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Resistance to cancer therapy via upregulation of the NRP2/VEGF-C axis can be neutralized by ATYR2810

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Abstract

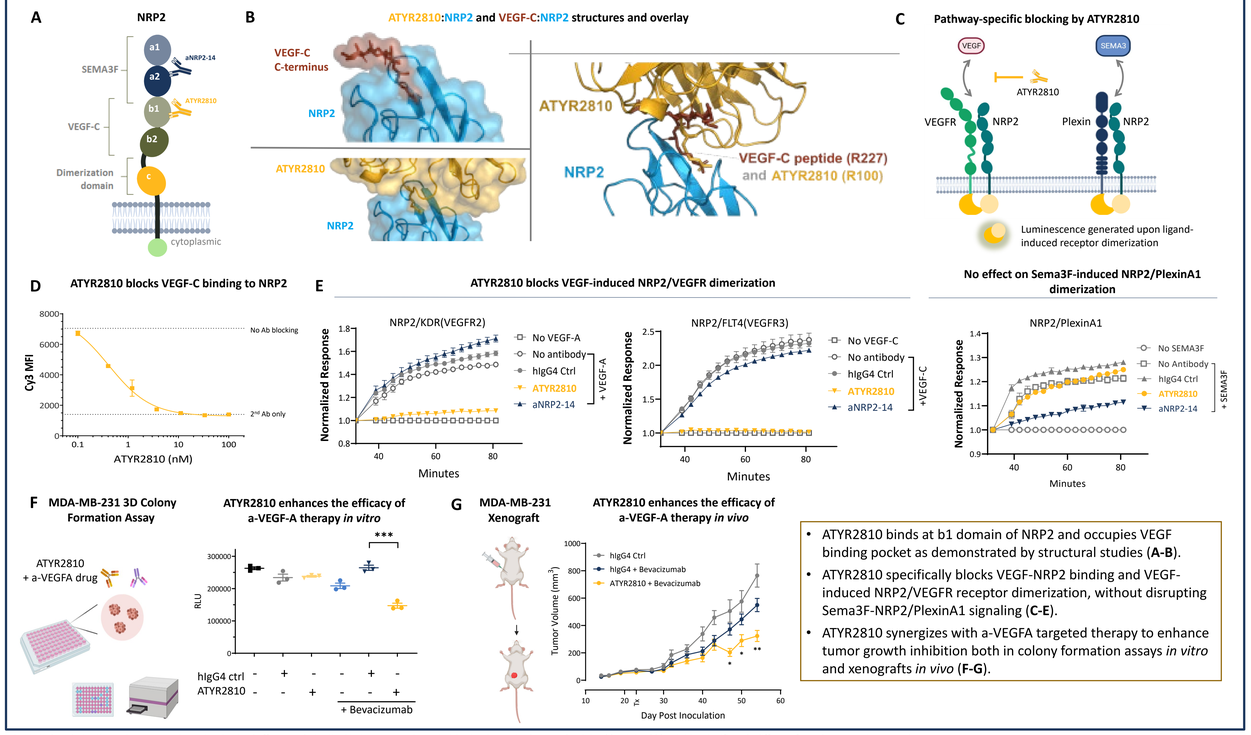
INTRODUCTION: Intrinsic or acquired resistance to cancer therapy is a major problem faced by many current cancer therapeutics. Therapy-resistant cancer cells are able to evade the effect of cancer drugs, resulting in highly aggressive clones that can cause poor long-term prognosis. Neuropilin-2 (NRP2) is a cell surface receptor that acts as a co-receptor for vascular endothelial growth factor (VEGF) and has been shown to be associated with therapy resistance[1]. The VEGF-C signaling pathway has also been linked to chemoresistance in several cancer types including breast, bladder, and gastric cancer[2-4]. Previously we have shown that ATYR2810—a highly specific humanized monoclonal antibody that blocks NRP2/VEGF-C signaling—sensitizes triple negative breast cancer (TNBC), a highly aggressive and chemoresistant subtype of breast cancer, to chemotherapy and targeted anti-VEGF therapy both *in vitro* and *in vivo*[5-6]. In this study we examined the role of enhanced VEGF-C signaling in tumor growth and therapeutic resistance, and the ability of ATYR2810 to neutralize these effects using *in vivo* models for lung and kidney cancers.

