ATYR2810, a Neuropilin-2 antibody, selectively blocks the NRP2/VEGFR signaling axis and sensitizes aggressive cancers to chemotherapy

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Abstract

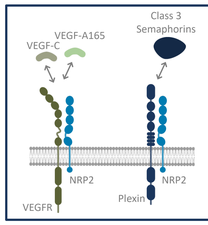
INTRODUCTION: Neuropilin-2 (NRP2) is a cell surface receptor that can interact with multiple ligands and co-receptors to influence their functional roles, among which are vascular endothelial growth factors (VEGFs). High NRP2 expression or concurrent NRP2 and VEGF expression is associated with worse outcomes in many forms of cancer. For example, a growing body of evidence has shown that the increased expression of NRP2 is associated with aggressive breast tumors, including triple-negative breast cancer (TNBC), and that NRP2/VEGF signaling contributes to chemotherapy resistance and tumor recurrence. However, a major challenge to exploit NRP2 as a therapeutic target against cancer has been the lack of highly selective anti-human NRP2 monoclonal antibodies (mAbs) that specifically block NRP2/VEGF signaling. aTyr Pharma has generated a panel of high quality, high affinity, NRP2 mAbs that are being developed for the clinical management of diseases that involve NRP2 signaling, including aggressive solid tumors.

RESULTS: We have characterized the aTyr-generated NRP2 mAbs in binding, ligand blocking and epitope mapping studies. A specific anti-human NRP2 mAb, ATYR2810, was shown to bind to the b1 domain of NRP2, which encompasses the VEGF binding site. Mutational studies indicated that ATYR2810 binds to the opening of the VEGF binding pocket, thus blocking ligand access. ATYR2810 selectively blocked VEGF-induced dimerization of NRP2 with VEGF receptors, but did not affect Semaphorin 3F (Sema3F)-induced dimerization of NRP2 and PlexinA1, demonstrating the specificity of ATYR2810 for blocking the NRP2/VEGF pathway.

In certain *in vitro* and *in vivo* cancer models, ATYR2810 administered in combination with widely used anti-cancer therapeutics, including chemotherapy, increased the anti-tumor effects of the therapeutic agents. To identify biomarkers that can predict the tumor response to ATYR2810, we further characterized TNBC cell lines and patient-derived organoids (PDOs) that were responsive or non-responsive to ATYR2810 treatment *in vitro*, as well as TNBC cell line xenografts and TNBC patient-derived xenografts (PDXs) *in vivo*. In some responsive TNBC models, ATYR2810 down-regulated a number of critical epithelial-mesenchymal transition (EMT) and cancer stem cells (CSC) genes, which may be a mechanism that mediates its anti-tumor effects.

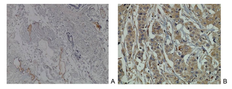
CONCLUSIONS: These results demonstrate the selectivity of ATYR2810 in blocking NRP2/VEGF signaling, and the efficacy of ATYR2810 in combination with anti-cancer therapeutics in *in vitro* and *in vivo* cancer models. The data suggest that in certain tumors, ATYR2810, by blocking NRP2 function, may inhibit EMT and CSC properties thereby rendering the tumors more sensitive to the treatment regimens. Therefore, the targeting of NRP2/VEGF signaling by ATYR2810 may provide a potential therapeutic option with improved efficacy in aggressive cancers.

Introduction



- NRP2 is a multi-functional receptor that exerts its cellular effects by dimerizing with co-receptors, the most well characterized of which are VEGFRs and Plexins, upon binding of ligands such as VEGF and Class 3 Semaphorins.
- High expression of NRP2 has been correlated with poor prognosis in several cancer types, including TNBCs [Ref1].
- NRP2/VEGF signaling has been shown to promote stem-like traits in breast cancer cells, and is associated with proliferation and survival of CSCs [Ref2].
- NRP2 expression is enriched in TNBC PDOs that are chemo-resistant as compared to patient-derived organoids that are chemo-sensitive [Ref3].

NRP2 expression in breast carcinoma tissue

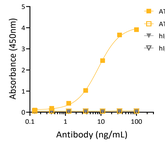


Normal breast epithelium | Breast carcinoma tissue

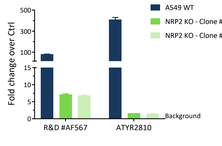
Results

Figure 1: ATYR2810 is a specific and high affinity antibody against NRP2

A **ATYR2810 binding by ELISA**

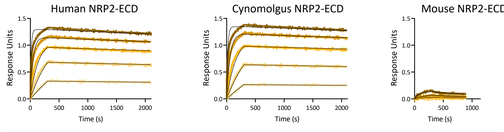


B **Binding to A549 WT or NRP2 KO cells**



- ATYR2810 binds to recombinant human NRP2 extracellular domain (ECD), but not to the homologous NRP1-ECD in ELISA.
- ATYR2810 binds to A549 wild-type (WT) cells that express endogenous NRP2, but not to NRP2 knockout (KO) A549 clonal cells. ATYR2810 shows better sensitivity and specificity than a commercial a-NRP2 antibody (R&D #AF567).

