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Subcutaneous Delivery of a Natural Asp-tRNA Synthetase Fragment Targeting Myofibroblasts Shows Strong Exposure and Reduced Markers of Lung Inflammation

KEYSTONE SYMPOSIA
Fibrosis: Cross Organ Pathology and Pathways to Clinical Development, 2026

Ryan A. Adams[1]*, Ying-Ting Wang[1], Alison Barber[1], Lauren Guy[1], David Siefker[1], Angelica Villanueva[1], Clara Polizzi[1], Jasmine Stamps[1], Max Pastenes[1], Annie Wang[1], Zhiwen Xu[1], Christoph Burkart[1], and Leslie Nangle[1]

[1]aTyr Pharma, San Diego, CA, USA

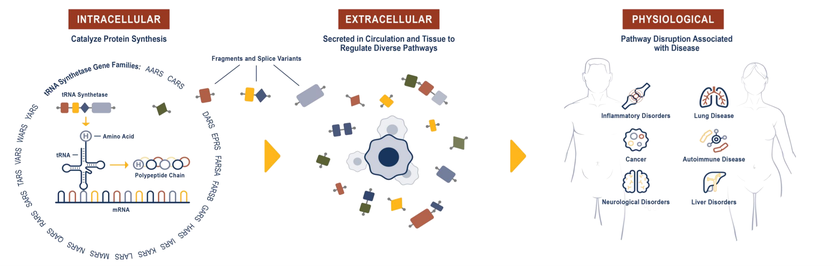
*Contact: radams@atyrpharma.com

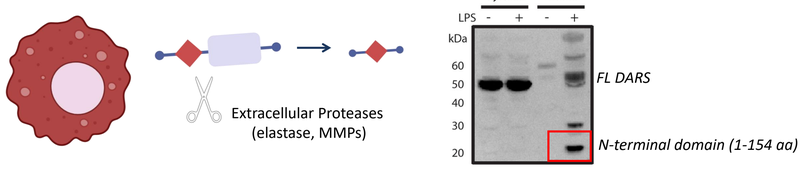
Introduction

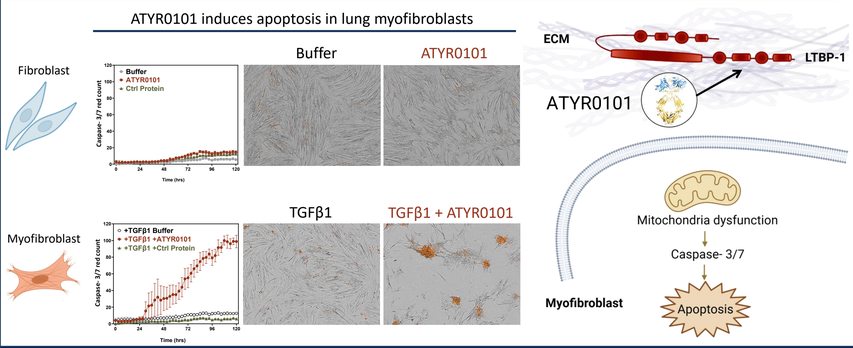
Background: Chronic fibrosis is driven by a vicious cycle where persistent inflammation activates fibroblasts to produce excessive scar tissue, which in turn fuels further inflammation. Despite being a major worldwide health crisis, available therapies for fibrotic conditions are not disease modifying, leaving a desperate need for more effective treatments. Novel approaches to address chronic fibrosis may be found within the aminoacyl-tRNA synthetase (aaRS) family of enzymes, which regulate physiological and pathological states through novel domains which are liberated via splicing or proteolytic cleavage from their catalytic core. Previous work has demonstrated that a splice variant of His-tRNA synthetase resulted in the development of a therapeutic drug candidate, efzofitimod, which effectively reduces proinflammatory activity of myeloid cells thereby disrupting the cycle of chronic inflammation and fibrosis. This candidate is currently being evaluated in the treatment of pulmonary sarcoidosis and systemic sclerosis-ILD patients. To further uncover the therapeutic potential intrinsic within members of the aaRS family, we developed ATYR0101, a novel proteolytic fragment from human Asp-tRNA synthetase (DARS), which targets latent-transforming growth factor beta binding protein-1 leading to the specific induction of apoptosis in myofibroblasts. Prior studies have demonstrated anti-fibrotic activity of ATYR0101 in murine models of lung and kidney fibrosis using standard intravenous (IV) or intraperitoneal (IP) delivery methods.

