Neuropilin-2 as a Therapeutic Target in Osteosarcoma: Spatial Transcriptomics and In Vivo Testing of a Clinic-Ready Anti-Neuropilin-2 Antibody

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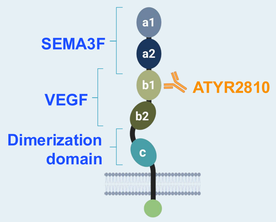
BACKGROUND

- **Osteosarcoma (OS)** is the most common primary malignant bone tumor in children and young adults.
- **Pulmonary metastasis** is the major cause of poor outcomes in patients with OS.
- **Neuropilin-2 (NRP2),** a transmembrane co-receptor, has been implicated in tumor angiogenesis and metastasis.



Human NRP2 protein domains and binding sites of NRP2 ligands and ATYR2810

- NRP2 inhibition reduces OS tumor growth and lung metastasis in preclinical models. Recently, a clinic-ready fully humanized NRP2 antibody has been developed. The purpose of this study is to further **explore its effect in the context of OS.**

METHODS

In Vitro studies:

- ATYR2810 binding specificity for human NRP2 versus NRP1 was assessed by ELISA using a human IgG4 isotype control. Binding kinetics and affinity were measured by surface plasmon resonance (SPR).

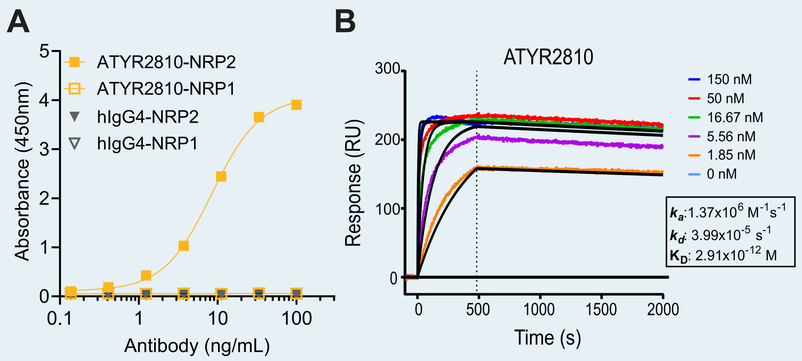
In Vivo Studies:

- **Spatial transcriptomics**
  - Paired primary OS tumors and lung metastases from two Rb1/Trp53 double-knockout mice were analyzed using the Visium spatial transcriptomics platform (10X Genomics). Reference-based deconvolution estimated cell-type proportions within individual capture spots and linear regression assessed associations between NRP2 expression and estimated cell populations.
- **Therapeutic targeting of NRP2**
  - **Xenograft models:** Human 143B OS cells were implanted into tibiae of **immunodeficient** SCID mice. ATYR2810 (10 mg/kg, intraperitoneal) or PBS was administered twice weekly from day 7 (n=5 per group). The patient derived B-265 tumor was orthotopically implanted and treated using the same protocol, starting on day 14.
  - **Syngeneic model:** Murine F420 cells were implanted into the tibiae of **immunocompetent** C57BL/6 mice. ATYR28v4, anti-mouse NRP2 antibody (n=5) or PBS (n=6) was administered twice weekly from day 7. Treatment ended on day 25 and was followed by continued monitoring.

Outcome assessment: Primary tumor growth was monitored using caliper measurements. At the experimental endpoint, lungs were harvested and stained with Bouin's solution to visualize and count lung surface nodules.

