Preclinical activity of seribantumab in gastrointestinal cancers with *NRG1* fusions

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Abstract

Background. Oncogenic rearrangements of the neuregulin 1 gene (NRG1) consist of a 5' partner fused to a 3' *NRG1* sequence that retains the epidermal growth factor (EGF)-like domain, and are found in ≈0.2% of solid tumors including lung, breast, and gastrointestinal (GI) cancers.[1] Carcinomas of GI origin, including pancreatic and cholangiocarcinoma, represent around 20% of solid tumors harboring NRG1 fusions[2] and there is no approved therapy for this group of cancers. The chimeric NRG1 oncoproteins bind to human epidermal growth factor receptor 3 (HER3/ERBB3) leading to trans-activation of other ERBB family members and trigger a signaling cascade that culminates in oncogenesis. Although targeting HER3 represents a rational therapeutic strategy for cancers harboring NRG1 fusions, this has remained relatively unexplored for GI malignancies with *NRG1* alterations. In this study, we investigated the efficacy of the anti-HER3 monoclonal antibody seribantumab in preclinical models of NRG1-driven GI cancers.

Methods. We developed models of isogenic pancreatic cancer cells with NRG1 fusions by lentiviral-mediated cDNA expression of ATP1B1-NRG1 and SLC3A2-NRG1 fusions in immortalized human pancreatic ductal cells (H6c7). Seribantumab efficacy was evaluated in isogenic cell lines and in patient-derived xenograft (PDX) models of pancreatic adenocarcinoma (**CTG-0943**, *APP-NRG1* fusion) and intrahepatic cholangiocarcinoma (**CH-07-0068**, *RBPMS-NRG1* fusion). Western blotting analysis was used to evaluate protein phosphorylation and expression. The presence of *NRG1* fusions was confirmed by reverse transcription polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS).

Results. Expression of NRG1 fusions in H6c7 cells resulted in enhanced phosphorylation of HER3 and AKT when compared with empty vector control cells (H6c7-EV). Treatment of H6c7-ATP1B1-NRG1 and H6c7-SLC3A2-NRG1 pancreatic cells with seribantumab resulted in a dose-dependent inhibition of HER3 and AKT phosphorylation. Tumor growth inhibition was observed after administration of 5 mg or 10 mg twice weekly [BIW] seribantumab to a PDX mouse model of pancreatic adenocarcinoma with an *APP-NRG1* rearrangement (**CTG-0943**). The two doses of seribantumab were more effective than afatinib (5 mg/kg QD), a pan-ERBB inhibitor, in this model, causing tumor shrinkage of up to 55% (23-77% range). There was no regression of afatinib-treated pancreatic PDX tumors. After treatment, residual **CTG-0943** tumors were extracted for Western blotting analysis (day 24 for vehicle, and day 31 or 32 for the seribantumab and afatinib-groups, respectively). Loss of phosphorylated and total EGFR, HER2 and HER3, cyclin D1, etc. in seribantumab-treated tumors at the end of the study suggests loss of the majority of human tumor cells in the xenograft tumors. This was confirmed using a human-specific GAPH antibody.

Seribantumab was further evaluated in an intrahepatic cholangiocarcinoma PDX model with an *RPBMS-NRG1* fusion as well as mutations in ERBB4 and IDH1 (**CH-17-0068**). While monotherapy seribantumab (5 mg and 10 mg per dose, BIW) was equally effective as afatinib (5 mg/kg once daily [QD]) in this model, enhanced tumor regression was observed with combination therapy. The triple combination of seribantumab 10 mg BIW with afatinib and AG-120, an IDH inhibitor, led to regressions in the majority of tumors. Allometric scaling (based on FDA guidelines) indicates that 5 mg/kg afatinib in mice is equivalent to a human dose of approximately 50 mg daily.

Conclusions. Our results here suggest that seribantumab is effective at reducing tumor growth in preclinical models of GI cancers with *NRG1* fusions. These data support the use of monotherapy seribantumab to treat GI and other cancers uniquely driven by an NRG1 fusion in the ongoing phase 2 CRESTONE study (NCT#04383210).

Prevalence of *NRG1* fusions in GI tumors

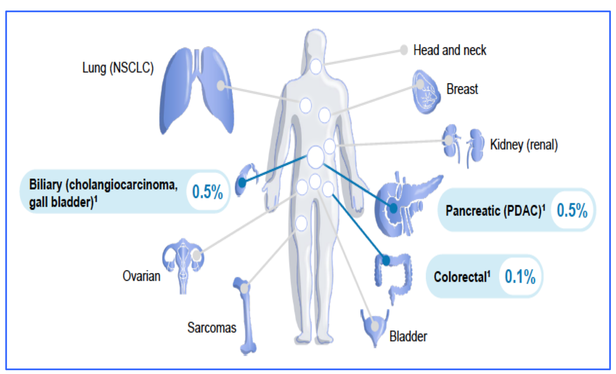


Figure 1. Pancreatic cancer, biliary cancer (cholangiocarcinoma, gall bladder), and colorectal cancer represent 7%, 6%, and 5% of solid tumors harboring NRG1 fusions, respectively.[1,2,3] NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma.