Aim: In the current proof-of-concept study, we assessed the potential of subcutaneous (SC) delivery of ATYR0101 to disrupt the self-perpetuating cycle of chronic fibrosis and inflammation by examining its exposure, antigenicity, and ability to impact the chronic immune response downstream of tissue injury.

Figure 1: tRNA Synthetase Drug Discovery Platform



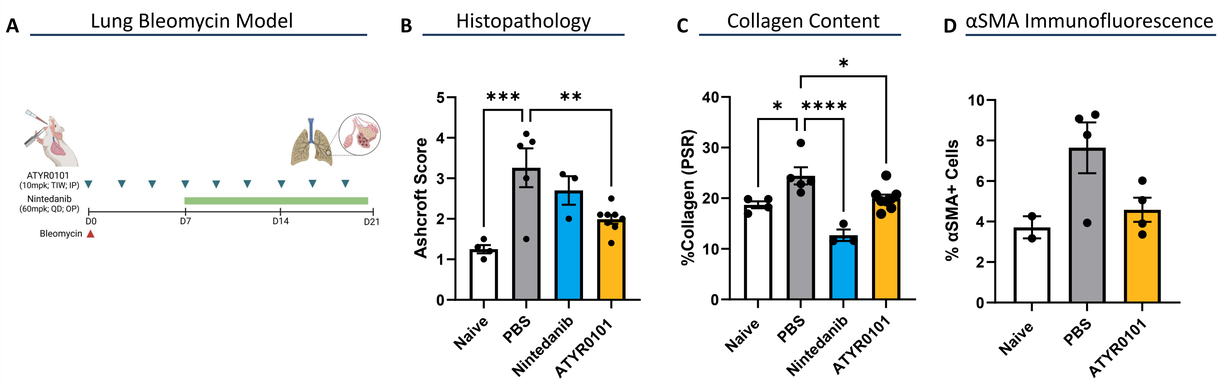




- Unique protein domains found in tRNA synthetases can be liberated via splicing or proteolysis and have been identified in extracellular spaces, where they have functions separate from their essential role in protein synthesis.
- aTyr has built an intellectual property portfolio covering >300 protein compositions derived from all 20 tRNA synthetase genes and is engaged in the discovery and development of potential first-in-class medicines based on newly discovered pathways effected by extracellular tRNA synthetases.
- An N-terminal fragment from Asp-tRNA synthetase was identified in macrophages stimulated with LPS. This fragment was subsequently found to induce myofibroblast apoptosis.
- Prior studies have demonstrated LTBP-1 as a novel binding partner of an Asp-tRNA synthetase fragment (ATYR0101) leading to the specific induction of apoptosis in myofibroblasts.
- In this work, ATYR0101 demonstrates significant potential as a novel anti-fibrotic therapeutic agent with potentially broad applicability across different delivery approaches.

