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A domain-specific antibody to NRP2 down-regulated epithelial-mesenchymal transition genes and enhanced efficacy of standard-of-care therapeutics for aggressive breast cancer

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Abstract

INTRODUCTION: A growing body of evidence suggests that increased expression of the vascular endothelial growth factor (VEGF) co-receptor Neuropilin-2 (NRP2) is associated with aggressive breast cancers and that VEGF/NRP2 signaling contributes to clinical resistance to chemotherapy and tumor recurrence, making NRP2 a promising therapeutic target. A major limitation that has hampered the development of such a therapy, however, has been the lack of availability of high-quality anti-human NRP2 monoclonal antibodies (mAbs) that specifically block VEGF/NRP2 signaling.

aTyr has generated a panel of high-quality, anti-human NRP2 mAbs that have the potential to be developed for the clinical management of diseases that involve NRP2 signaling. Among them, ATYR2810 has been characterized to bind to the b1 domain of NRP2 that encompasses the VEGF binding sites. It completely blocks the binding of VEGF to NRP2, and VEGF-induced NRP2/VEGFR dimerization. Importantly, ATYR2810 has no effect on Semaphorin 3F (Sema3F) induced NRP2/PlexinA1 dimerization, demonstrating its specificity for blocking the VEGF/NRP2 pathway. We have previously shown that ATYR2810, but not a Sema3F-blocking mAb has tumor-inhibitory effects on triple negative breast cancer (TNBC) cell lines or patient-derived organoids.

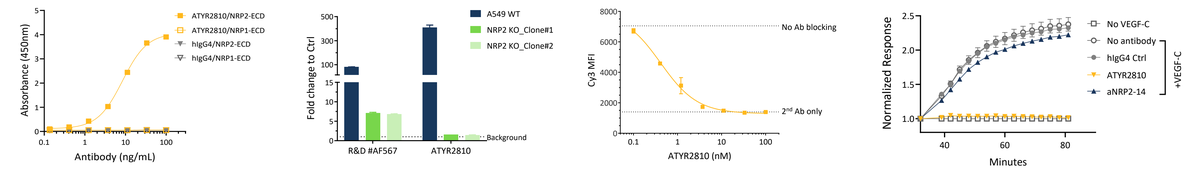
We subsequently further evaluated the efficacy of ATYR2810 in combination with standard-of-care anti-cancer therapeutics including Cisplatin and Bevacizumab (anti-VEGF-A blocking antibody).

RESULTS: In *in vitro* 3D methylcellulose colony formation assays, ATYR2810 sensitized TNBC cells to Cisplatin or Bevacizumab and considerably reduced colony formation in combination with these treatments. In an *in vivo* TNBC xenograft cancer model (MDA-MB-231), ATYR2810 augmented the anti-tumor effects of Cisplatin or Bevacizumab. To explore the underlying molecular mechanism, we performed gene expression profiling with samples treated by ATYR2810 alone and the combo therapy with Cisplatin or Bevacizumab. A number of gene markers of cancer stem cells (CSC) and/or epithelial-mesenchymal transition (EMT) were found to be down-regulated by ATYR2810 treatment in TNBC patient-derived organoids, including a key EMT transcription factor ZEB1. We also confirmed the reduction of ZEB1 protein expression by ATYR2810 treatment in TNBC cells.

CONCLUSIONS: These results demonstrate the efficacy of ATYR2810 in combination with anti-cancer therapeutics in *in vitro* and *in vivo* TNBC models, and suggest its activity is mediated through inhibiting both EMT and cellular dedifferentiation that renders tumors more sensitive to the treatment regimes. The targeting of VEGF/NRP2 signaling by ATYR2810 may provide a new therapeutic option, and lead to the identification of new treatment biomarkers, which could offer improved efficacy and reduced toxicity in aggressive breast cancers.

Introduction

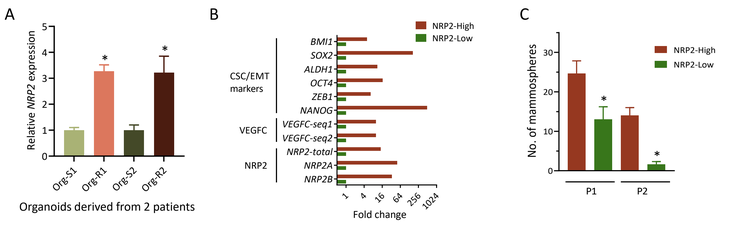
Figure 1. ATYR2810 specifically binds NRP2 and selectively blocks VEGF binding and VEGF-induced receptor dimerization



