LB085

ATYR2810, a fully humanized monoclonal antibody targeting the VEGF-NRP2 pathway sensitizes highly aggressive and chemoresistant TNBC subtypes to chemotherapy

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Abstract

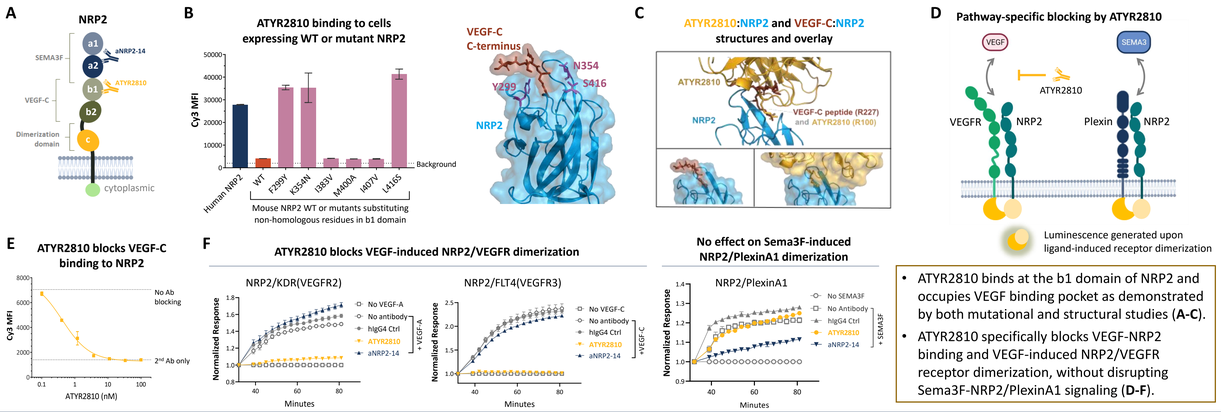
INTRODUCTION: Triple negative breast cancer (TNBC) represents 15% of all breast cancers[1], and is a notably aggressive subtype that is prone to early recurrence following initial diagnosis[2] with a median survival of only 9 months following recurrence. One of the few treatment options for patients with advanced and metastatic TNBC is platinum-based chemotherapy. However, not all tumors respond and those that do often become refractory during the course of treatment[3]. The cell surface receptor Neuropilin-2 (NRP2), which acts as a co-receptor for vascular endothelial growth factors (VEGFs), has been shown to be both highly expressed and associated with therapeutic resistance in TNBC[4]. NRP2 also serves as a co-receptor for semaphorins, which may play tumor-suppressor functions in breast cancer. We have previously demonstrated that ATYR2810, a highly specific humanized monoclonal antibody which effectively blocks NRP2/VEGF signaling without disrupting NRP2/Semaphorin 3F signaling, down-regulated cancer stem cell (CSC) and epithelial-mesenchymal transition (EMT) genes such as *ZEB1* and sensitizes TNBC to chemotherapy both *in vitro* and *in vivo*[5].

RESULTS: In this study we have further characterized the breast cancer subtypes that are most responsive to ATYR2810 treatment. Using *in vitro* 3D colony formation assays (CFAs), we have interrogated 14 breast cancer cell lines covering luminal, HER2+ and TNBC, for responsiveness to ATYR2810 treatment in combination with chemotherapy. The responsive cell lines were associated with the more mesenchymal TNBC with enriched NRP2 expression. Additionally, we screened patient derived xenografts (PDXs) and again found that the responsive PDXs were associated with the more aggressive TNBC. To gain insight into the potential mechanism for enhanced chemosensitivity by ATYR2810 treatment, we performed RNAseq on the PDX tumors. Interestingly, the chemokine receptor *CXCR4*, which is known to promote drug resistance and metastatic potential, was found to be significantly downregulated in responder PDX tumors treated with ATYR2810 in combination with cisplatin as compared to those treated with cisplatin alone. Further, gene set enrichment analysis (GSEA) showed that *CXCR4* was found in numerous downregulated pathways enriched in the PDX responder tumors treated with ATYR2810/cisplatin, suggesting that *CXCR4* may be driving the responder phenotype of the PDXs. Flow cytometry analysis of TNBC cells treated with ATYR2810 monotherapy *in vitro* resulted in a reduced frequency of cells expressing *CXCR4*. Furthermore, ATYR2810 monotherapy inhibited spontaneous lung metastasis in experimental models of mesenchymal TNBC.

