An Ex Vivo Organoid Study to Identify Biomarkers of Sensitivity to ST316, a Clinical-Stage β-catenin Antagonist Peptide

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SAPIENCE THERAPEUTICS

Abstract #2361

Background. The WNT/β-catenin pathway is a key oncogenic driver in various cancers and has long been deemed undruggable. Historically, attempts at targeting its components resulted in toxicity due to β-catenin's role in homeostatic functions such as intestinal stem cell renewal and bone osteogenesis. ST316 is a β-catenin antagonist peptide that disrupts its interaction with co-activator BCL9, targeting oncogenic signaling and immune exclusion while preserving homeostatic functions. ST316 reduces β-catenin transcriptional activity and tumor growth in vitro and in vivo, yet GLP-toxicology studies and Phase 1 clinical trial data confirm no WNT-inhibition-associated toxicity. ST316 has progressed into a Phase 2 clinical study in patients with colorectal cancer (CRC), an indication with a high prevalence of WNT-related mutations (NCT05848739). Here, we evaluated the anti-tumor activity of ST316 in 60 tumor-derived organoids with diverse genetic backgrounds to identify pathways associated with treatment responses. RNA sequencing and qRT-PCR identified transcriptional signatures of cell lines treated with ST316 or vehicle. Combination studies were performed with ST316 and the BRAF inhibitor encorafenib in vitro and in vivo to support findings from the organoid study.

Results. Study of sensitivity of 60 organoids to ST316 identified genetic backgrounds linked to ST316 responses, with TP53 WT models showing the highest sensitivity. Further analysis revealed higher ST316 sensitivity in models with BRAF, NRAS, and NF1 mutations, suggesting co-dependency between MAPK and WNT/β-catenin pathways. Given the importance of MAPK signaling in cancer, we evaluated dual inhibition of β-catenin and BRAF by combining ST316 with the encorafenib in BRAF-mutant organoids and LIM2405 CRC cell line. This combination showed additive/synergistic anti-tumor effects, highlighting new therapeutic opportunities for BRAFV600E-mutant CRC, a subgroup with limited treatment options.

Conclusions. ST316 preserves WNT/β-catenin homeostasis while targeting oncogenic and immune-suppressive β-catenin/BCL9 signaling. Analysis of 60 patient-derived tumor organoids revealed that tumors with TP53 WT and mutations in the MAPK pathway significantly correlate with sensitivity to ST316. Additionally, combining ST316 with encorafenib showed additive/synergistic effects in BRAFV600E-mutant CRC organoids and LIM2405 cells. In an in vivo BRAF mutant CRC model, combination of subpharmacologic ST316 and encorafenib displayed enhanced activity compared to either single agent alone ($p<0.05$). β-catenin has been linked to resistance to BRAF inhibitors, and combination therapies targeting both β-catenin and BRAF are proposed to overcome resistance. Given the poor prognosis and limited treatment options for BRAFV600E-mutant CRC, ST316 offers a promising new therapeutic approach for this difficult-to-treat cancer. Our findings support rational combinations of ST316 with clinically relevant MAPK inhibitors.

Non-clinical Data Supports ST316 Antagonism of Wnt/β-catenin Signaling

ST316 anti-tumor activity was validated in pre-clinical studies. By disrupting the β-catenin/BCL9 complex, ST316 antagonizes the Wnt/β-catenin pathway in CRC cells, leading to reduced expression of key β-catenin target genes (Fig. 1A). As a result, ST316 demonstrates potent anti-tumor activity in vitro (IC50 of 1.3 μM; not shown) and in vivo (Fig. 1B; $p<0.01$ vs control) in a HCT116 CRC tumor model harboring an activating mutation in β-catenin.

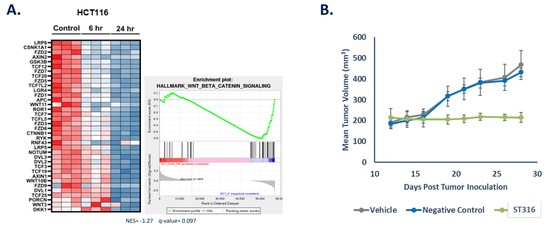


Fig 1. ST316 impacts the Wnt/β-catenin pathway. (A) Exposure of HCT116 CRC cells to ST316 results in antagonism of the Wnt/β-catenin transcriptional signature. Heat map indicates decreased expression of β-catenin target genes after 6hr and 24h of ST316 treatment compared to control, confirmed by GSEA (NES=-1.27, q-value=0.097). **(B)** ST316 treatment results in anti-tumor activity *in vivo*, in HCT116 tumor model. HCT116 cells were inoculated subcutaneously and dosing started when Tumor Volume (TV) reached ~200 mm^3. ST316 was administered S.C. 1x/week at 10 mg/kg.