Seribantumab inhibits phospho-HER3 and phospho-AKT activated by overexpression of NRG1 fusions in immortalized H6C7 human pancreatic ductal epithelial cells

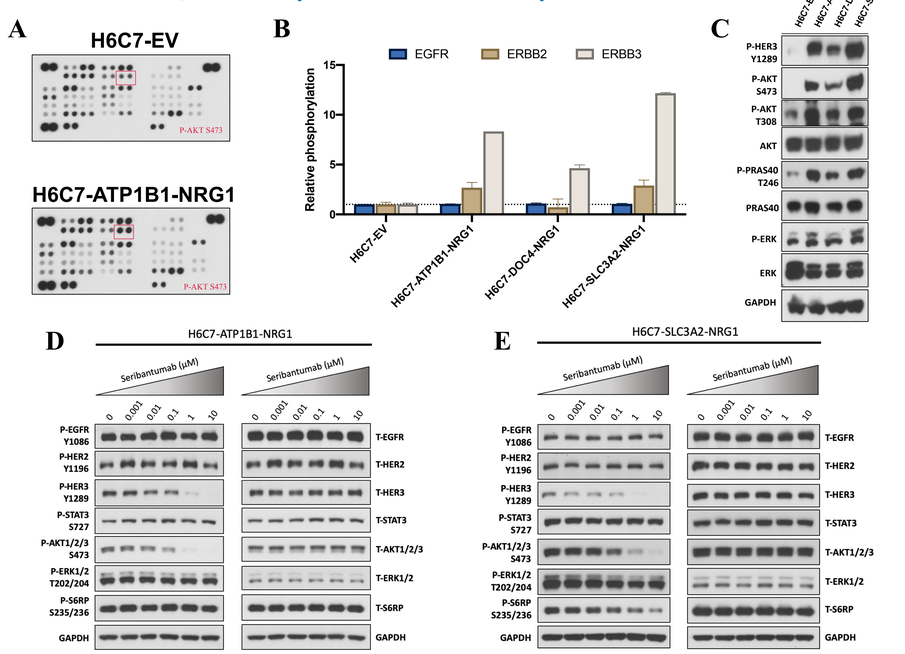


Figure 2. A. H6C7-EV (empty vector) and H6C7-ATP1B1-NRG1 cells were profiled for activated intracellular kinases using phospho-proteomic arrays. B. H6C7-EV and H6C7 cells with the indicated NRG1 fusions were profiled for activated receptor tyrosine kinases (RTK) using phospho-RTK arrays. Quantitation of phosphorylated EGFR, HER2, and HER3 is shown. C. Western blot analysis of cell extracts from H6C7-EV and H6C7 cells expressing NRG1 fusions. D and E. Cells were treated with the indicated concentrations of seribantumab and then whole-cell lysates profiled for the indicated phospho- and total proteins by Western blotting. All cells were serum-started for 24 hours prior to experimentation.

Seribantumab inhibits growth of *NRG1*-rearranged pancreatic adenocarcinoma PDX model *(CTG-0943, APP-NRG1)*

