KEYSTONE SYMPOSIA
Fibrosis Pathogenesis and Resolution: From Mechanisms to Therapies, 2023

# Identification of latent-transforming growth factor beta-binding protein 1 (LTBP-1) as a binding partner of aspartyl-tRNA synthetase

**Tarsis F. Brust[1], Andrew Imfeld[1], Ying-Ting Wang[1], Yeeting E. Chong[1], Kristina Hamel[1], Alison G. Barber[1], Ann L. Menefee[1], Cory Soto[1], Gennyne Walker[1], Eileen Sun[1], Ryan A. Adams[1] and Leslie A. Nangle[1]**

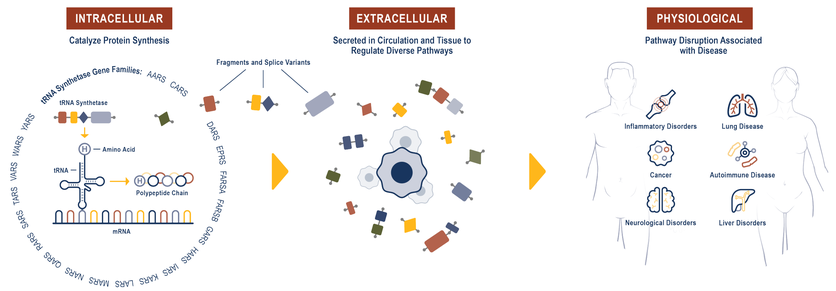
[1]aTyr Pharma, San Diego, CA USA *Contact: radams@atyrpharma.com

## Introduction

**Background:** Proteolytic cleavage and alternative splicing give rise to unique tRNA synthetase fragments, which can reach the extracellular space and interact with cell surface and extracellular proteins.[1, 2] As a result, these fragments have functions that are distinct from the canonical roles of tRNA synthetases in protein synthesis and present the potential for the development of novel therapeutic approaches. Proof of concept for this approach has recently been established for efzofitimod, a Fc-fused fragment of histidyl-tRNA synthetase (HARS). Efzofitimod, which binds to neuropilin-2, induced dose-related improvements on multiple measures of efficacy in a phase 1b/2a trial in patients with pulmonary sarcoidosis, with a confirmatory Phase 3 study underway.[3, 4]

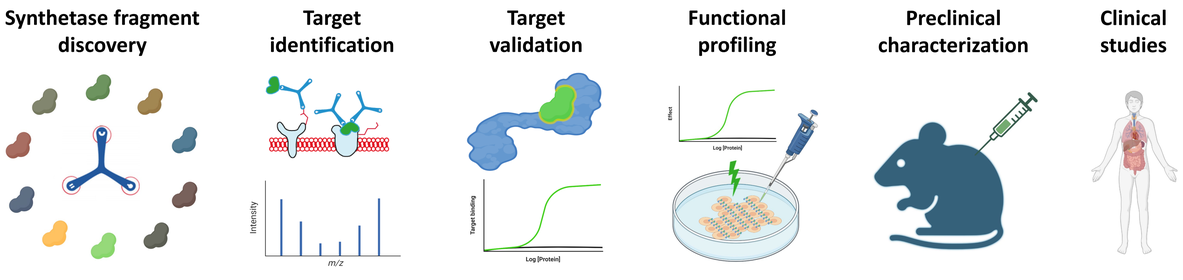
**Aim:** Aspartyl-tRNA synthetase (DARS) is known to be released from cells and detected in serum. To identify binding partners for secreted DARS, we employed a ligand-receptor capture screen[5] that identified latent-transforming growth factor beta-binding protein 1 (LTBP-1) as a binding partner for the DARS fragment ATYR0101.

