Efzofitimod, a first in-in class NRP2-targeting immunomodulator, ameliorates rheumatoid arthritis and associated lung fibrosis in preclinical models

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Abstract

Background/Purpose:

Efzofitimod, a novel immunomodulator, has shown clinical proof-of-concept in a Phase 1b/2a clinical trial in patients with pulmonary sarcoidosis and is currently enrolling a global Phase 3 clinical trial. By selectively binding to its target neuropilin-2 (NRP2), a membrane protein that is strongly upregulated on myeloid cells during inflammation, efzofitimod reduces inflammation and fibrosis in a range of animal models of interstitial lung disease (ILD). NRP2 appears to also play a role in other inflammatory diseases such as arthritis [1,2]. In this work, we confirm that connection utilizing a genetic NRP2 knock-out (KO) mouse model. Based on efzofitimod's anti-inflammatory effects and NRP2's role in the pathology of arthritis, we tested its therapeutic potential in experimental models of rheumatoid arthritis.

Methods:

The collagen-induced arthritis (CIA) model was performed in NRP2 WT and KO animals utilizing the Hooke Kit™ for CIA induction. Efzofitimod was dosed at 1 mg/kg weekly intravenously upon emergence of clinical signs. Study animals were scored every other day until termination. Terminal serum samples were analyzed for pro-inflammatory cytokines. To test the effect of efzofitimod in rheumatoid arthritis-associated ILD (RA-ILD), we utilized the SKG mouse model of RA-ILD. The disease was induced via IP injection of 5 mg of zymosan. Efzofitimod was dosed at 3 mg/kg weekly intravenously starting one day prior to disease induction. At termination, lung single cell suspensions were immunophenotyped and lung sections were analyzed for fibrosis.

Results:

NRP2 KO animals exhibited heightened sensitivity to disease induction in the CIA model, resulting in excessive mortality. Analysis of serum samples from these animals demonstrated elevated levels of pro-inflammatory cytokines, indicating a deficiency in the negative regulation of the immune response. Based on the impaired immune regulation observed in NRP2 KO animals, we hypothesized that efzofitimod may produce therapeutic benefit in the CIA model via modulation of NRP2. Encouragingly, we observed improved clinical scores in 37.5% of the study animals treated with efzofitimod, compared to only 12.5% in the control group. Additionally, efzofitimod displayed activity in the SKG model of RA-ILD. Treatment with efzofitimod led to a reduction in the number of immune cell populations in the lungs and exhibited a noteworthy reduction in RA-induced lung fibrosis.

Conclusion:

The data presented here indicate a critical role for NRP2 in modulating immune responses in autoimmune diseases, such as rheumatoid arthritis and RA-ILD. By targeting NRP2 with efzofitimod, we observed improved disease outcomes, reduced inflammation, and mitigated lung fibrosis, suggesting the potential of efzofitimod as a therapeutic intervention for rheumatoid arthritis and potentially other immune mediated diseases.

1. Fassold, A. *et al.* Soluble neuropilin-2, a nerve repellent receptor, is increased in rheumatoid arthritis synovium and aggravates sympathetic fiber repulsion and arthritis. *Arthritis Rheum.* 60, 2892–2901 (2009).
2. 2. Shoda, J. *et al.* Semaphorin 3G exacerbates joint inflammation through the accumulation and proliferation of macrophages in the synovium. *Arthritis Res. Ther.* 1–12 (2022)

tRNA synthetase drug discovery platform

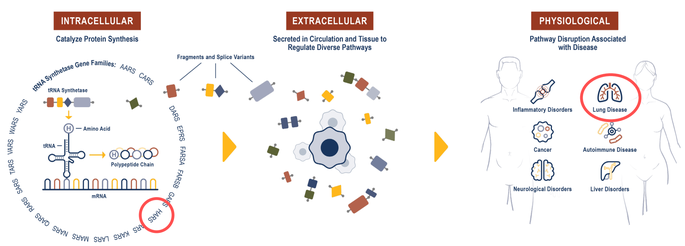

Figure 1. Extracellular tRNA synthetases have non-canonical immuno-modulating properties. The canonical role of tRNA synthetases is to catalyze protein synthesis within the cell. Splice variants and proteolytic fragments of tRNA synthetases have been identified in extracellular spaces, where they have novel functions. aTyr Pharma is engaged in the discovery and development of potential first-in-class medicines based on newly discovered pathways effected by extracellular tRNA synthetases.