RESULTS: We generated a stable VEGF-C overexpressing non-small cell lung cancer (NSCLC) cell line and found that tumor growth was enhanced by increased VEGF-C signaling. Importantly, ATYR2810 monotherapy counteracted this VEGF-C-driven enhanced tumor growth as tumor growth inhibition was increased in the VEGF-C overexpressing tumors compared to control tumors. Further, VEGF-C overexpression was also found to enhance resistance to platinum-based chemotherapy in this model. However, sensitivity to chemotherapy was restored when ATYR2810 was used in combination with cisplatin. In clear cell renal cell carcinoma (ccRCC), the use of sunitinib—a small molecule inhibitor that inhibits the signaling of several receptor tyrosine kinases (RTKs), including vascular endothelial growth factor receptors (VEGFRs)—has reportedly been associated with the upregulation of VEGF-C and NRP2 expression as well as increased metastasis[7]. In line with this, we found that the use of sunitinib leads to increased levels of circulating VEGF-C in the serum of ccRCC xenograft mice. The use of ATYR2810 in combination with sunitinib was found to act synergistically to inhibit tumor growth in ccRCC xenograft tumors leading to complete tumor regression in some animals.

CONCLUSIONS: Here we demonstrate that high levels of VEGF-C expression increase tumor dependency on the VEGF-C/NRP2 signaling axis thereby promoting tumor growth and chemoresistance. ATYR2810 counteracts the aggressive traits of tumors expressing high levels of VEGF-C resulting in enhanced tumor growth inhibition and sensitivity to both chemotherapy and targeted therapy.

Introduction

Figure 1. ATYR2810 blocks VEGF-NRP2 binding and synergizes with VEGF-targeting agents *in vitro* and *in vivo*

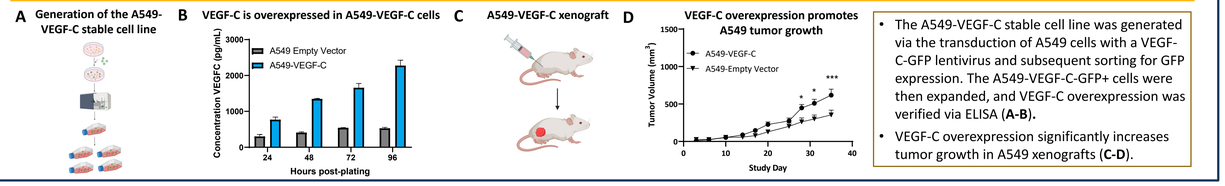


- ATYR2810 binds at b1 domain of NRP2 and occupies VEGF binding pocket as demonstrated by structural studies (**A-B**).
- ATYR2810 specifically blocks VEGF-NRP2 binding and VEGF-induced NRP2/VEGFR receptor dimerization, without disrupting Sema3F-NRP2/PlexinA1 signaling (**C-E**).
- ATYR2810 synergizes with a-VEGFA targeted therapy to enhance tumor growth inhibition both in colony formation assays *in vitro* and xenografts *in vivo* (**F-G**).

Statistical analysis: Fig. 1F One-way ANOVA; Fig 1G Mixed effects analysis; Fig. 2D Two-way ANOVA; Fig 3C, 4B, 4C Unpaired t-test *p<0.05, **p<0.01, ***p<0.001 significantly different from the respective control.