- ATYR2810 selectively binds to the recombinant NRP2 extracellular domain (ECD), but not to the homolog NRP1-ECD in ELISA (Fig. 1A)
- ATYR2810 specifically binds to A549 wildtype (WT) cells that express endogenous NRP2, but not to NRP2 knockout (KO) A549 clonal cells. ATYR2810 shows better sensitivity and specificity than commercial a-NRP2 antibodies such as R&D #AF567 (Fig. 1B).
- ATYR2810 entirely blocks VEGF-C binding to Expi293 cells overexpressing NRP2 on the cell surface (Fig. 1C).
- ATYR2810 completely blocks VEGF-C induced NRP2/FLT4 (VEGFR3) dimerization, which is not observed with a Sema3F-blocking antibody (aNRP2-14) (Fig. 1D). ATYR2810 shows similar blocking effect on VEGF-A induced NRP2/KDR (VEGFR2) dimerization, but not on Sema3F induced NRP2/PlexinA1 dimerization [Ref 1].

Results

Figure 2. Chemo-resistant TNBC organoids are NRP2-enriched, and NRP2-high TNBC cells have higher expressions of CSC/EMT markers



- In TNBC patient-derived organoids, *NRP2* gene expression is enriched in cisplatin-resistant (R) organoids compared to those sensitive (S) to cisplatin (Fig. 2A).
- TNBC BT-549 cells were sorted as NRP2-high and NRP2-low populations. The NRP2-high cells showed much higher expressions of CSC and EMT marker genes (Fig. 2B), and formed more mammospheres than NRP2-low cells (Fig. 2C)
- VEGF-NRP2 signaling has been shown to promote stem-like traits in breast cancer cells [Ref 2-4]. The current results further support its involvement in EMT and chemoresistance.

Statistical analysis: Fig. 2A, 2C, 4D, 5E by Student's *t* test, Fig. 3A, 3B by One-way ANOVA, Fig. 5B, 5C, 5F by Two-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significantly different from the respective control.

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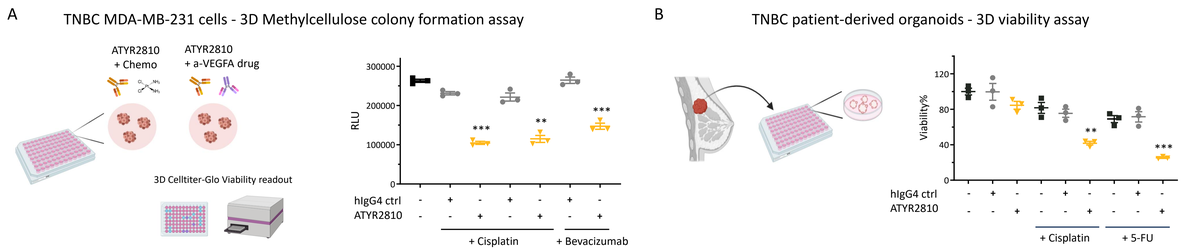
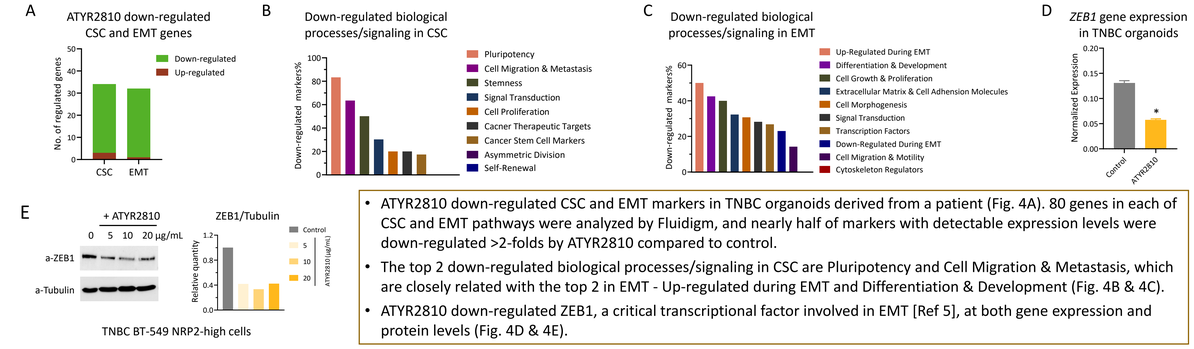
Figure 3. ATYR2810 sensitized TNBC organoids and cells to chemo or anti-VEGFA cancer therapeutics *in vitro*