C **ATYR2810 binding affinities measured by Octet BLI**

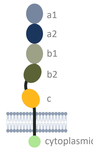


	Human NRP2-ECD	Cyno NRP2-ECD	Mouse NRP2-ECD
k_a ($M^{-1}s^{-1}$)	$4.39x10^5$	$3.70x10^5$	$8.44x10^4$
k_d (s^{-1})	$3.70x10^{-5}$	$3.05x10^{-5}$	$7.68x10^{-4}$
K_D (pM)	84	87	9099

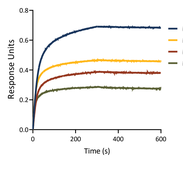
- ATYR2810 demonstrates high affinity binding against human and cynomolgus NRP2 but very weak binding to mouse NRP2.

Figure 2: ATYR2810 binds to the VEGF binding pocket of NRP2

A



B **ATYR2810 binding to recombinant NRP2 domains**



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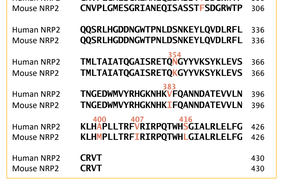
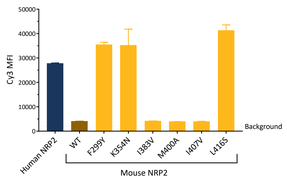


D **Binding to cells expressing WT or mutant NRP2**



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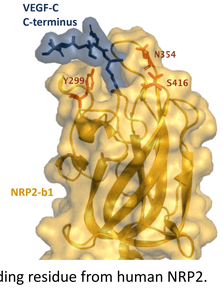
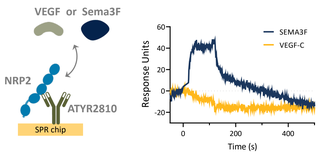


- ATYR2810 binds to the b1 domain of human NRP2, within amino acid residues 276-430.
- Mouse and human NRP2 differ by 6 amino acids in the b1 domain. Each of these residues in mouse NRP2 were individually mutated to the corresponding residue from human NRP2. Binding of ATYR2810 to these mutant mouse NRP2 proteins was assayed by flow cytometry.
- Mutation of residues 299, 354, and 416 in mouse NRP2 to their human counterparts resulted in a gain of ATYR2810 binding towards the mutant mouse NRP2. Mutation of residues 383, 400 and 407 had no effect.
- Based on previous structural studies[Ref4], these three amino acids surround a binding pocket for the C-terminal tail of VEGF. Binding of ATYR2810 at this location is likely to block binding of VEGF to NRP2.

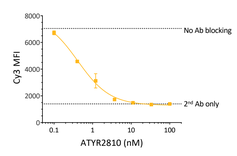
Results

Figure 3: ATYR2810 selectively blocks dimerization of NRP2 with VEGFR

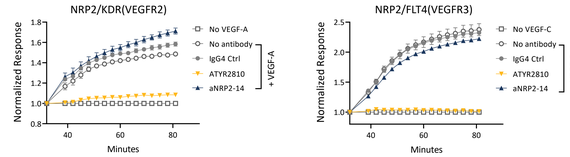
A **Ligand binding following ATYR2810 binding to NRP2**