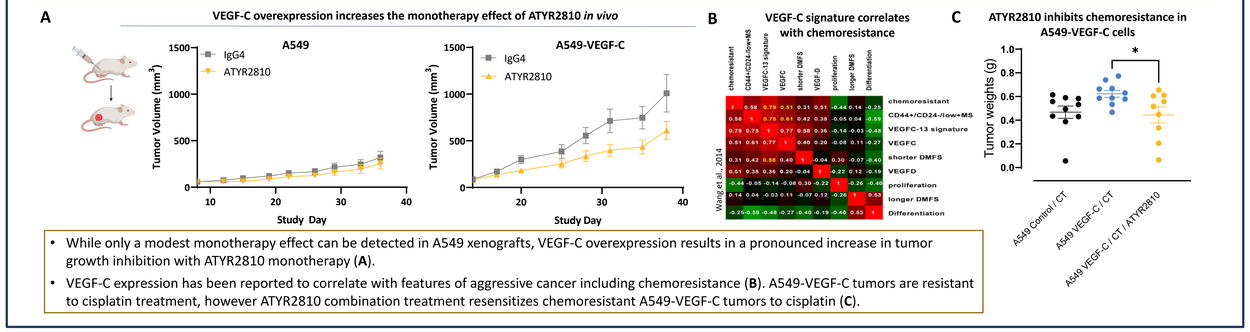
Results

Figure 2. VEGF-C stable A549 cells exhibit increased tumor growth



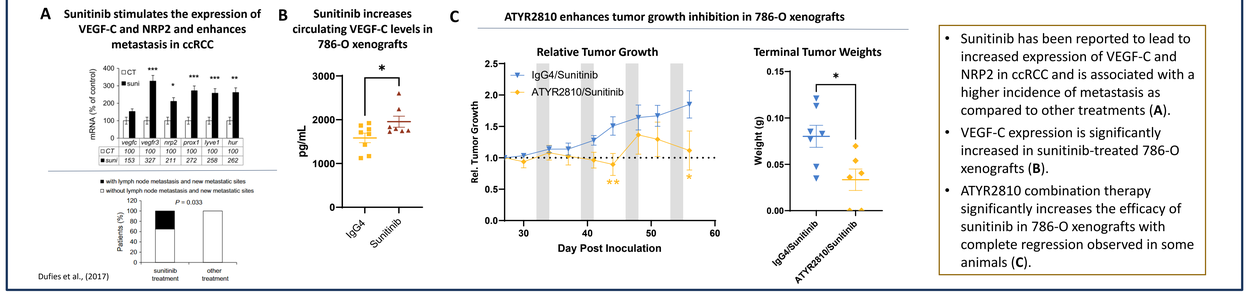
- The A549-VEGF-C stable cell line was generated via the transduction of A549 cells with a VEGF-C-GFP lentivirus and subsequent sorting for GFP expression. The A549-VEGF-C-GFP+ cells were then expanded, and VEGF-C overexpression was verified via ELISA (**A-B**).
- VEGF-C overexpression significantly increases tumor growth in A549 xenografts (**C-D**).

Figure 3. ATYR2810 enhances tumor growth inhibition in A549-VEGF-C xenografts and inhibits VEGF-C-driven chemoresistance



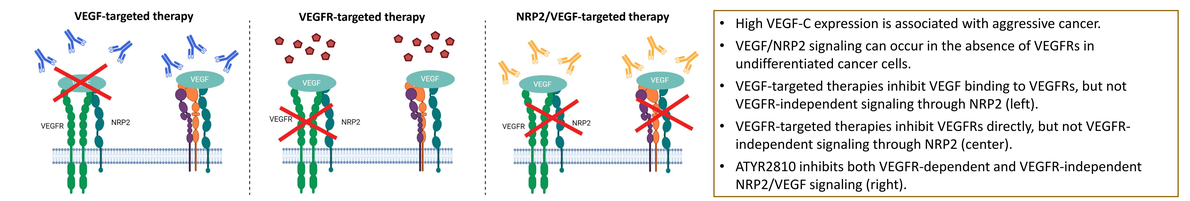
- While only a modest monotherapy effect can be detected in A549 xenografts, VEGF-C overexpression results in a pronounced increase in tumor growth inhibition with ATYR2810 monotherapy (**A**).
- VEGF-C expression has been reported to correlate with features of aggressive cancer including chemoresistance (**B**). A549-VEGF-C tumors are resistant to cisplatin treatment, however ATYR2810 combination treatment resensitizes chemoresistant A549-VEGF-C tumors to cisplatin (**C**).