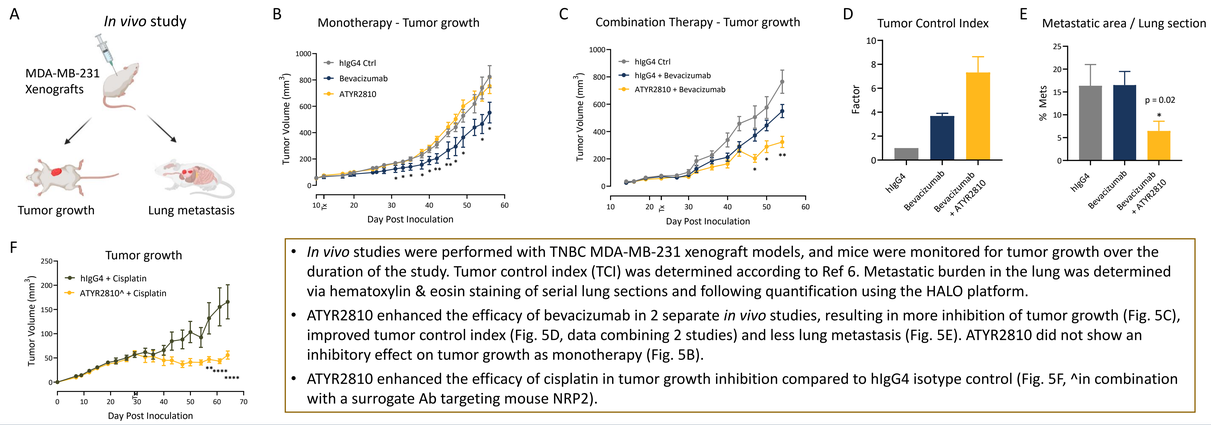
- ATYR2810 sensitized TNBC MDA-MB-231 cells to treatment by cisplatin or the a-VEGFA drug (bevacizumab) in the 3D methylcellulose colony formation assay.
- ATYR2810 sensitized TNBC patient-derived organoids to the treatment by chemo drugs cisplatin or 5-FU in the 3D viability assay.

Figure 4. Mechanism-of-action explorations for ATYR2810 efficacy in TNBC organoids and cells



- ATYR2810 down-regulated CSC and EMT markers in TNBC organoids derived from a patient (Fig. 4A). 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-folds by ATYR2810 compared to control.
- The top 2 down-regulated biological processes/signaling in CSC are Pluripotency and Cell Migration & Metastasis, which are closely related with the top 2 in EMT - Up-regulated during EMT and Differentiation & Development (Fig. 4B & 4C).
- ATYR2810 down-regulated ZEB1, a critical transcriptional factor involved in EMT [Ref 5], at both gene expression and protein levels (Fig. 4D & 4E).

Figure 5. ATYR2810 enhanced efficacy of anti-VEGFA or chemo therapeutics in *in vivo* TNBC models



- *In vivo* studies were performed with TNBC MDA-MB-231 xenograft models, and mice were monitored for tumor growth over the duration of the study. Tumor control index (TCI) was determined according to Ref 6. Metastatic burden in the lung was determined via hematoxylin & eosin staining of serial lung sections and following quantification using the HALO platform.
- ATYR2810 enhanced the efficacy of bevacizumab in 2 separate *in vivo* studies, resulting in more inhibition of tumor growth (Fig. 5C), improved tumor control index (Fig. 5D, data combining 2 studies) and less lung metastasis (Fig. 5E). ATYR2810 did not show an inhibitory effect on tumor growth as monotherapy (Fig. 5B).
- ATYR2810 enhanced the efficacy of cisplatin in tumor growth inhibition compared to hIgG4 isotype control (Fig. 5F, ^in combination with a surrogate Ab targeting mouse NRP2).

Conclusions

- **ATYR2810 specifically binds NRP2, blocks VEGF binding to NRP2, and selectively inhibits VEGF-induced NRP2/VEGFR dimerization.**
- **ATYR2810 sensitized TNBC organoids and cells to the treatment by chemo or a-VEGFA cancer therapeutics *in vitro.***
- **ATYR2810 down-regulated CSC and EMT markers in TNBC organoids, including ZEB1 and markers of pluripotency.**
- **ATYR2810 enhanced the efficacy of a-VEGFA or chemo therapeutics in *in vivo* TNBC models, suggesting the targeting of VEGF/NRP2 signaling by ATYR2810 may provide a new therapeutic option with improved efficacy in aggressive breast cancers.**

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