# Identification of Key Fibrotic Extracellular Targets for Alanyl- and Aspartyl-tRNA Synthetases

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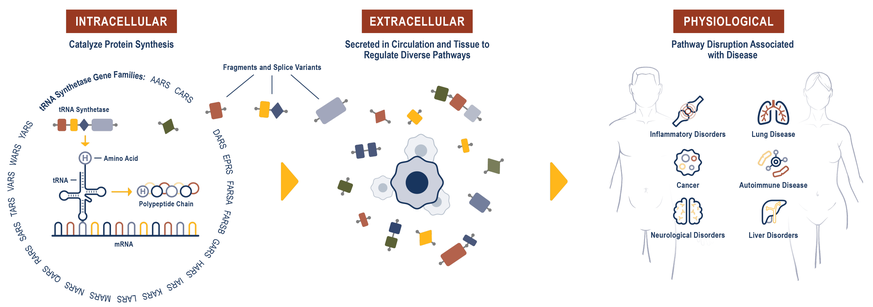
**AARS 2023**
June 4-9, 2023
Grand Bend, Ontario, Canada

## Introduction

**Background:** Throughout the course of evolution, aminoacyl-tRNA synthetases have acquired diverse functional domains capable of impacting an expansive range of biology. These signaling activities often result from the extracellular locality of these domains generated as either splice variants or proteolytic fragments from full-length synthetases. Efforts to characterize a splice variant of Histidyl-tRNA synthetase (HARS) have resulted in a therapeutic molecule, shown to interact with the extracellular receptor Neuropilin-2 (NRP2), which is currently engaged in a Phase 3 trial for Pulmonary Sarcoidosis, a form of inflammatory and fibrotic lung disease. (ClinicalTrials.gov Identifier: NCT05415137)

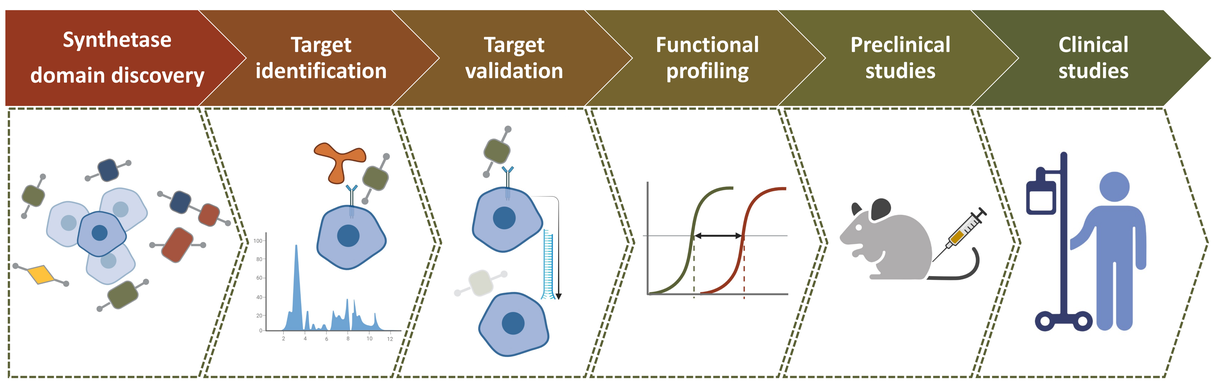
**Aim:** In an effort to discover novel functions from other members of the aminoacyl-tRNA synthetase family, we set out to identify extracellular interacting targets for domains from alanyl- (AARS) and aspartyl- (DARS) tRNA synthetases. To uncover these novel targets, we initially identified cell types that bound domains from AARS and DARS. Utilizing a ligand-based receptor-capture mass spectrometry technique on these bound cells, we identified Fibroblast Growth Factor Receptor 4 (FGFR4) as a receptor for a domain of AARS and Latent-Transforming Growth Factor Beta-binding Protein 1 (LTBP-1) as a binding partner for DARS. These interactions were confirmed through siRNA knockdown experiments, immunocytochemistry for colocalization and AlphaLISA for direct protein-protein interactions. Ongoing functional characterization of these interactions has revealed potential novel fibrotic mechanisms that underscore the therapeutic potential within the tRNA synthetase family of molecules.

## tRNA Synthetase Drug Discovery Platform



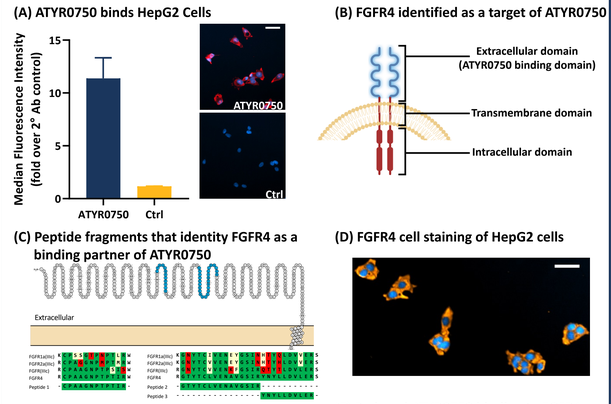

- Unique protein domains found in tRNA synthetases can be liberated via splicing or proteolysis and have been identified in extracellular spaces, where they have functions separate from their essential role in protein synthesis.
- aTyr has built an intellectual property portfolio covering >300 protein 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.
- 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.
- In this work we characterize domains from DARS (ATYR0101) and AARS (ATYR0750) and identify novel binding partners for these synthetases.

