# ATYR1923 Specifically Binds To Neuropilin-2, A Novel Therapeutic Target For The Treatment Of Immune-mediated Diseases

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## Abstract

**INTRODUCTION:** We have previously discovered that a splice variant of the histidyl-tRNA synthetase (HARS) gene has an immuno-modulatory effect. ATYR1923 is a fusion protein comprised of this splice variant, which aTyr termed iMod, fused to human IgG1 Fc. ATYR1923 inhibits cytokines and chemokines involved in the regulation of inflammatory and fibrotic responses and reduces inflammation and fibrosis in animal models of interstitial lung diseases (ILD), a group of immune-mediated lung disorders that can result in lung fibrosis. ATYR1923 is being evaluated in a Phase 1b/2a study in patients with pulmonary sarcoidosis, a major form of ILD. We sought to characterize the molecular basis for ATYR1923's immuno-modulatory properties.

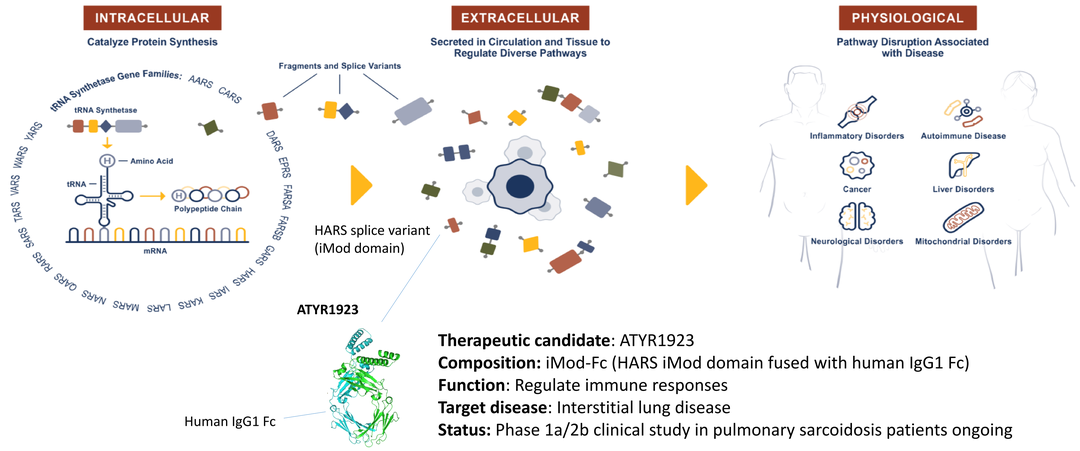
**RESULTS:** Cell microarray screening technology was used to assess ATYR1923 binding to a library of >4500 human plasma membrane proteins, and identified the membrane-bound splice isoforms of Neuropilin-2 (NRP2) as specific, selective hits. Notably, no binding was detected for the related and structurally similar receptor Neuropilin-1 (NRP1). Specific binding of ATYR1923 to NRP2 was confirmed by surface plasmon resonance (SPR) and also by flow cytometry analysis of HEK293 cells over-expressing NRP2. Furthermore, ATYR1923 also bound to cells that endogenously express NRP2 on the surface (such as A549, THP-1 polarized M1 macrophages).

To map the binding sites of ATYR1923 on NRP2, we constructed a number of deletion mutants of the extracellular region of NRP2, and the binding was narrowed to the a2b1b2 fragment that encompasses binding contacts for vascular endothelial growth factors (VEGFs) and semaphorins. Domain swapping studies in which individual domains of cell-surface expressed NRP2 were replaced with the corresponding, structurally similar domain from NRP1 demonstrated that b1 is the key domain for binding, while the a2 and b2 domains likely also provide contacts with ATYR1923. In addition, we solved the crystal structure of the protein complex of ATYR1923 with an anti-iMod antibody that blocks its binding to NRP2, demonstrating the loop region within iMod to be the key interaction site for ATYR1923 with NRP2.

**CONCLUSIONS**: ATYR1923 specifically and selectively binds to NRP2 on the cell surface. ATYR1923 has previously demonstrated potent immunomodulatory activity *in vitro* and *in vivo*. NRP2 is a pleiotropic cell surface receptor known to be expressed on a number of different immune cell types and may play a key role in regulating inflammatory responses. These findings indicate that modulation of the NRP2 signaling pathway with ATYR1923 could be a novel therapeutic approach to immune-mediated diseases such as pulmonary sarcoidosis and other interstitial lung diseases.

