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The Neuropilin-2 targeting antibody ATYR2810 inhibits non-small cell lung cancer tumor growth in monotherapy and combination therapy

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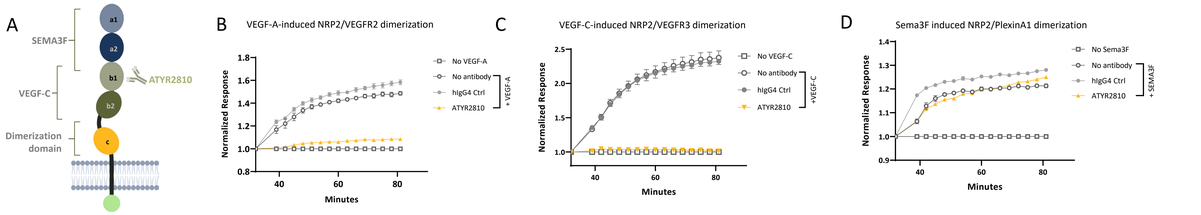
Abstract

Lung cancer remains one of the leading cancer types and is the foremost cause of cancer-related deaths, yet treatment options for advanced lung cancer remain limited. Novel therapies targeting factors critical to the progression of lung cancer are essential. Neuropilin-2 (NRP2) acts as a co-receptor for VEGF and has been correlated with aggressive cancer growth and metastasis[1, 2]. The expression of NRP2 has been shown to be upregulated in several cancers, including non-small cell lung cancer (NSCLC), and the expression of NRP2 in NSCLC is associated with increased invasive tumor growth *in vitro* as well as a significant decrease in patient survival[2, 3-6]. As shown previously, aTyr has developed ATYR2810, a domain-specific anti-human monoclonal antibody to NRP2 which blocks its binding to VEGF. Importantly, ATYR2810 blocks VEGF binding in a highly specific manner and does not affect the binding of Semaphorin-3F or subsequent Semaphorin-3F-induced dimerization of NRP2 and PlexinA1. In this study, we have used ATYR2810 to examine the effects of blocking VEGF-mediated NRP2 signaling both as monotherapy and in combination therapy. Using *in vitro* 3D colony formation assays, we found that ATYR2810 used as monotherapy led to a significant reduction in colony formation in the A549 NSCLC cell line. The effects of ATYR2810 were also examined in NSCLC xenografts, where the use of ATYR2810 as a single agent was found to result in the inhibition of tumor growth. Interestingly, the tumor growth inhibition observed with ATYR2810 as monotherapy was comparable to that of cisplatin monotherapy, suggesting that ATYR2810 may be a potent inhibitor of tumor growth in the A549 xenograft model. The effects of ATYR2810 combination therapy in NSCLC xenografts was also examined, and we found that the use of ATYR2810 in combination with either 5-FU or cisplatin enhanced tumor growth inhibition as compared to the use of either chemotherapeutic agent alone. Taken together, these results suggest that NRP2 may be an important target in aggressive NSCLC and that the use of our novel ATYR2810 antibody to block VEGF-mediated NRP2 signaling may serve as a valuable therapeutic agent both as monotherapy as well as in combination therapy.

Background

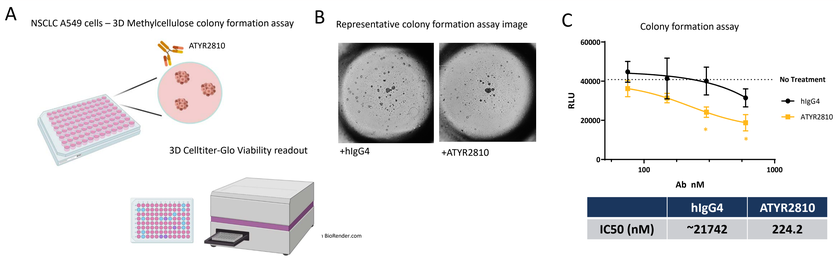
Figure 1: ATYR2810 Blocks VEGF-Induced NRP2 Dimerization



- ATYR2810 is a highly specific antibody which binds to the b1 extracellular domain of NRP2 (Fig 1A).
- ATYR2810 completely blocked VEGF-A-induced NRP2/KDR (VEGFR2) dimerization as well as VEGF-C-induced NRP2/FLT4 (VEGFR3) dimerization (Fig 1B-C, ref 7-8).
- ATYR2810 is specific for VEGF-induced dimerization and has no effect on Sema3F induced NRP2/PlexinA1 dimerization (Fig 1D, ref 7-8).