### tRNA synthetase drug discovery platform




| | SYNTHETASE FRAGMENT DISCOVERY | TARGET ID, VALIDATION, AND FUNCTIONAL PROFILING | PRECLINICAL CHARACTERIZATION | CLINICAL STUDIES |
|---|---|---|---|---|
| Efzofitimod | | | | |
| AARS (ATYR0750) | | | | |
| DARS (ATYR0101) | | | | |
| Other tRNA synthetases | | | | |

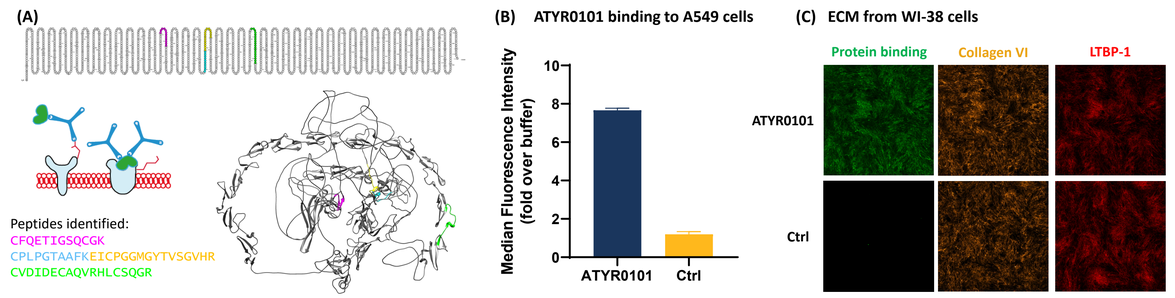
- Unique protein domains found in tRNA synthetases can be liberated via splicing or proteolysis and have been identified in extracellular spaces, where they have functions separate from their essential role in protein synthesis.
- aTyr has built an intellectual property portfolio covering >300 protein 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.
- Our tRNA synthetase platform has produced efzofitimod, an Fc-fused fragment of HARS, which has recently initiated a global Phase 3 clinical study in patients with pulmonary sarcoidosis.
- We recently reported the identification of fibroblast growth factor receptor 4 (FGFR4) as a binding partner of a fragment derived from alanyl-tRNA synthetase (AARS).[6]
- In this work we identify LTBP-1 as a binding partner of a DARS fragment (ATYR0101).




**References**:
1. Kwon NH, et al. Aminoacyl-tRNA synthetases as therapeutic targets. Nat Rev Drug Discov 2019.
2. Adams RA, et al. Serum-circulating His-tRNA synthetase inhibits organ-targeted immune responses. Cell Mol Immunol 2019.
3. Xu Z., et al. ATYR1923 specifically binds to neuropilin-2, a novel therapeutic target for the treatment of immune-mediated diseases. Am J Respir Crit Care Med 2020.
4. Sporn PHS, et al. Safety and Efficacy ATYR1923, a Novel Immunomodulator for Pulmonary Sarcoidosis: Results of a Phase 1b/2a Randomized Placebo Controlled Trial. Am J Respir Crit Care Med 2022.
5. Dualsystems Biotech AG.
6. Adams RA, et al. Identification of key extracellular binding proteins implicate role in inflammation and fibrosis for alanyl- and aspartyl-tRNA synthetases. Keystone Symposia: Tissue Fibrosis and Repair, 2022.