Results

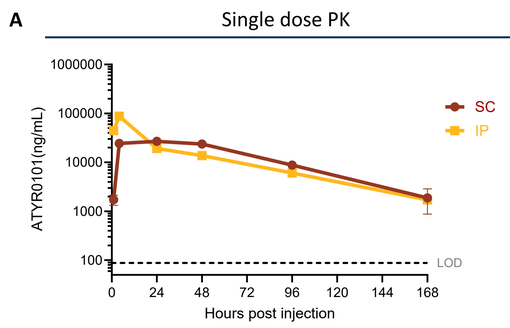
Figure 2: ATYR0101 Exhibits Anti-fibrotic Activity in a Lung Fibrosis Model with Systemic Administration

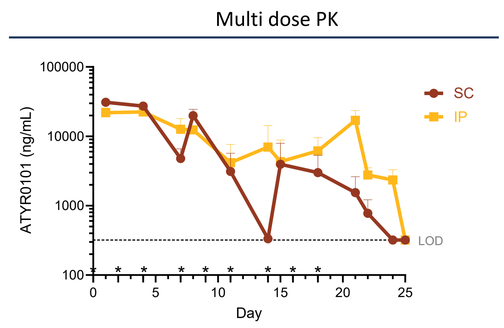


ATYR0101 reduces fibrosis and the frequency of αSMA+ cells in the bleomycin lung fibrosis model. (A) The prophylactic bleomycin lung fibrosis efficacy study, wherein mice were induced with bleomycin on day 0 and received 10mg/kg ATYR0101 or PBS via IP injection three times per week beginning on day 0. Nintedanib served as the positive control and the study terminated on day 21. ATYR0101 treatment led to a significant reduction in (**B**) Ashcroft score, **(C)** collagen content and **(D)** the frequency of α-smooth muscle actin positive (αSMA+) cells. *P < 0.05, **P < 0.01, ***P<0.001, ****P<0.0001 by ordinary one-way ANOVA.

Results

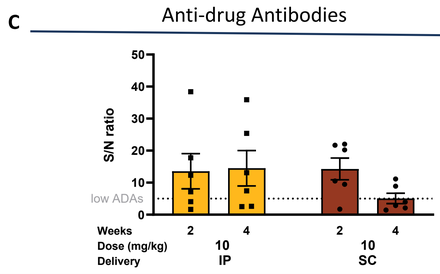
Fig 3: Comparable Pharmacokinetic (PK) Profiles and Anti-Drug Antibody (ADA) Levels Between IP and SC Dosing of ATYR0101





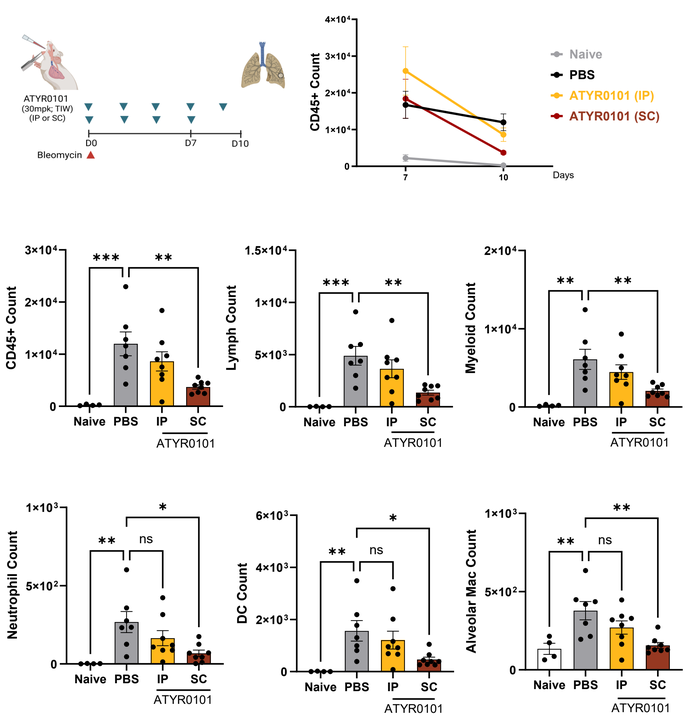
Dose (mg/kg)	Route	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$– Lambda-z (h)	AUClast (ng/ml*h)	C (av) (ng/ml)
10	IP	87,272	4	39.98	2,435,835	14,499 (161 nM)
10	SC	26,844	24	32.75	2,322,387	13,824 (154 nM)