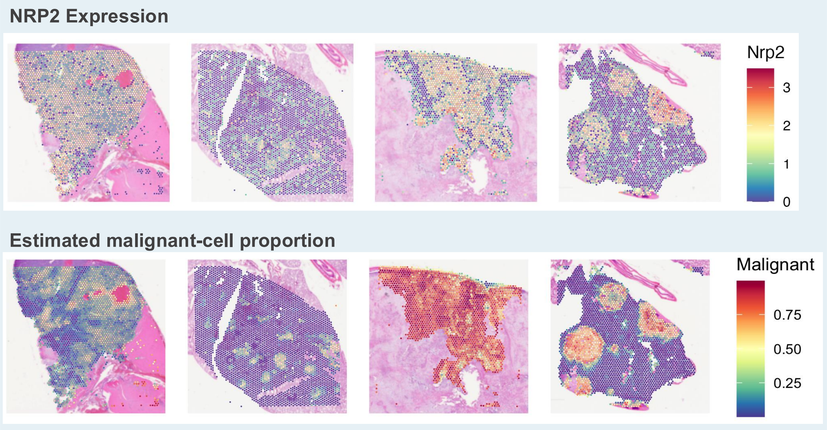
RESULTS

Figure 1: ATYR2810 specifically binds human NRP2 with picomolar affinity



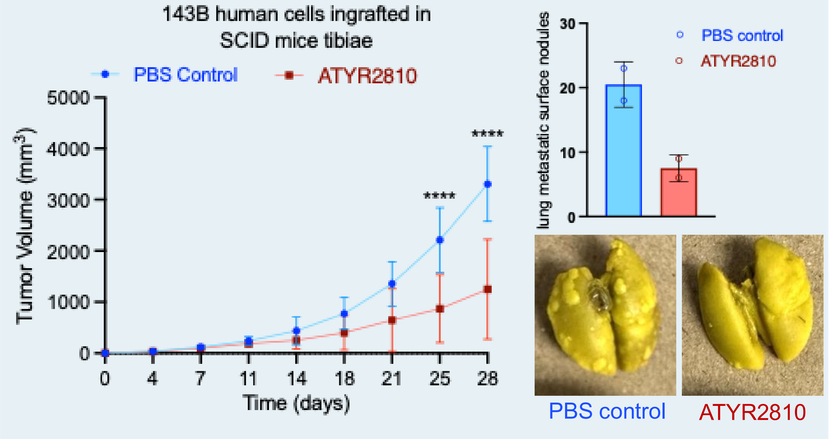
A. Binding of ATYR2810 to extracellular domains (ECD) of human NRP2, but not to ECD of the homolog NRP1, as demonstrated by ELISA. hIgG4 serves as the isotype control of ATYR2810. **B.** Representative SPR sensorgram measuring ATYR2810 binding kinetics to human NRP2 ECD. Mean K_D = 27.1 ± 1.8 pM (n = 14 independent runs; k_a, on rate; k_d, off rate; K_D, dissociation constant)

Figure 2: Spatial distribution of Nrp2 expression and malignant cells in primary OS and lung metastases in mice



Spatial transcriptomics revealed heterogeneous Nrp2 expression in primary tumors and lung metastases from two mice. Regions with higher Nrp2 expression visually overlapped with areas of higher estimated malignant-cell proportions, particularly in Mouse 2. These patterns suggest a spatial association between Nrp2 expression and malignant-cell enrichment.

Figure 3: ATYR2810 treatment reduces OS primary tumor growth and the number of lung nodules in orthotopic model using human OS cell line



RESULTS

Figure 4: ATYR2810 treatment reduces OS primary tumor growth and number of lung nodules in PDX model

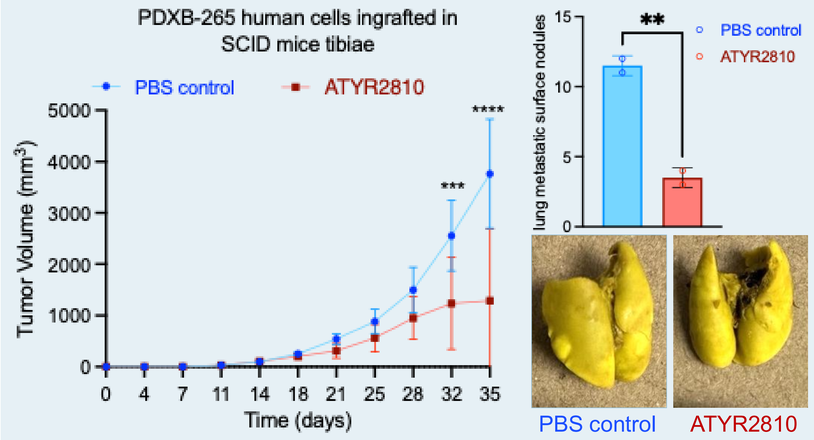
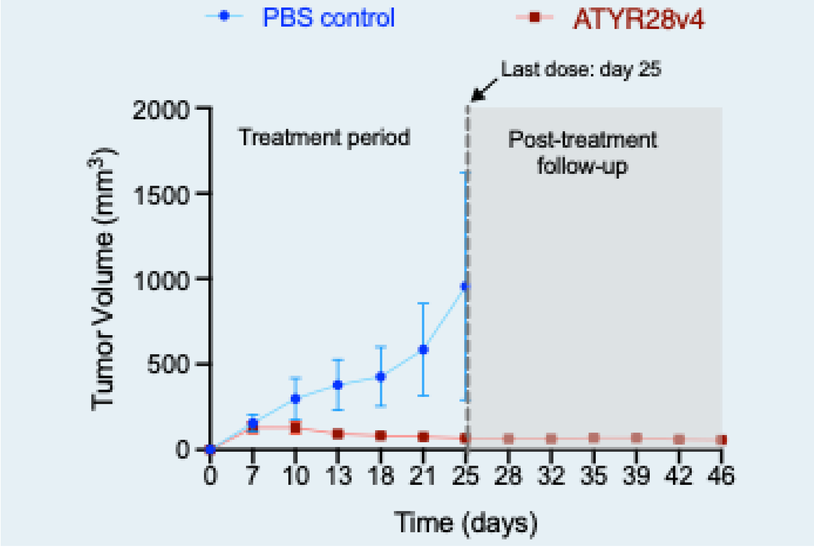


Figure 5: ATYR28v4 Treatment shows no tumor growth during post-treatment follow-up in immunocompetent mice



CONCLUSION

- NRP2 inhibition with ATYR2810 was associated with **reduced tumor growth and metastasis** in preclinical OS models, with no overt toxicity observed.
- ATYR2810 demonstrated **high specificity** and **strong affinity** to NRP2.
- Spatial transcriptomic identified **greater Nrp2 expression in more advanced disease**, suggesting it plays a role in tumorigenesis.
- Within an immunocompetent model, **effect appears to be sustained even after treatment is discontinued.**
- These findings provide a strong rationale for advancing ATYR2810 into **clinical trials** for OS patients.

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