Neuropilin-2 (NRP2) is a pleiotropic co-receptor with an emerging role in the regulation of inflammatory responses. NRP2 was identified by aTyr as the sole binding partner for ATYR1923, a fusion protein combining a novel immunomodulatory domain from histidyl-tRNA synthetase (HARS) and a human IgG1 Fc. ATYR1923 is in early clinical testing for pulmonary granulomas with positive signals predominantly localized within the inner structure of the granuloma where Langhans’ giant cells (myeloid origin) reside. This finding was confirmed in both lung and skin samples from sarcoidosis patients. We thus sought to investigate which immune cells express NRP2.

In vitro, we showed that NRP2 is highly inducible in key immune cells following immunomodulatory insults such as lipopolysaccharide (LPS) or other toll-like-receptor ligands (TLR). NRP2 expression on macrophages was only induced following activation of toll-like receptors bound on the cell surface (TLR2, TLR4, TLR5, TLR9, TLUR), but not by endosomal TLR ligands (TLR3, TLR7/8, TLR9). In addition to detecting NRP2 on the cell surface of macrophages, we also confirmed its presence on dendritic cells, T cells and regulatory cells using other live cell or primary cells.

In vivo, multi-color flow cytometry was used to immunophenotype either lungs or spleens isolated from LPS challenged animals. We confirmed our in vitro findings demonstrating that expression of NRP2 is present and inducible on the cell surface of various immune cells (macrophages, dendritic cells, neutrophils, and T cells).

We report for the first time that NRP2 is expressed in samples obtained from lung and skin of sarcoidosis patients. We clearly show that NRP2 expression can be detected on key immune cells known to play an important role in inflammation and granuloma formation. These findings highlight the potential of ATYR1923 to act on various immune cells directly related to the pathology of the target patient population.

**Abstract**

**Introduction**

Sarcoidosis is an inflammatory disorder of unknown etiology.

- The disease involves granuloma formation (clumps of inflammatory cells) in affected organs, which can lead to fibrosis and irreversible organ damage. Lungs are the most commonly affected organ (>90% of cases) and this is referred to as pulmonary sarcoidosis.
- We previously demonstrated that ATYR1923, a fusion protein combining a novel immunomodulatory domain from histidyl-tRNA synthetase (HARS) and a human IgG1 Fc binds to the NRP2 receptor in human and mouse overexpressing cells (Paz et al., Keystone 2019; Xu et al., ATS 2020).
- Little is known about NRP2's role in immune regulation and disease/granuloma progression.

**Experimental Procedure**

Figure 1. Granuloma Formation in Pulmonary Sarcoidosis

**Figure 2. Schematic Representation showing the experimental procedures utilized to measure NRP2 mRNA expression in skin and lung biopsy samples.**

**Figure 3. Generation of Human Primary Macrophages**

**Figure 4. Generation of Human Macrophages from THP-1 Cell Line**

**Figure 5. Generation of Human Primary Dendritic Cells**

**Figure 6. Generation of Primary Human Inducible T Regulatory Cells (iTregs)**

**Figure 7. NRP2 Expression in Sarcoid Samples**

**Figure 8. NRP2 Expression on Primary Macrophages**

**Figure 9. NRP2 Expression on Human THP-1 Cell Line**

**Figure 10. NRP2 Expression on Human Primary Dendritic Cells**

**Figure 11. NRP2 Expression on Human Inducible Treg Cells**

**Figure 12. In vitro NRP2 Expression**

**Figure 13. NRP2 Expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 14. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 15. Representative image of NRP2 ISH staining (red dots) in lung granulomas (A) and skin samples (B)**

**Figure 16. NRP2 Expression on Human Primary Dendritic Cells**

**Figure 17. NRP2 Expression in Granulomas from Sarcoid and Langerhans’ Disease**

**Figure 18. NRP2 Expression on Primary Macrophages**

**Figure 19. NRP2 Expression on Primary Dendritic Cells**

**Figure 20. NRP2 Expression on Human THP-1 Cell Line**

**Figure 21. NRP2 Expression on Human Primary Dendritic Cells**

**Figure 22. In vitro NRP2 Expression**

**Figure 23. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 24. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 25. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 26. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 27. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 28. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 29. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 30. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 31. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 32. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 33. NRP2 expression on human primary monocytes and neutrophils in response to inflammatory stimuli**

**Figure 34. Schematic representation showing the experimental procedures to generate human immune cells.**

**Conclusions**

**References**

Neuropilin-2, the Specific Binding Partner To ATYR1923, is Expressed In Sarcoid Granulomas and Key Immune Cells

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- NRP2 was identified as specific binding partner for ATYR1923 (Paz et al.; Keystone Poster 2019; Xu et al.; ATS Poster AP1173)
- NRP2 was expressed within the granulomas of both lung and skin biopsies obtained from sarcoidosis patients, predominantly localized within the inner structure of the granuloma where Langhans’ giant cells (myeloid origin) reside.
- NRP2 was highly expressed following immunomodulation (TLR agonists) in both in vitro and in vivo systems.
- NRP2 expression on monocytes, dendritic cells and Tregs, which are all cell types involved in granuloma formation.
- NRP2 expression on monocytes, dendritic cells and Tregs can be detected on key immune cells known to play an important role in inflammation and granuloma formation. These findings highlight the potential of ATYR1923 to act on various immune cells directly related to the pathology of the target patient population.