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Efzofitimod Is An Immunomodulator of Myeloid Cell Function and Novel Therapeutic Candidate for Interstitial Lung Diseases

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Abstract/Aims

Interstitial lung disease (ILD) represents a diverse group of inflammatory and fibrotic conditions with limited treatments available. Myeloid cells are becoming increasingly appreciated as drivers of inflammation and fibrosis in ILD. Efzofitimod is a myeloid cell immunomodulator currently in a Phase 3 clinical trial for pulmonary sarcoidosis. It selectively binds to neuropilin-2 (NRP2), a membrane protein upregulated at sites of inflammation, most notably on myeloid cells, and has been shown to reduce inflammation and fibrosis across various murine ILD models. An efzofitimod Phase 1b/2a trial in pulmonary sarcoidosis patients showed dose dependent trends of improvement in lung function and reduced pro-inflammatory serum biomarkers[1]. Given these promising results, we probed more deeply into the underlying molecular mechanism that is the basis for efzofitimod's activity and therapeutic potential in ILD.

Here, we investigated the mechanism of action of efzofitimod using samples from ILD patients. Expression of target protein NRP2 in ILD patient cells and tissue samples was probed by flow cytometry, public RNA sequencing datasets, immunohistochemistry (IHC) and immunofluorescence techniques. The morphology change of primary monocyte-derived macrophages (MDM) by efzofitimod treatment was monitored using an automated live cell imaging and analysis platform. Transcriptome regulation of macrophages and PBMC from sarcoidosis patients was analyzed by RNA sequencing and gene set enrichment analysis. Results from these studies demonstrate that efzofitimod acts on myeloid cells in ILD patients to diminish inflammatory potential. Given the central role of myeloid cells in the pathology of numerous ILDs and other chronic inflammatory and fibrotic diseases, efzofitimod may have broad therapeutic potential in ILD and beyond.

Evolution of Non-Catalytic Domains of tRNA Synthetases for Novel Functions

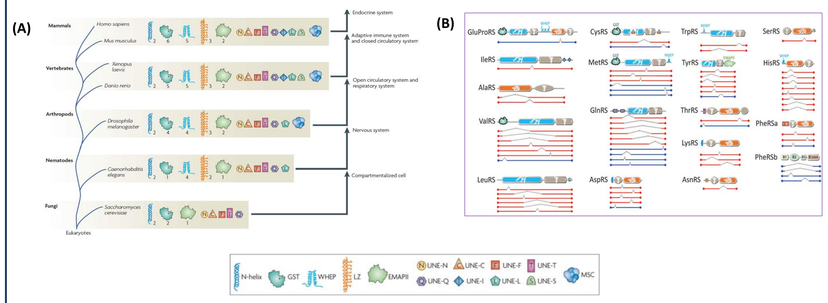


Figure 1. The catalytic domains of tRNA synthetases are ancient and conserved from bacteria to humans. (A) As life became more complex, tRNA synthetases evolved additional non-catalytic domains with novel functions that coincide with the appearance of higher organ systems[2]. (B) About 80% of tRNA synthetase splice variants do not possess the aminoacylation catalytic domains and are instead liberated novel signaling domains[3].

tRNA Synthetase Drug Discovery Platform

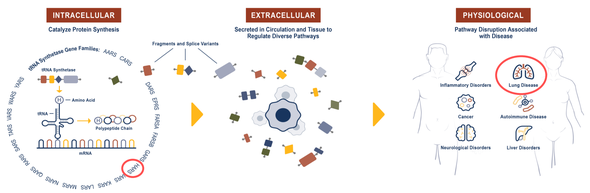


Figure 2. Splice variants and proteolytic fragments of tRNA synthetases have been identified in extracellular spaces, where they have novel functions. aTyr Pharma is engaged in the discovery and development of potential first-in-class medicines based on newly discovered pathways effected by extracellular tRNA synthetases.

Efzofitimod, a Novel Ligand for Neuropilin-2 (NRP2) Receptor

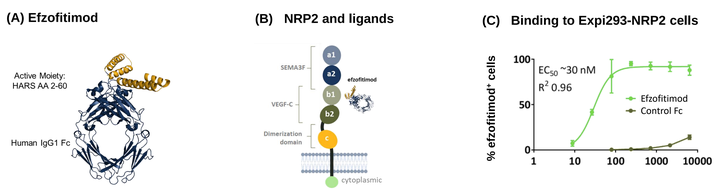


Figure 3. Efzofitimod binds to the NRP2 receptor. (A) Efzofitimod is a fusion protein consisting of amino acids 2-60 of histidyl-tRNA synthetase (HARS) and human IgG1 Fc. (B) Schematic of NRP2 domains and ligands. (C) Efzofitimod binds to Expi293F-NRP2 cells.