Efzofitimod, a novel ligand for neuropilin-2 (NRP2)

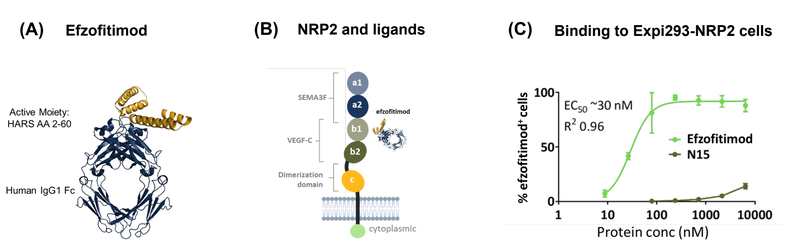

Figure 2. Efzofitimod binds to NRP2 receptor. (A) Efzofitimod is a fusion protein consisting of amino acids 2-60 of Histidyl-tRNA synthetase (HARS) and human IgG1 Fc. (B) Schematic of NRP2 domains and ligands. (C) Efzofitimod binds to Expi293F-NRP2 cells.

NRP2 is a negative regulator of the inflammatory response

NRP2-deficiency exacerbates pathology in preclinical models of inflammatory disease

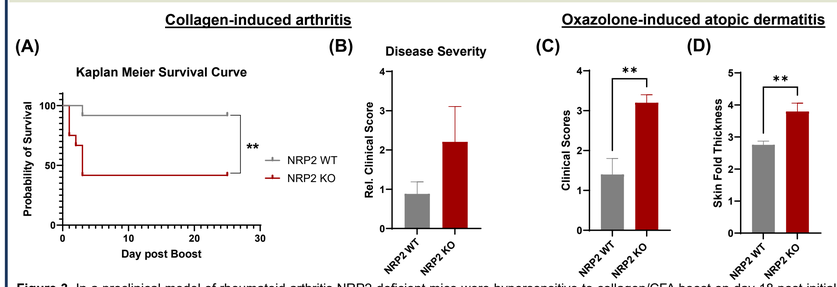

Figure 3. In a preclinical model of rheumatoid arthritis NRP2-deficient mice were hypersensitive to collagen/CFA boost on day 18 post initial collagen immunization (A) leading to increased mortality. At study termination NRP2-deficient animals (n=4) showed a non-significant increase in relative clinical scores compared to wildtype animals (n=11) (B). An enhanced immune response was also observed in a model of chronic atopic dermatitis. Disease severity as determined by clinical observations (C) and terminal skin fold measurement on day 17 (D) was increased in NRP2-deficient mice.

NRP2-deficiency enhances the inflammatory response in CIA

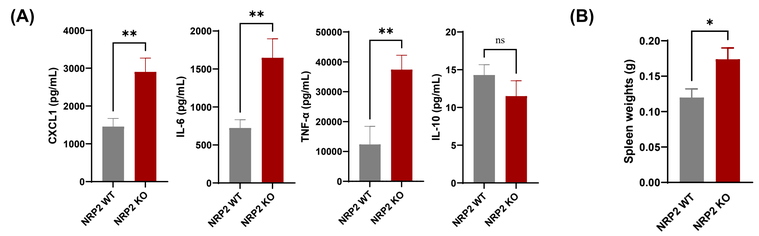

Figure 4. (A) Pro-inflammatory cytokines/chemokines are increased in the serum of NRP2-deficient animals 48h post collagen/CFA boost. (B) Spleen hyperplasia, a sign of an overactive immune response, observed in NRP2 KO mice at study termination.

NRP2 is expressed on immune cells of the myeloid lineage in RA

Macrophages and synovial lining cells are the main cell types with NRP2 expression

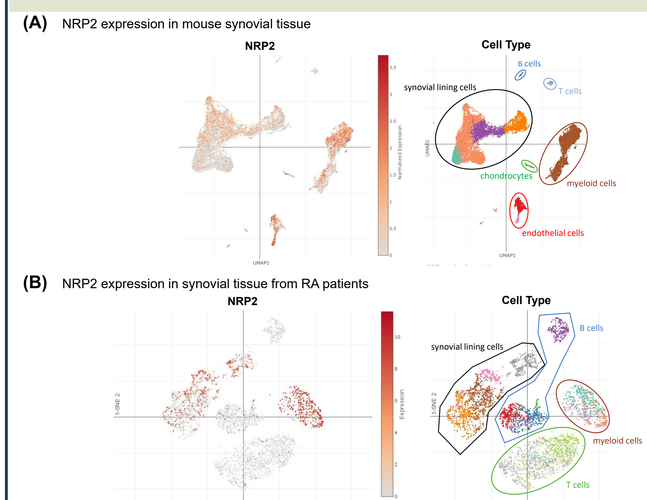

Figure 5. (A) Publicly available mouse scRNAseq from a preclinical model of RA was analyzed for Nrp2 expression (study accession: GSE145286). (B) Publicly available scRNAseq data from RA patients was analyzed for NRP2 expression (data available at ImmPort: accession code SDY998 and SDY999).