Figure 4. ATYR2810 synergizes with sunitinib in the ccRCC xenograft model



- Sunitinib has been reported to lead to increased expression of VEGF-C and NRP2 in ccRCC and is associated with a higher incidence of metastasis as compared to other treatments (**A**).
- VEGF-C expression is significantly increased in sunitinib-treated 786-O xenografts (**B**).
- ATYR2810 combination therapy significantly increases the efficacy of sunitinib in 786-O xenografts with complete regression observed in some animals (**C**).

Figure 5. ATYR2810 in combination with established VEGF-targeting agents can generate complete blockade of VEGF-signaling



- High VEGF-C expression is associated with aggressive cancer.
- VEGF/NRP2 signaling can occur in the absence of VEGFRs in undifferentiated cancer cells.
- VEGF-targeted therapies inhibit VEGF binding to VEGFRs, but not VEGFR-independent signaling through NRP2 (left).
- VEGFR-targeted therapies inhibit VEGFRs directly, but not VEGFR-independent signaling through NRP2 (center).
- ATYR2810 inhibits both VEGFR-dependent and VEGFR-independent NRP2/VEGF signaling (right).

Conclusions

- **ATYR2810 specifically and functionally blocks VEGF-NRP2 binding and VEGF-induced NRP2/VEGFR dimerization.**
- **High levels of VEGF-C expression are known to be associated with key features of aggressive cancer including therapy resistance.**
- **ATYR2810 monotherapy inhibits VEGF-C-induced tumor growth.**
- **ATYR2810 synergizes with chemotherapy and anti-VEGF(R) therapies to enhance tumor growth inhibition.**

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References:

1. Islam, R., Mishra, J., Bodas, S., Bhattacharya, S., Batra, S. K., Dutta, S., & Datta, K. (2022). Role of Neuropilin-2-mediated signaling axis in cancer progression and therapy resistance. *Cancer and Metastasis Reviews*. https://doi.org/10.1007/s10555-022-10048-0
2. Wang, C. A., Harrell, J. C., Iwanaga, R., Jedlicka, P., & Ford, H. L. (2014). Vascular endothelial growth factor C promotes breast cancer progression via a novel antioxidant mechanism that involves regulation of superoxide dismutase 3. *Breast Cancer Research*, *16*(1), 1–17. https://doi.org/10.1186/s13058-014-0462-2
3. Zhu, H., Yun, F., Shi, X., & Wang, D. (2015). VEGF-C inhibition reverses resistance of bladder cancer cells to cisplatin via upregulating maspin. *Molecular Medicine Reports*, *12*(2), 3163–3169. https://doi.org/10.3892/mmr.2015.3684
4. Jun Cho, H., Kim, I. K., Park, S. M., Eun Baek, K., Nam, I. K., Park, S. H., Ryu, K. J., Choi, J., Ryu, J., Hong, S. C., Jeong, S. H., Lee, Y. J., Ko, G. H., Won Kim, J., Won Lee, C., Soo Kang, S., & Yoo, J. (2014). VEGF-C mediates RhoGDI2-induced gastric cancer cell metastasis and cisplatin resistance. *International Journal of Cancer*, *135*(7), 1553–1563. https://doi.org/10.1002/ijc.28801
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. Xu, Z. and Barber, A. et al., ATYR2810, a fully humanized monoclonal antibody targeting the VEGF-NRP2 pathway sensitizes highly aggressive and chemoresistant TNBC subtypes to chemotherapy. 2022 AACR Annual Meeting (Poster #LB085)
7. Dufies, M. et al, (2017). Sunitinib stimulates expression of VEGFC by tumor cells and promotes lymphangiogenesis in clear cell renal cell carcinomas. *Cancer Research*, *77*(5), 1212–1226. https://doi.org/10.1158/0008-5472.CAN-16-3088