Methods and Results

NRP2 is Highly Expressed in Circulating Monocytes and Tissue Macrophages from Pulmonary Sarcoidosis and SSc-ILD Patients

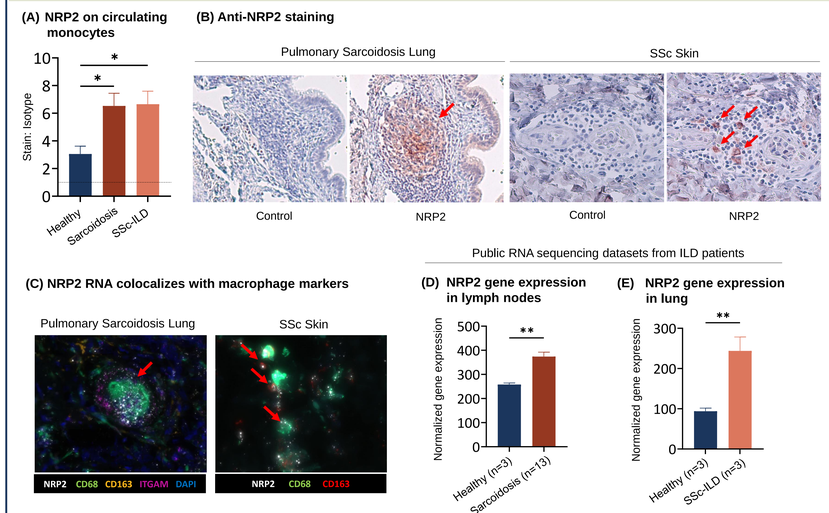


Figure 4. (A) NRP2 expression was determined by flow cytometry on CD14+ monocytes in unstimulated PBMC from healthy donors (n=6), pulmonary sarcoidosis patients (n=7), and SSc-ILD patients (n=5). (B) NRP2 expression (brown) is detectable by IHC within granulomas of lung from a pulmonary sarcoidosis patient and skin from a scleroderma patient. (C) NRP2 RNA (white spots) co-localizes with macrophage markers CD68 (green) and CD163 (orange/red). (D,E) Public RNA sequencing datasets were utilized to compare NRP2 expression in tissues from ILD patients and controls (GSE157671, GSE166036).

Promotion of MDM Differentiation by Efzofitimod Observed in Healthy and ILD Donors

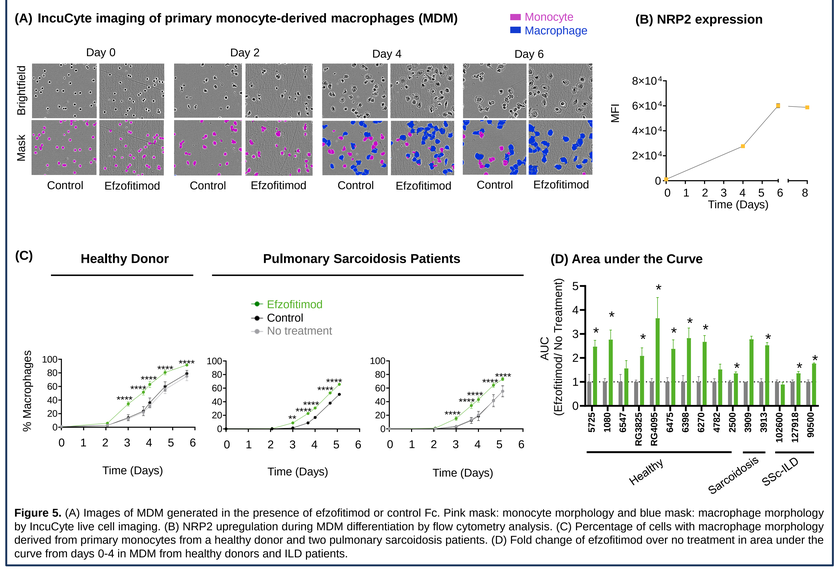


Figure 5. (A) Images of MDM generated in the presence of efzofitimod or control Fc. Pink mask: monocyte morphology and blue mask: macrophage morphology by IncuCyte live cell imaging. (B) NRP2 upregulation during MDM differentiation by flow cytometry analysis. (C) Percentage of cells with macrophage morphology derived from primary monocytes from a healthy donor and two pulmonary sarcoidosis patients. (D) Fold change of efzofitimod over no treatment in area under the curve from days 0-4 in MDM from healthy donors and ILD patients.