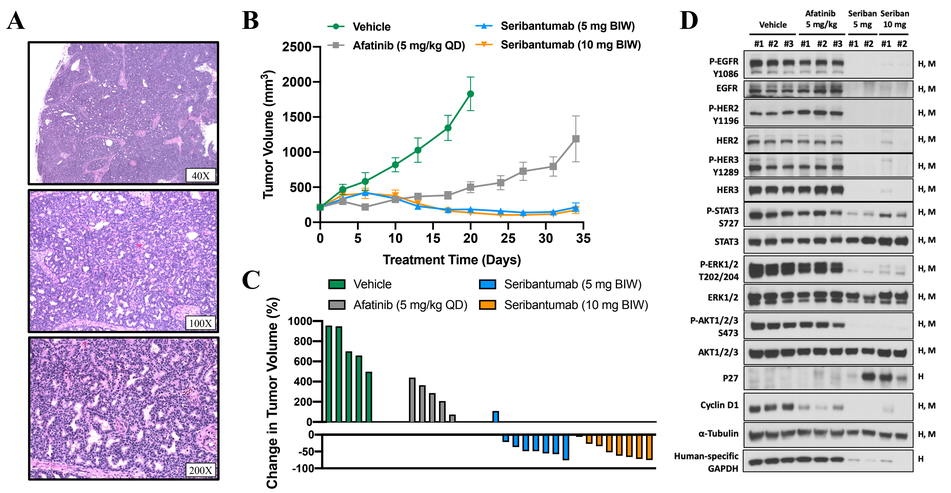
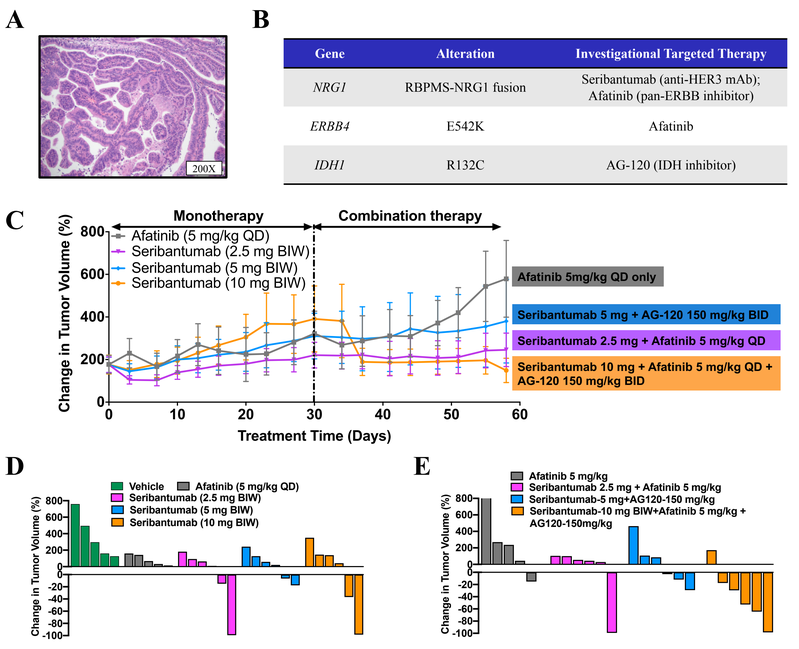


Figure 3. Mice bearing CTG-0943 PDX tumors (5-8 mice per group) were treated with indicated agents. A. Representative H&E-stained slides of a vehicle-treated tumor. B. Tumor volume, results represent the mean ± SEM. Animals in the seribantumab 5 mg and 10 mg groups were administered seribantumab 5 mg/kg and 10 mg/kg, respectively, for the first two doses. C. Change in the volume of individual tumors (vehicle: day 20; treated groups: day 31) compared with day 0 (%). D. Western blot analysis of vehicle, and afatinib and seribantumab-treated tumors. Tumor residues extracted day 24 for vehicle-, day 31 for seribantumab-, and day 32 for afatinib-treated groups. Antibody reactivity with human (H) and/or mouse (M) proteins is indicated.

Targeted combinations inhibit growth of NRG1-rearranged cholangiocarcinoma PDX model harboring additional known driver alterations (CH-17-0068, *RBPMS-NRG1*)



Gene	Alteration	Investigational Targeted Therapy
NRG1	RBPMS-NRG1 fusion	Seribantumab (anti-HER3 mAb); Afatinib (pan-ERBB inhibitor)
ERBB4	E542K	Afatinib
IDH1	R132C	AG-120 (IDH inhibitor)

Figure 4. A. Representative H&E-stained CH-17-0068 PDX tumor. B. Genomic alterations identified by RNAseq and corresponding investigational targeted therapies. C. Mice bearing CH-17-0068 PDX tumors (5-6 mice per group) were treated with seribantumab or afatinib monotherapy for 30 days. Afatinib (5 mg/kg QD) or AG-120 (isocitrate dehydrogenase [IDH] inhibitor, 150 mg/kg twice daily [BID]) were then added to the indicated groups. Results represent the mean ± SEM. Change in the volume of individual tumors on day 30 (D) or at the end of the study (E).

Summary and Conclusion

- *NRG1* fusions are rare but recurrent oncogenic drivers in GI cancers.[1,2]
- Overexpression of NRG1 fusions in immortalized human pancreatic ductal epithelial H6C7 cells activated HER3 and AKT.
- Seribantumab inhibits HER3 and AKT phosphorylation in H6C7 cells with NRG1 fusions.
- Treatment of NRG1 fusion-positive pancreatic PDX model with seribantumab inhibits tumor growth at clinically achievable doses. Residual tumor xenografts show depleted human tumor cell content when assessed by Western blotting.
- Investigation of a cholangiocarcinoma PDX model with three genomic alterations (NRG1 fusion, and ERBB4 and IDH1 mutations) suggests that treatment of NRG1 fusion-driven tumors harboring additional oncogenic drivers may require combination therapy to address the contribution of each genomic alteration in disease progression.
- These data support the use of monotherapy seribantumab to treat GI and other cancers uniquely driven by an NRG1 fusion in the ongoing phase 2 CRESTONE study (NCT#04383210).

References

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