CONCLUSIONS: Our data suggest that ATYR2810 enhances chemosensitivity and reduces metastasis in highly aggressive TNBC, and that this response may be mediated through the downregulation of genes known to be associated with aggressive cancer states such as CSC/EMT genes like *ZEB1* and *CXCR4*. ATYR2810 may therefore serve as a novel therapeutic agent for the treatment of advanced and metastatic TNBC and potentially other aggressive cancer types.

Introduction

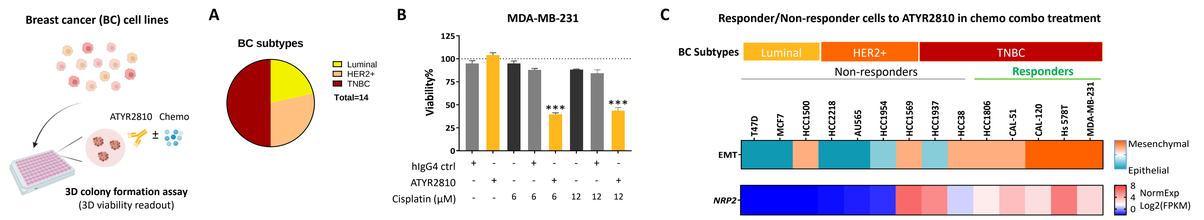
Figure 1. ATYR2810 specifically blocks VEGF-NRP2 binding and VEGF-induced NRP2/VEGFR receptor dimerization



- ATYR2810 binds at the b1 domain of NRP2 and occupies VEGF binding pocket as demonstrated by both mutational and structural studies (**A-C**).
- ATYR2810 specifically blocks VEGF-NRP2 binding and VEGF-induced NRP2/VEGFR receptor dimerization, without disrupting Sema3F-NRP2/PlexinA1 signaling (**D-F**).

Results

Figure 2. ATYR2810 enhances chemosensitivity of more mesenchymal TNBC cells with enriched NRP2 expression



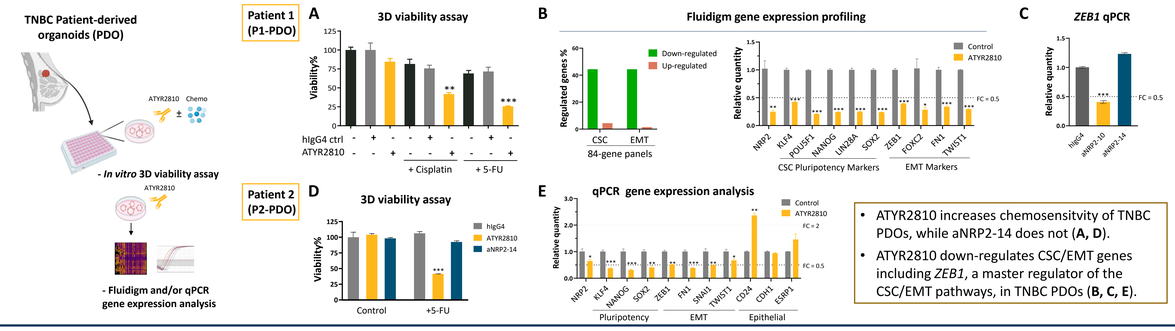
- Screening of 14 cell lines representing different subtypes of breast cancer **(A)** in 3D methylcellulose colony formation assay (CFA) demonstrates that ATYR2810 increases chemosensitivity of more mesenchymal TNBC cells with enriched NRP2 expression (**B**: Representative CFA data, **C**: EMT color scheme based on EMT scores by Le et al.[6], *NRP2* expression from CCLE database).