## Introduction

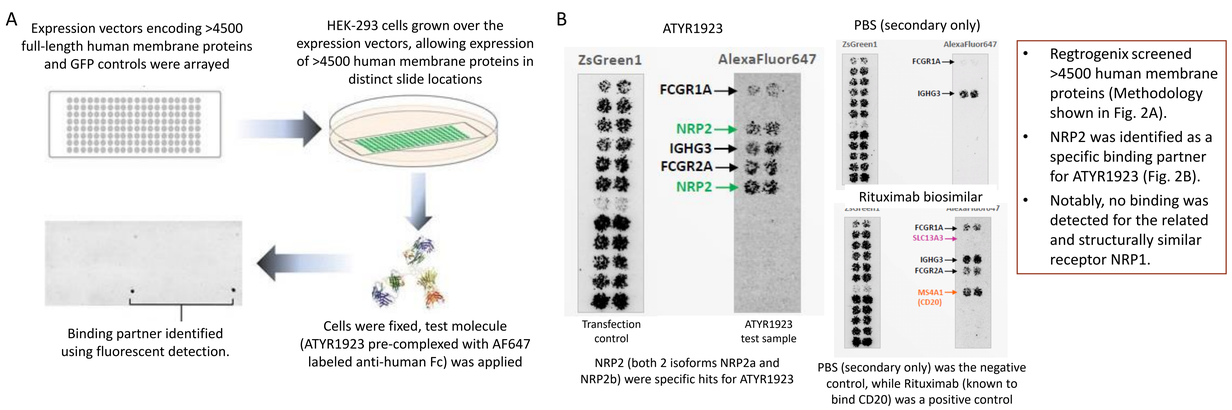
### Figure 1. Extracellular tRNA Synthetase Biology and Development of ATYR1923 as A Therapeutic Candidate




**Therapeutic candidate**: ATYR1923
**Composition:** iMod-Fc (HARS iMod domain fused with human IgG1 Fc)
**Function**: Regulate immune responses
**Target disease**: Interstitial lung disease
**Status:** Phase 1a/2b clinical study in pulmonary sarcoidosis patients ongoing

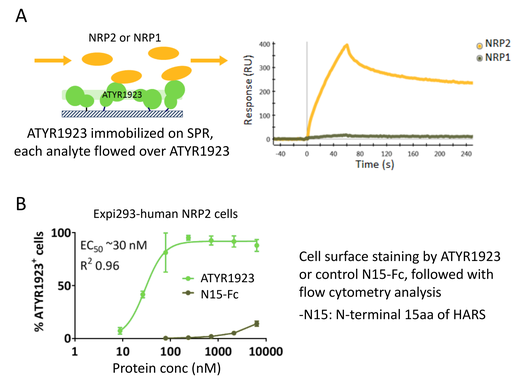
## Results

### Figure 2. Screening of Human Membrane Proteins at Retrogenix and Identification of NRP2 As a Specific ATYR1923-Binding Partner



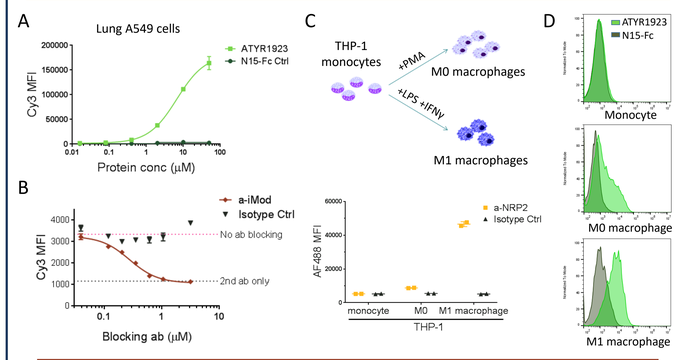

- Regtrogenix screened >4500 human membrane proteins (Methodology shown in Fig. 2A).
- NRP2 was identified as a specific binding partner for ATYR1923 (Fig. 2B).
- Notably, no binding was detected for the related and structurally similar receptor NRP1.