Efzofitimod shows therapeutic activity in model of RA

Efzofitimod regulates pro-inflammatory genes involved in pathogenesis of RA

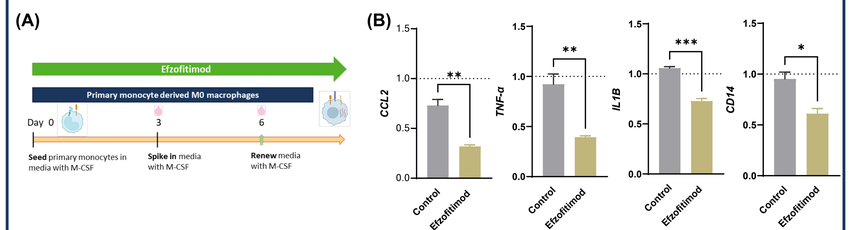

Figure 6. (A) Monocyte-derived macrophages were generated in the presence or absence of 1uM efzofitimod. (B) Efzofitimod reduced gene expression of pro-inflammatory genes in *in vitro* generated macrophages. Gene expression relative to untreated cells shown.

Efzofitimod improves remission in CIA model

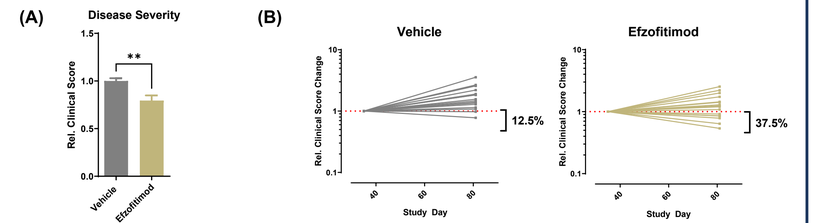

Figure 7. In the collagen-induced arthritis (CIA) model efzofitimod, dosed once weekly at 1 mg/kg via the IV route, led to a significantly lower clinical score at termination than the vehicle group (A). Longitudinal analysis of the clinical scores shows improvement of clinical signs in 37.5% of the efzofitimod-treated animals versus only 12.5% in the vehicle control group (B).

Efzofitimod ameliorates lung fibrosis in RA-ILD Model

Efzofitimod modulates the immune response leading to reduced fibrosis

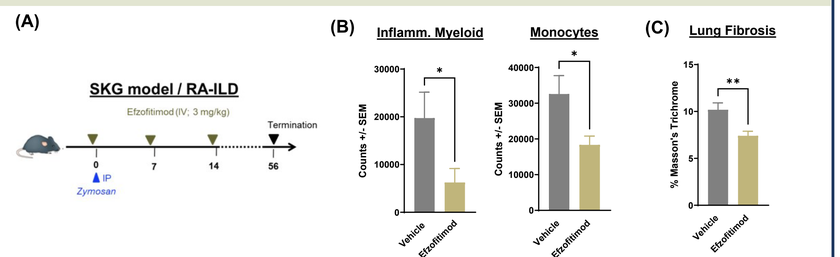

Figure 8. In the SKG model of RA-ILD (A), a significant decrease in monocytes and inflammatory myeloid cells in the lung was observed in efzofitimod treated animals (B). Histological analysis of the lungs via Masson's Trichrome staining showed reduced collagen deposition (C).

Conclusions

Efzofitimod is a first-in-class immunomodulator that has demonstrated POC in a Phase 1b/2a trial in patients with pulmonary sarcoidosis.

- NRP2, the sole binding partner to efzofitimod, is a negative regulator of the immune response.
- NRP2 is highly expressed on myeloid cells within the synovium of RA patients.
- Efzofitimod is a novel ligand to NRP2 and demonstrates immune regulatory function leading to reduced inflammation, mitigated lung fibrosis and improved disease outcomes in inflammatory disease models.
- Data support further investigation of efzofitimod in RA and RA-related ILD.