Alanyl-tRNA synthetase fragment binds to FGFR4 and induces morphological changes and downstream signaling in liver cells with functional similarities to FGF2

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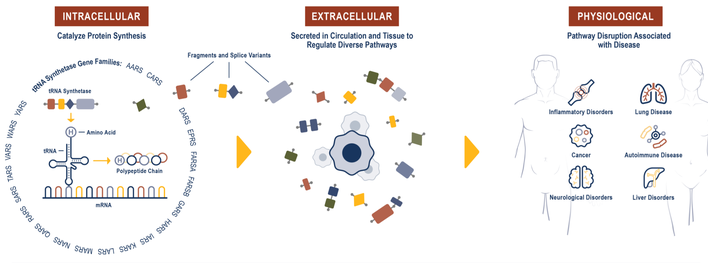
GRC 2024
March 24-29, 2024
Galveston, Texas, USA

Introduction

Background: Through posttranslational modifications and alternative splicing, tRNA synthetases have evolved unique domains, which when released from cells, interact with extracellular or membrane bound targets to evoke novel signaling pathways. A fragment of alanyl tRNA synthetase, termed ATYR0750, has been shown to interact with fibroblast growth factor receptor 4 (FGFR4) which is highly expressed in the liver. This unique interaction may complement known FGFR4 signaling via canonical fibroblast growth factors leading to a multitude of cellular effects including proliferation, survival, differentiation, and migration. The primary goal of this project was to further characterize new functional effects of ATYR0750 signaling through its target receptor FGFR4.

Methods: ATYR0750 was recombinantly expressed and purified from mammalian cells. Direct protein-protein interactions were detected with AlphaLISA technology and morphological changes were observed and quantified through live cell imaging by Incucyte®. FGFR dependence was demonstrated by inhibition of FGFR signaling, additionally RNA sequencing analysis of a liver derived cell line (HepG2) was utilized to characterize gene expression changes upon treatment with ATYR0750 and FGFs.

tRNA Synthetase Drug Discovery Platform



PROGRAM	tRNA SYNTHETASE	TARGET/MOA	INDICATION	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3
			Pulmonary Sarcoidosis[1]		efzo-fit		
Efzofitimod	HARS	NRP2 modulator	SSc-ILD		efzo-connect		
			Other ILD (CTD-ILD; CHP)				
ATYR0101	DARS	LTBP1 modulator	Fibrosis				
ATYR0750	AARS	FGFR4 modulator	Liver Disorders				
tRNA Synthetase Candidates[2]							

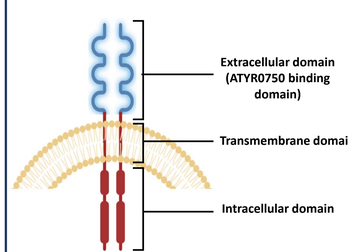
(1) In partnership with Kyorin Pharmaceutical Co., Ltd. for the development and commercialization of efzofitimod for ILD in Japan
(2) Pipeline candidates in development based on additional tRNA synthetases from IP portfolio
ODD = orphan drug designation; SSc-ILD = Scleroderma-related ILD; CTD-ILD = Connective Tissue Disease-ILD; CHP = Chronic Hypersensitivity Pneumonitis

- 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.
- aTyr's drug discovery platform has identified additional synthetase fragments, resulting in ATYR0750 and ATYR0101 early-stage programs.
- In this study, we characterize a fragment from AARS (ATYR0750) and explore novel functionality of this fragment as it compares to two known FGFR ligands in human liver cells.

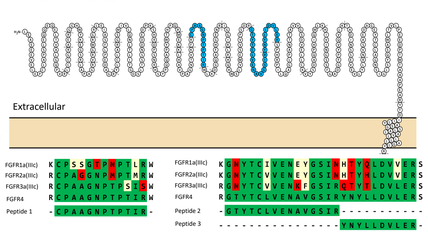
Synthetase fragment discovery → Target identification → Target validation → Functional profiling → Preclinical studies → Clinical studies

AARS Fragment (ATYR0750) Binds to FGFR4

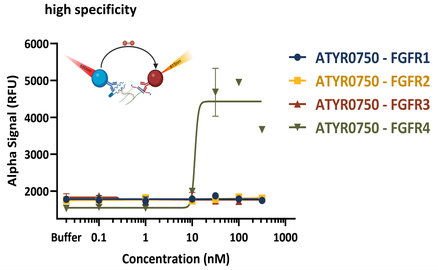
(A) FGFR4 identified as a target of ATYR0750



(B) Peptide fragments that identify FGFR4 as a binding partner of ATYR0750



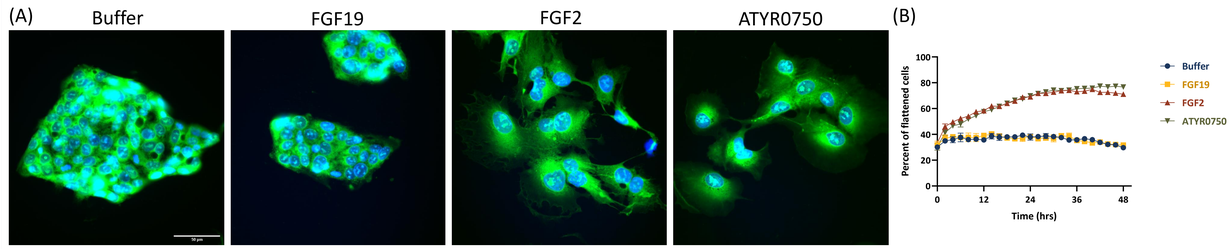
(C) ATYR0750-FGFR4 interactions detected by AlphaLISA with high specificity



(A) Structure of ATYR0750 binding partner, FGFR4. (B) Specific FGFR4 peptides identified in a binding screen are highlighted in blue (top) and are specifically derived from FGFR4's sequence versus closely related FGFR isoforms (bottom). (C) ATYR0750 interacts with recombinant human FGFR4 (R&D Systems Cat# 11120-FR; 100nM) in the presence of 2.5ug/mL heparan sulfate proteoglycans (Millipore Sigma Cat# H4777-0.1mg) detected through AlphaLISA technology (FGFR1a (IIIC) R&D Systems Cat# 11118-FR, FGFR2a (IIIC) R&D Systems Cat# 11119-FR and FGFR3a (IIIC) G&P Biosciences Cat# FCL075; 100nM).