Results

Figure 2: ATYR2810 Monotherapy Inhibits Tumor Growth *In vitro*

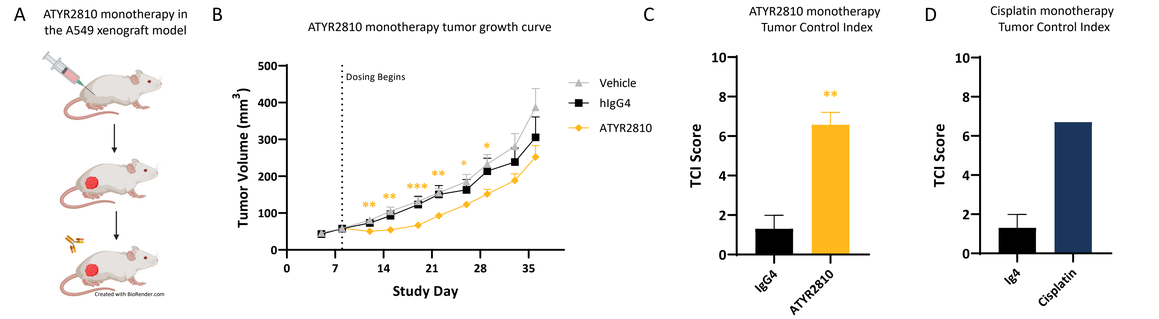


	hIgG4	ATYR2810
IC50 (nM)	~21742	224.2

- 3D Methylcellulose colony formation assays were used to determine the response to ATYR2810 monotherapy in the A549 non-small cell lung cancer cell line (Fig 2A).
- A549 colony formation was significantly inhibited following ATYR2810 treatment (Fig 2B-C).

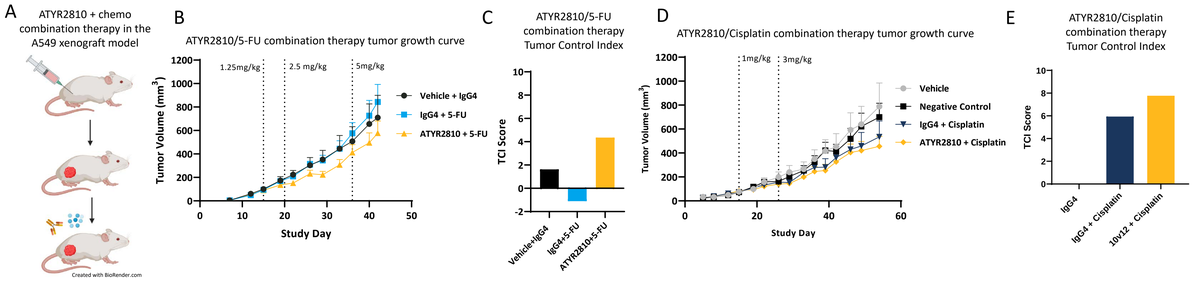
Results

Figure 3: ATYR2810 Monotherapy Inhibits Tumor Growth *In vivo*



- The effects of ATYR2810 on tumor growth *in vivo* were determined using an A549 xenograft model in which immunocompromised NU/J (nude) mice were inoculated subcutaneously in the right flank with A549 cells and monitored for tumor establishment. Mice with measurable, growing tumors were randomized into treatment groups on Day 8 and dosed with either a vehicle control, hIgG4 negative control (25mg/kg), or ATYR2810 (25mg/kg) biweekly (Fig 3A).
- ATYR2810 monotherapy treatment resulted in a significant inhibition of tumor growth (Fig 3B-C).
- The tumor control index (TCI)—a quantitative standard to assess treatment outcomes based on tumor inhibition, tumor rejection, and tumor stability—for ATYR2810 monotherapy (TCI 6.6; three independent studies) was comparable to that of cisplatin monotherapy (TCI 6.7; one study) in A549 xenografts (Fig 3C-D, ref 9).

Figure 4: ATYR2810 Combination Therapy Inhibits Tumor Growth *In vivo*



- The effects of ATYR2810 in combination with chemotherapy on tumor growth *in vivo* were determined using an A549 xenograft model in the manner previously described. Following randomization into treatment groups, mice were dosed with either a vehicle control, hIgG4 negative control, chemo monotherapy, or ATYR2810/chemo combination therapy (Fig 4A). ATYR2810 and the hIgG4 control were dosed at 25mg/kg biweekly, 5-FU was administered triweekly and Cisplatin was administered biweekly at the doses indicted in Fig 4B and Fig 4D, respectively.
- ATYR2810/5-FU combination therapy (TCI 4.4) enhanced tumor growth inhibition when combined with suboptimal 5-FU dose levels (TCI -1.1) (Fig 4B-C).
- ATYR2810/Cisplatin combination therapy (TCI 7.8) lead to a 32% increase in tumor control as compared to Cisplatin monotherapy (TCI 5.9) (Fig 4D-E).

Conclusions

- **ATYR2810 is a highly specific antibody to NRP2 that completely blocks VEGF-induced NRP2/VEGFR dimerization.**
- **ATYR2810 monotherapy significantly inhibits A549 colony formation *in vitro.***
- **ATYR2810 monotherapy significantly inhibits tumor growth in the A549 xenograft model *in vivo*.**
- **ATYR2810 combination therapy enhances tumor growth inhibition as compared to either 5-FU or Cisplatin monotherapy in the A549 xenograft model.**
- **ATYR2810 may serve as a valuable therapeutic agent for NSCLC both in monotherapy and in combination therapy.**

Acknowledgements

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