### Figure 3. ATYR1923 Specifically Binds to NRP2 but Not NRP1



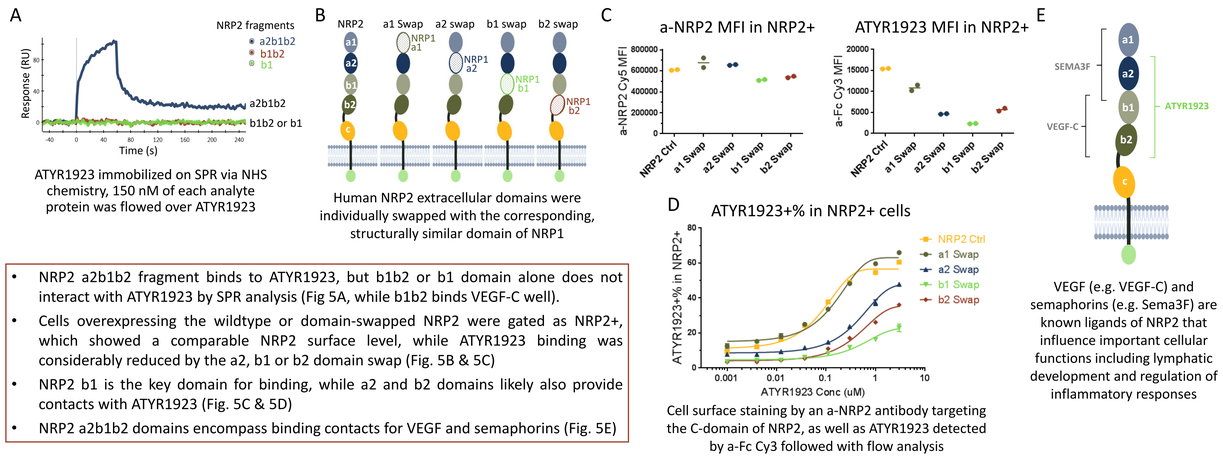

- ATYR1923 binds to NRP2 but not NRP1 by SPR measurement (Fig. 3A)
- ATYR1923 binds to NRP2 overexpressed on Expi293 cells (Fig. 3B).
- NRP2 is a pleiotropic receptor with important functional roles in cardiovascular, nervous and immune systems (Guo HF et al., 2015)

### Figure 4. ATYR1923 Binds to Cells That Express Endogenous NRP2



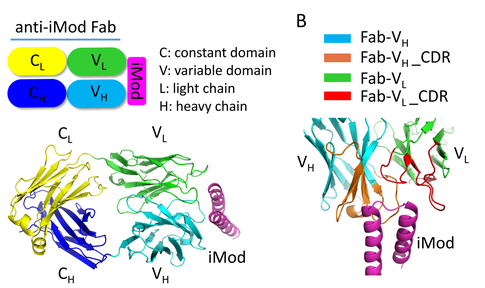

- ATYR1923 binds to lung A549 cells known to express endogenous NRP2 (Fig. 4A), which is entirely blocked by an anti-iMod antibody (Fig. 4B)
- Cell surface NRP2 level was up-regulated by the THP-1 differentiation, especially on M1 macrophages (Fig. 4C).
- ATYR1923 binds to THP-1 macrophages, which is in accordance with the up-regulation of surface NRP2 level (Fig. 4D)

### Figure 5. Delineation of ATYR1923 Binding Region on NRP2 at a2b1b2 Domains




- NRP2 a2b1b2 fragment binds to ATYR1923, but b1b2 or b1 domain alone does not interact with ATYR1923 by SPR analysis (Fig 5A, while b1b2 binds VEGF-C well).
- Cells overexpressing the wildtype or domain-swapped NRP2 were gated as NRP2+, which showed a comparable NRP2 surface level, while ATYR1923 binding was considerably reduced by the a2, b1 or b2 domain swap (Fig. 5B & 5C)
- NRP2 b1 is the key domain for binding, while a2 and b2 domains likely also provide contacts with ATYR1923 (Fig. 5C & 5D)
- NRP2 a2b1b2 domains encompass binding contacts for VEGF and semaphorins (Fig. 5E)

### Figure 6. Complex Structure of iMod with a Blocking Antibody




- iMod domain of ATYR1923 adopts a helix-turn-helix structure
- The middle loop of iMod interacts with NRP2 (Fig. 6A & 6B)

## Conclusions

- **NRP2, a pleiotropic cell surface receptor that is expressed and may play a key role in inflammatory responses, was identified as a specific binding target for ATYR1923**
- **NRP2 extracellular a2b1b2 domains encompass binding sites for ATYR1923**
- **ATYR1923 binds to overexpressed and endogenous NRP2 on human cell surface**
- **The middle loop region of iMod domain in ATYR1923 interacts with NRP2**

## References

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