Methods and Results

Novel Efzofitimod-induced Macrophage Signature Observed in MDM from Sarcoidosis Patient

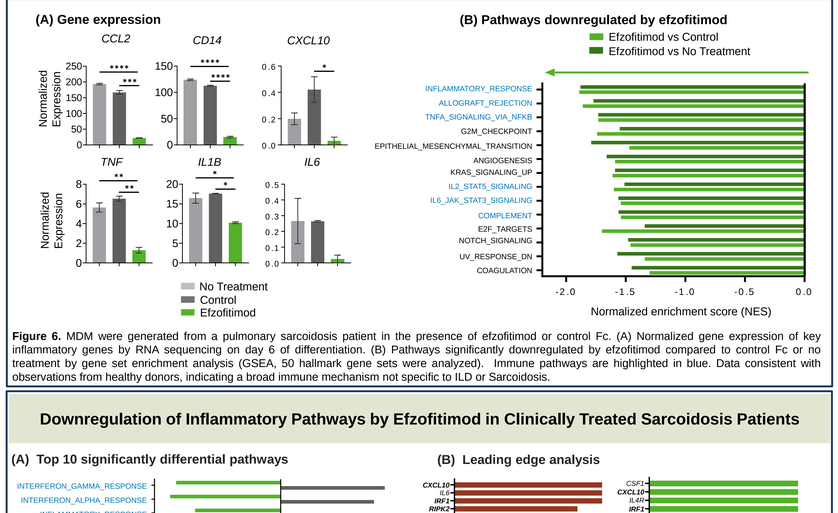
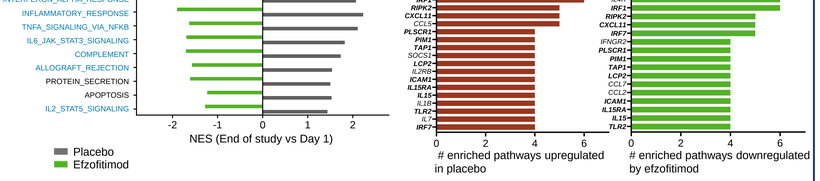


Figure 6. MDM were generated from a pulmonary sarcoidosis patient in the presence of efzofitimod or control Fc. (A) Normalized gene expression of key inflammatory genes by RNA sequencing on day 6 of differentiation. (B) Pathways significantly downregulated by efzofitimod compared to control Fc or no treatment by gene set enrichment analysis (GSEA, 50 hallmark gene sets were analyzed). Immune pathways are highlighted in blue. Data consistent with observations from healthy donors, indicating a broad immune mechanism not specific to ILD or Sarcoidosis.

Downregulation of Inflammatory Pathways by Efzofitimod in Clinically Treated Sarcoidosis Patients



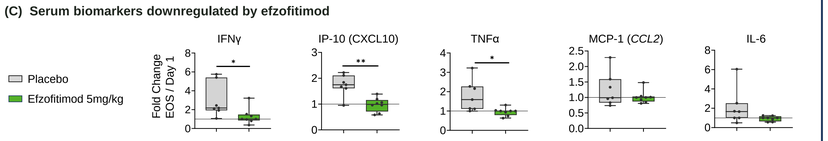
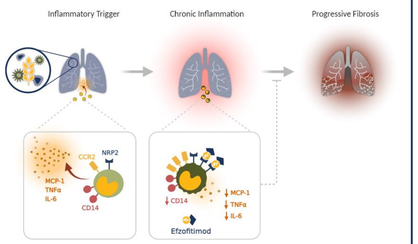


Figure 7. (A) Top 10 pathways differentially regulated at the end of study (EOS) compared to baseline (Day 1) by efzofitimod versus placebo. Immune pathways are highlighted in blue. (B) Core enriched genes by GSEA leading edge analysis. (C) Fold change in serum biomarkers at end of study compared to baseline.

Conclusions

- NRP2, the binding partner to efzofitimod, is highly expressed in cells and tissues from patients with ILD.
- NRP2 is upregulated during monocyte-derived macrophage differentiation, which corelates with efzofitimod's effect on promoting macrophages with an anti-inflammatory profile.
- Efzofitimod downregulates inflammatory genes and pathways in macrophages and PBMC from pulmonary sarcoidosis patients.
- Data support investigation of efzofitimod in ILD and other inflammatory and fibrotic diseases that involve myeloid cells in pathology.



Disclosures:
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References:
[1]Culver D, et. al. Efzofitimod for the Treatment of Pulmonary Sarcoidosis. *Chest*. 163 (4) (2023)
[2]Guo, Yang, et. al. New Functions of Aminoacyl-tRNA Synthetases beyond Translation. *Nat Rev Mol Cell Biol*. 11(9)(2010)
[3]Lo, Wing-Sze, et. al. Human tRNA synthetase catalytic nulls with diverse functions. *Science*. 345(6194)(2014)