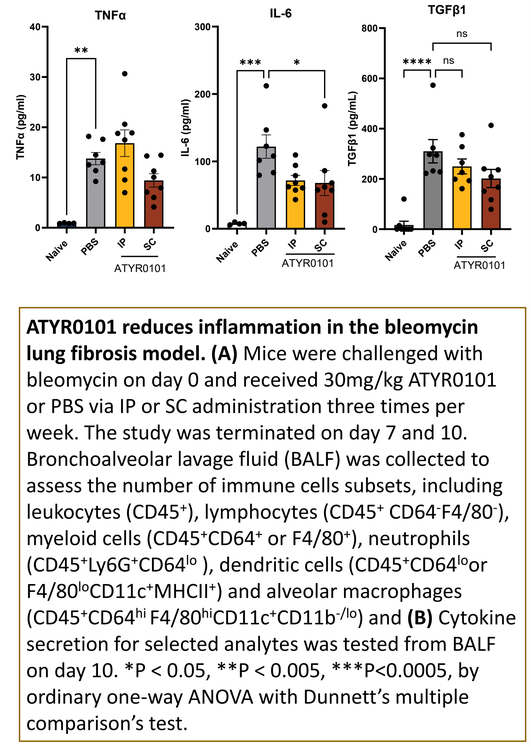
Dose (mg/kg)	Route	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$– Lambda-z (h)	AUClast (ng/ml*h)	C (av) (ng/ml)
10	IP	22,462	96	91.16	4,086,921	15,480
10	SC	30,929	24	26.92	4,373,819	18,224



Equivalent exposure with comparable immunogenicity between IP and SC delivery. (A) Mice received a single dose of 10mg/kg ATYR0101 via IP or SC delivery. Pharmacokinetic (PK) profiles indicate the levels of ATYR0101 within serum over the course of one week. **(B-C)** Mice received 10mg/kg ATYR0101 via IP or SC delivery three times per week for a total of three weeks period. Asterisk (*) indicated the dosing day. **(B)** PK profiles and **(C)** anti-drug antibodies (ADA) against ATYR0101 in serum at week 2 and 4 were evaluated.

Fig 4: ATYR0101 Reduces Immune Cell Infiltration and Attenuated Pro-inflammatory Cytokines in a Lung Fibrosis Model





ATYR0101 reduces inflammation in the bleomycin lung fibrosis model. (A) Mice were challenged with bleomycin on day 0 and received 30mg/kg ATYR0101 or PBS via IP or SC administration three times per week. The study was terminated on day 7 and 10. Bronchoalveolar lavage fluid (BALF) was collected to assess the number of immune cells subsets, including leukocytes ($CD45^+$), lymphocytes ($CD45^+$ $CD64^-F4/80^-$), myeloid cells ($CD45^+CD64^+$ or $F4/80^+$), neutrophils ($CD45^+Ly6G^+CD64^{lo}$), dendritic cells ($CD45^+CD64^{lo}$or $F4/80^{lo}CD11c^+MHCII^+$) and alveolar macrophages ($CD45^+CD64^{hi}$ $F4/80^{hi}CD11c^+CD11b^{-/lo}$) and **(B)** Cytokine secretion for selected analytes was tested from BALF on day 10. *P < 0.05, **P < 0.005, ***P<0.0005, by ordinary one-way ANOVA with Dunnett's multiple comparison's test.

Conclusions

- ATYR0101 demonstrates significant potential as a novel anti-fibrotic therapeutic agent with systemic exposure.
- Subcutaneous delivery of ATYR0101 yielded good exposure, low ADAs and comparable activity in reducing lung inflammation.
- Continued therapeutic development of ATYR0101 with subcutaneous delivery modes and more detailed exploration of effects on downstream fibrosis in preclinical models may provide additional opportunities for clinical applications in patients suffering from fibrosis.

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