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

References:

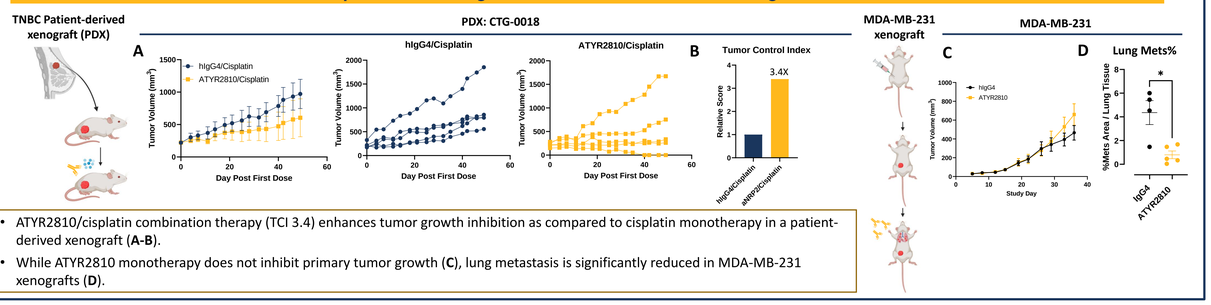
1. Qiu, J. et al., Comparison of Clinicopathological Features and Prognosis in Triple-Negative and Non-Triple Negative Breast Cancer. J Cancer 7, 167-73 doi: 10.7150/jca.10944 (2016).
2. Harbeck, N., et al. Breast cancer. *Nature Reviews Disease Primers* (Vol. 5, Issue 1). doi.org/10.1038/s41572-019-0111-2 (2019).
3. Marra, A., et al. Practical classification of triple-negative breast cancer: intratumoral heterogeneity, mechanisms of drug resistance, and novel therapies. *Npj Breast Cancer*, *6*(1), 1–16. doi.org/10.1038/s41523-020-00197-2 (2020).
4. Goel, H. L. & Mercurio, A. M. VEGF Targets The Tumour Cell. Nat Rev Cancer 13, 871-882, doi:10.1038/nrc3627 (2013).
5. Xu Z et al. A domain-specific antibody to NRP2 down-regulated epithelial-mesenchymal transition genes and enhanced efficacy of standard-of-care therapeutics for aggressive breast cancer. 2021 AACR Annual Meeting (Poster #LB095).
6. Le. A. V-P et al. DNA Methylation Profiling of Breast Cancer Cell Lines along the Epithelial Mesenchymal Spectrum-Implications for the Choice of Circulating Tumour DNA Methylation Markers. Int J Mol Sci . 2018 Aug 28;19(9):2553.

Figure 3. ATYR2810 increases chemosensitivity of TNBC patient-derived organoids and down-regulates key CSC/EMT genes



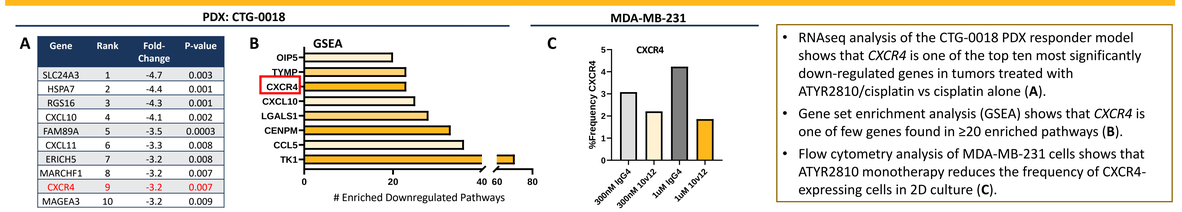
- ATYR2810 increases chemosensitivity of TNBC PDOs, while aNRP2-14 does not (**A, D**).
- ATYR2810 down-regulates CSC/EMT genes including *ZEB1*, a master regulator of the CSC/EMT pathways, in TNBC PDOs (**B, C, E**).