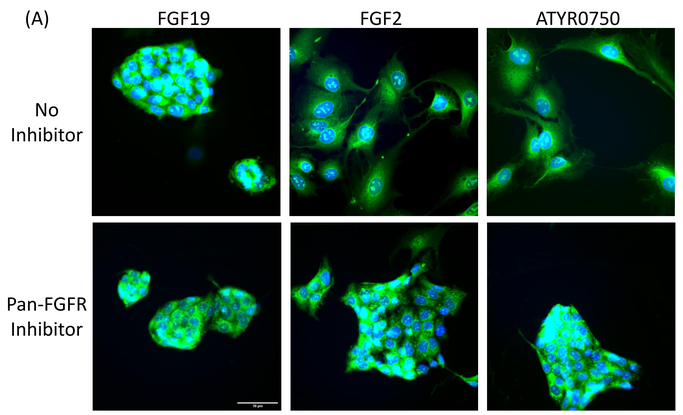
Results

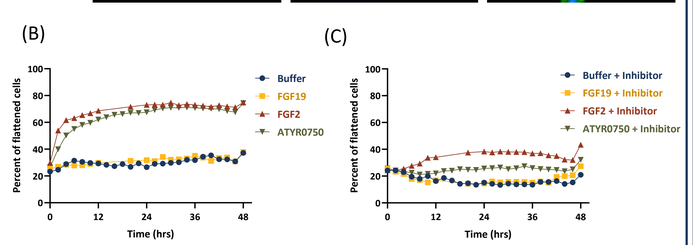
ATYR0750 and FGF2 Specifically Induce Morphologic Changes in HepG2 Cells in a Manner Distinct from FGF19



(A) 40x representative images of HepG2 cell morphology after treatment with FGF19 (R&D Systems Cat# 969-FG; 2.1ug/mL), FGF2 (R&D Systems Cat# 3718-FB; 1.6ug/mL) or ATYR0750 (1uM) in low serum media. Treated cells were fixed with methanol and membrane stained with CellBrite 488 (Biotium Cat# 30090) and counterstained with Hoechst 33342 (Invitrogen Cat# H3520). (B) Percentage of cells exhibiting a flattened membrane, increased size and reduced clumping normally seen with HepG2 cells. Calculated using Incucyte® software.

ATYR0750 Induced Morphologic Changes Lost with FGFR Inhibition

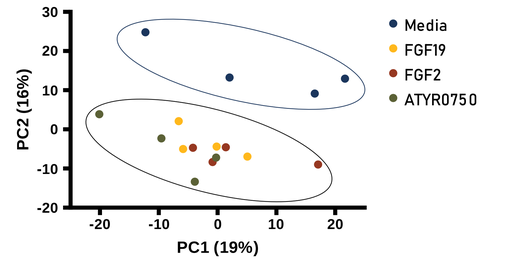




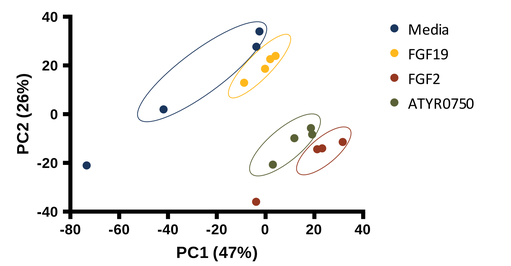
A) 40x representative images of HepG2 morphology after 48 hrs of FGF19 (2.1ug/mL), FGF2 (1.6ug/mL) and ATYR0750 (1uM) treatment. Cells were preincubated with a pan-FGFR inhibitor (PD161570, Tocris Cat# 3724; 6uM) or buffer for 1 hour prior to treatment. Percent of cells exhibiting a flattened membrane and increased size in the HepG2 cell line without (B) or with (C) a pan-FGFR inhibitor (PD161570). Calculated using the Incucyte® software.

Similar Transcriptomic Profiles of ATYR0750 & FGF2

(A) Transcriptional profile of treated HepG2 cells (4hrs)



(B) Transcriptional profile of treated HepG2 cells (24hrs)



Principal component analysis (PCA) from transcriptional profiling of HepG2 cells treated with FGF2 (5ug/mL), FGF19 (5ug/mL) or ATYR0750 (1uM) at 4 hours (A) and 24 hrs (B).

Conclusions

- ATYR0750 demonstrates similar FGFR-dependent functional effects to FGF2, as shown by morphologic changes, signaling inhibition and transcriptional changes which is unlike another known FGFR4 ligand FGF19.
- ATYR0750 binds selectively to FGFR4 resulting in phenotypic and functional signaling events on liver cells, suggesting a novel signaling pathway originating from an extracellular tRNA synthetase fragment.

Acknowledgements: This work was supported by aTyr Pharma, Inc. The authors would like to thank Dualsystems Biotech for assistance with the initial target screening.