
  - Reduced lung collagen content
- 1- and 3-month GLP-compliant studies found no adverse test article–related findings. The NOAEL was 60 mg/kg in both species.

Conclusions

- ATYR1923 has been engineered to have a long duration of action and is efficacious in bleomycin-induced lung fibrosis preclinical models when administered weekly. Based on the preclinical data, a phase 1 clinical study is under way.

Acknowledgments

The authors thank the scientists at Charles River Laboratories (bleomycin-induced lung fibrosis experiment), Edinburgh, UK; Shin Nippon Biomedical Laboratories (cynomolgus monkey PK study), Seattle, WA, USA; MPI Research (toxicology), Mattawan, MI, USA; and SD Scientific, Inc. (PK predictions), San Diego, CA, USA, for the data that they contributed.