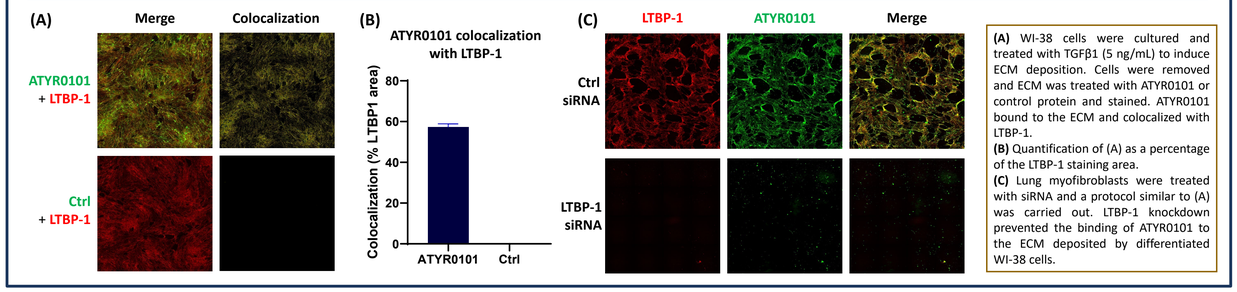
## Results

### LTBP-1 is a binding partner of ATYR0101



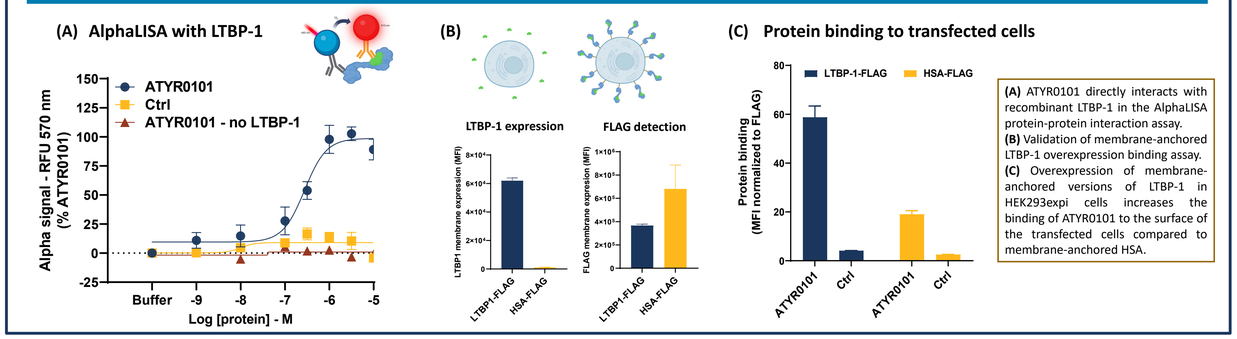

**(A)** A mass spectrometry-based receptor screen employing Dualsystems' ligand-receptor capture technology identified latent transforming growth factor beta-binding protein 1 (LTBP-1) as a binding partner of ATYR0101 in A549 and THP1 cells. Identified LTBP-1 peptides are highlighted in the sequence and AlphaFold structure (entry Q14766). **(B)** The binding of ATYR0101 to A549 cells was confirmed through flow cytometry. **(C)** WI-38 cells (human embryonic lung fibroblasts) were treated with TGFβ1 for 6 days to allow for ECM deposition. Cells were removed from the wells, leaving only the ECM. ATYR0101 or a control protein were labeled in green, collagen VI in orange, and LTBP-1 in red. ATYR0101 bound to the ECM deposited by WI-38 cells.

### ATYR0101 colocalizes with LTBP-1 in the extracellular matrix of WI-38 cells




**(A)** WI-38 cells were cultured and treated with TGFβ1 (5 ng/mL) to induce ECM deposition. Cells were removed and ECM was treated with ATYR0101 or control protein and stained. ATYR0101 bound to the ECM and colocalized with LTBP-1.
**(B)** Quantification of (A) as a percentage of the LTBP-1 staining area.
**(C)** Lung myofibroblasts were treated with siRNA and a protocol similar to (A) was carried out. LTBP-1 knockdown prevented the binding of ATYR0101 to the ECM deposited by differentiated WI-38 cells.

### ATYR0101 directly interacts with LTBP-1




**(A)** ATYR0101 directly interacts with recombinant LTBP-1 in the AlphaLISA protein-protein interaction assay.
**(B)** Validation of membrane-anchored LTBP-1 overexpression binding assay.
**(C)** Overexpression of membrane-anchored versions of LTBP-1 in HEK293expi cells increases the binding of ATYR0101 to the surface of the transfected cells compared to membrane-anchored HSA.

## Conclusions and future directions

- **LTBP-1 was confirmed as a binding partner of ATYR0101.**
- **Binding of ATYR0101 to LTBP-1 was validated using multiple methods:**
  - **Colocalization with LTBP-1 in the extracellular matrix deposited by WI-38 cells and binding was reduced by siRNA knockdown of LTBP-1.**
  - **Direct interaction with recombinant LTBP-1 in an AlphaLISA assay.**
  - **Increased binding to the cell surface of overexpressed membrane-anchored LTBP-1 in HEK293 cells.**
- **Preclinical development is ongoing to investigate the therapeutic potential of ATYR0101 in fibrosis treatment.**

**Acknowledgements**: This work was supported by aTyr Pharma, Inc. The authors would like to acknowledge Dualsystems Biotech for assistance with the receptor screening experiments.