B **VEGF-C binding to cell surface NRP2**

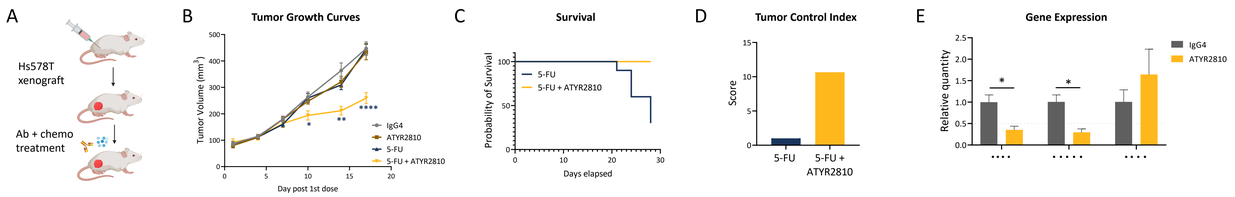


C **VEGF-induced NRP2/VEGFR receptor heterodimerization**



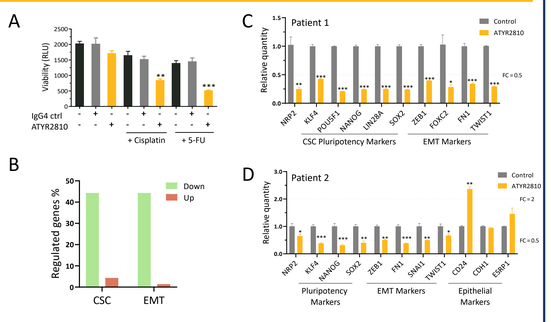
- ATYR2810 blocks VEGF-C binding to NRP2-ECD, but does not block Sema3F binding to NRP2-ECD.
- ATYR2810 entirely blocks VEGF-C binding to Expi293 cells overexpressing NRP2 on the cell surface.
- ATYR2810 completely blocks VEGF-A induced NRP2/KDR (VEGFR2) dimerization and VEGF-C induced NRP2/FLT4 (VEGFR3) dimerization. Blocking in these assays is not observed with a Sema3F-blocking antibody (aNRP2-14). ATYR2810 does not have any effect on Sema3F induced NRP2/PlexinA1 dimerization [Ref5].

Figure 4: ATYR2810 in combination with chemotherapy enhances *in vivo* efficacy in Hs578T TNBC xenograft model



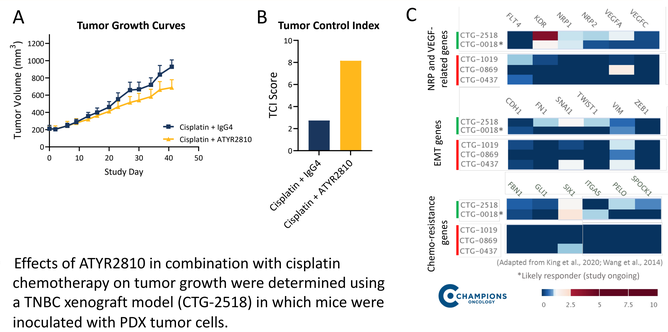
- Effects of ATYR2810 in combination with 5-fluorouracil (5-FU) chemotherapy on tumor growth were determined in a TNBC Hs578T xenograft model.
- ATYR2810 + 5-FU combination therapy enhanced efficacy in tumor growth inhibition and improved survival compared to 5-FU monotherapy in this model.
- ATYR2810 down-regulated gene expression of EMT (ZEB1) and CSC (NANOG) markers, and up-regulated epithelial (CDH1/E-cadherin) markers in tumor samples from the Hs578T xenograft model.

Figure 5: ATYR2810 down-regulates EMT and CSC markers in TNBC patient-derived organoids



- ATYR2810 enhanced chemosensitivity of TNBC patient-derived organoids.
- ATYR2810 down-regulated CSC and EMT markers in TNBC patient-derived organoids. 80 genes in each of CSC and EMT pathways were analyzed by Fluidigm, and nearly half of markers with detectable expression levels were down-regulated >2-fold by ATYR2810 compared to control.
- ATYR2810 down-regulated a number of critical CSC pluripotency markers and EMT markers in organoids derived from both TNBC Patient 1 and Patient 2, and up-regulated certain epithelial markers (e.g. CD24) in organoids derived from TNBC Patient 2.

Figure 6: ATYR2810 in combination with chemotherapy enhances efficacy in TNBC patient-derived xenografts



- Effects of ATYR2810 in combination with cisplatin chemotherapy on tumor growth were determined using a TNBC xenograft model (CTG-2518) in which mice were inoculated with PDX tumor cells.
- ATYR2810 + cisplatin combination therapy (TCI score of 8.2) enhanced tumor growth inhibition and led to a 3-fold increase in tumor control as compared to cisplatin monotherapy (TCI score of 2.7).
- Prior to treatment, the responsive TNBC PDX models (CTG-2518 and CTG-0018) showed increased expression of several NRP and VEGF-related genes as well as several EMT and chemo-resistance genes as compared to non-responsive models (CTG-1019, CTG-0869 and CTG-0473), suggesting that tumors with high expression of these genes may be responsive to ATYR2810 combination treatment.

Conclusions

- ATYR2810 is a highly specific and high affinity antibody against human NRP2, and binds to the b1 domain of NRP2 in a manner than blocks VEGF ligand binding.
- ATYR2810 selectively blocks VEGF-induced dimerization of NRP2 with VEGFR, while not affecting Sema3F-induced dimerization of NRP2 with PlexinA1.
- In combination with chemotherapeutics, ATYR2810 displays enhanced efficacy in TNBC xenograft models as compared to the chemotherapy alone.
- Gene expression data from TNBC xenograft samples and TNBC PDOs reveal down-regulation of several CSC and EMT markers following ATYR2810 treatment.
- ATYR2810 blocking of the NRP2/VEGF signaling pathway may sensitize chemo-resistant TNBC models to anti-cancer therapeutics.

References

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