Figure 4. ATYR2810 enhances chemosensitivity of TNBC patient-derived xenografts (PDX) and reduces spontaneous lung metastasis in MDA-MB-231 xenograft tumor model



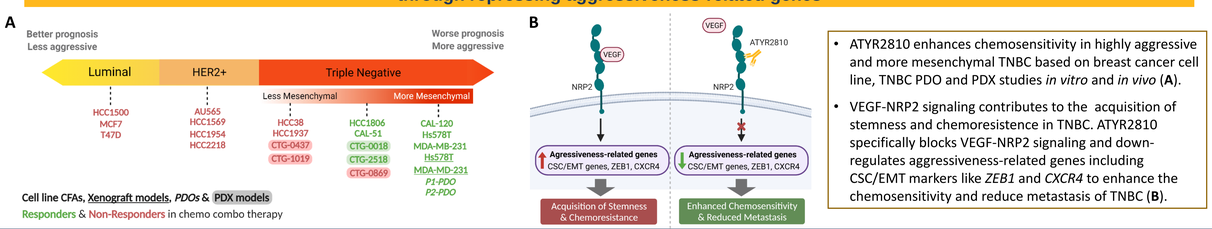
- ATYR2810/cisplatin combination therapy (TCI 3.4) enhances tumor growth inhibition as compared to cisplatin monotherapy in a patient-derived xenograft (**A-B**).
- While ATYR2810 monotherapy does not inhibit primary tumor growth (**C**), lung metastasis is significantly reduced in MDA-MB-231 xenografts (**D**).

Figure 5. ATYR2810 downregulates CXCR4 in TNBC PDX model and cells



- RNAseq analysis of the CTG-0018 PDX responder model shows that *CXCR4* is one of the top ten most significantly down-regulated genes in tumors treated with ATYR2810/cisplatin vs cisplatin alone (**A**).
- Gene set enrichment analysis (GSEA) shows that *CXCR4* is one of few genes found in ≥20 enriched pathways (**B**).
- Flow cytometry analysis of MDA-MB-231 cells shows that ATYR2810 monotherapy reduces the frequency of CXCR4-expressing cells in 2D culture (**C**).

Figure 6. ATYR2810 enhances chemosensitivity and reduces metastasis in highly aggressive TNBC through repressing aggressiveness-related genes



- ATYR2810 enhances chemosensitivity in highly aggressive and more mesenchymal TNBC based on breast cancer cell line, TNBC PDO and PDX studies *in vitro* and *in vivo* (**A**).
- VEGF-NRP2 signaling contributes to the acquisition of stemness and chemoresistence in TNBC. ATYR2810 specifically blocks VEGF-NRP2 signaling and down-regulates aggressiveness-related genes including CSC/EMT markers like *ZEB1* and *CXCR4* to enhance the chemosensitivity and reduce metastasis of TNBC (**B**).

Conclusions

- **A fully humanized anti-NRP2 antibody, ATYR2810, specifically blocks VEGF-NRP2 binding and VEGF-induced NRP2/VEGFR dimerization.**
- **ATYR2810 enhances chemosensitivity of more mesenchymal TNBC cells with enriched NRP2 expression.**
- **ATYR2810 increases chemosensitivity of TNBC patient-derived organoids (PDOs) *in vitro* and patient-derived xenografts (PDXs) *in vivo.***
- **ATYR2810 monotherapy reduces spontaneous lung metastasis in the TNBC MDA-MB-231 xenograft tumor model.**
- **ATYR2810 enhances chemosensitivity and reduces metastasis in highly aggressive TNBC subtypes by repressing aggressiveness-related genes, such as CSC/EMT pathway genes including the master regulator *ZEB1*, and *CXCR4* which is associated with drug resistance and metastatic potential.**

Funding support: This work was supported by aTyr Pharma, Inc.