| PROGRAM | TARGET/MOA | INDICATION | RESEARCH | PRECLINICAL | PHASE 1 | PHASE 2 | PHASE 3 |
|---|---|---|---|---|---|---|---|
| *tRNA synthetase programs* | | | | | | | |
| **Efzofitimod** | NRP2 modulator | Pulmonary Sarcoidosis | | | | efzo-fit | |
| | | SSc-ILD | | | | | |
| | | Other ILD (CTD-ILD; CHP) | | | | | |
| **ATYR0101** | LTBP1 modulator | Fibrosis | | | | | |
| **ATYR0750** | FGFR4 modulator | Liver Disorders | | | | | |



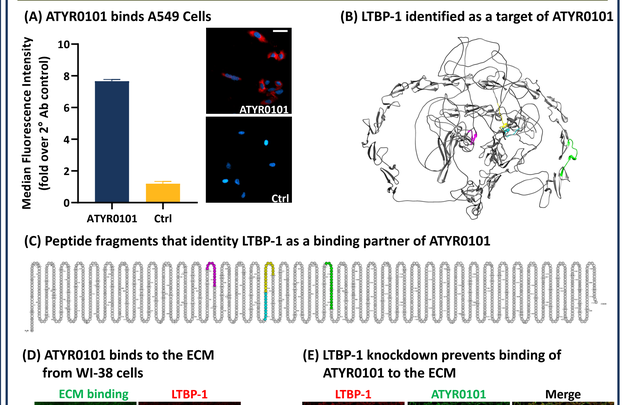

## Results

### AARS Domain (ATYR0750) Binds to FGFR4



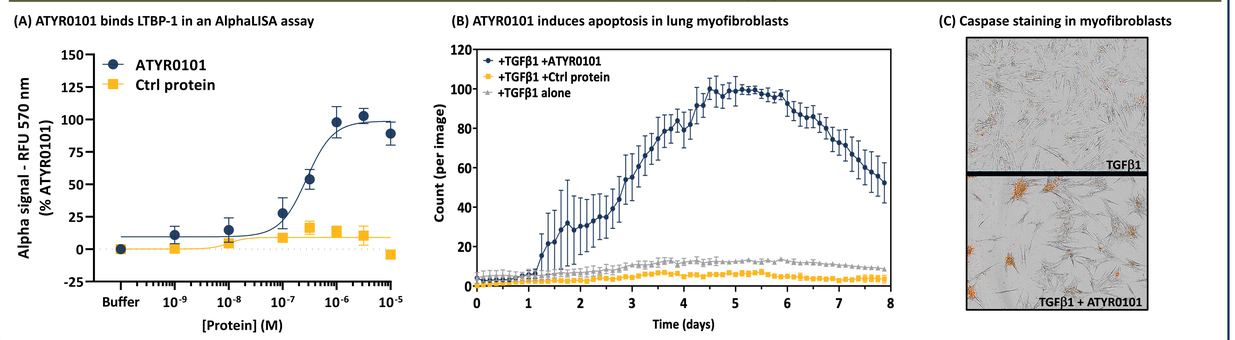

**(A)** ATYR0750 binds to the surface of HepG2 cells. Bar = 50µm. **(B)** FGFR4 is a transmembrane receptor tyrosine kinase. **(C)** The specific FGFR4 peptides identified in the screen are highlighted in blue (top) and are specifically derived from FGFR4's sequence versus other FGFR isoforms (bottom). **(D)** Immunocytochemistry assays show FGFR4 is highly expressed on the surface of HepG2 cells. Orange: FGR4; Blue: Nuclei. Bar=50µm. **(E)** Flow cytometry experiments showed a marked decrease in FGFR4 expression on HepG2 cells. **(F)** FGFR4 knockdown resulted in reduced ATYR0750 cell binding.

### DARS Domain (ATYR0101) Binds to LTBP-1




**(A)** ATYR0101 binds to the surface of A549 cells. Bar = 50µm. **(B)** LTBP-1 structure (AlphaFold entry Q14766). **(C)** LTBP-1 identified peptides are highlighted in the LTBP-1 sequence. **(D)** WI-38 cells were cultured and treated with TGFβ1 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. **(E)** Lung myofibroblasts were treated with siRNA in a protocol as in (D). LTBP-1 knockdown prevented the binding of ATYR0101 to the ECM deposited by WI-38 cells.

### ATYR0101 Induces Myofibroblast Apoptosis




**(A)** ATYR0101 directly interacts with recombinant LTBP-1 in an AlphaLISA protein-protein interaction assay. [LTBP-1] = 122nM **(B)** WI-38 fibroblasts treated with TGFβ1 in the presence of ATYR0101 or a control protein (600nM) were stained with a marker for activated Caspase 3/7. Positive cells were counted on an Incucyte instrument over a time course of incubation. **(C)** Images of activated myofibroblasts treated with TGFβ1 alone or in combination with ATYR0101 (600nM) and stained for Caspase 3/7 activity on day 5 of treatment.

## Conclusions and Future Directions

- ATYR0101 and ATYR0750 were shown to bind specifically on the surface of select human cell lines.
- Target identification experiments revealed that ATYR0750 bound to FGFR4 and ATYR0101 bound to LTBP-1 using a ligand-receptor capture technique and mass spectrometry to identify binding targets.
- Protein target interactions were validated through knockdown experiments resulting in significant decreases in target binding.
- Preclinical development is ongoing to investigate the therapeutic potential of ATYR0101 and ATYR0750 in fibrotic disease treatment.
- The methods utilized in this study can be further employed to identify and validate new molecular targets from aTyr Pharma's tRNA synthetase drug discovery platform.

Acknowledgements: This work was supported by aTyr Pharma, Inc. The authors would like to acknowledge Dualsystems Biotech for assistance with the target screening experiments. Select images created using BioRender.com.