Biomarker Analysis

The goal of this study was to determine the effectiveness of ST316 across various genetic backgrounds relevant in cancer. The projected outcomes of this project were identification of potential biomarkers of response and identifying and providing rationale for combination strategies.

For this reason, we evaluated the anti-tumor activity of ST316 across 60 patient-derived tumor organoids with diverse genetic deregulations, to identify biomarkers of response. Organoids were treated with escalating ST316 concentrations to determine IC50s. RNA-seq and WES data (untreated) were analyzed for transcriptional signatures, alterations in gene copy numbers, and gene mutations linked to high- and low-sensitivity models.

TP53 wild-type status (Fig. 2A) and MAPK-related mutations (Fig. 2B; BRAF, NRAS, NF1) were significantly associated with drug sensitivity. Specific mutations in MAPK pathway genes, such as BRAF, are typically mutually exclusive with TP53 mutations in cancer. As such, the coexistence of BRAF mutations and wild-type TP53, represents a clinically relevant genetic background to explore as putative biomarker of ST316 sensitivity.

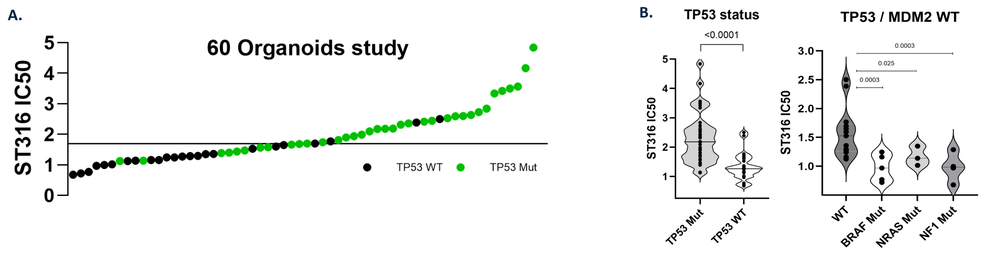


Fig 2. Biomarker discovery in ST316 treated organoids. Organoids were chosen to represent most relevant genetic deregulations for the subtypes of interest. **(A)** IC50s determination upon ST316 exposure for each model revealed TP53 and BRAF (not shown) mutations, being among the most significantly associated with responses. Of note, organoids were cultured in WNT-containing media, likely enhancing ST316 activity in models that lack WNT pathway activation by mutation. **(B)** Bioinformatics analysis of TP53WT organoids showed significant enrichment of MAPK pathway-related mutations (BRAFV600E, NRAS and NF1) in the most ST316-sensitive organoids. Study conducted in collaboration with Crown Biosciences.

Combination Studies – In Vitro

BRAF mutations occur in ~10% of CRC patients. Unlike melanoma, where BRAF–MEK inhibitors demonstrate significant efficacy, in CRC this combination shows limited effectiveness and new therapeutic options are needed. To address this unmet medical need, the combination of ST316 and encorafenib (BRAFi) was evaluated in three BRAF-mutant CRC organoid models. Checkerboard assays were performed in each to elucidate the combination effect (Fig. 3A). Results indicate enhanced activity with the combination at subpharmacologic ST316 and encorafenib concentrations that did not impact organoid viability alone.

β-catenin and BRAF co-inhibition showed enhanced anti-tumor activity that was identified as additive to synergistic in vitro in patient-derived CRC tumor organoids. Similar results were observed in BRAFV600E mutant LIM2405 CRC cells (Fig. 3B).

A.

Conc. Encorafenib (μM) \ Conc. ST316 (μM)	CR20012B 2.50	CR20012B 1.25	CR20012B 0.00	CR20043B 2.50	CR20043B 1.25	CR20043B 0.00	CR6863B 2.50	CR6863B 1.25	CR6863B 0.00
0.512	74.66	49.31	10.39	60.33	40.11	29.98	58.37	37.13	41.84
0.256	63.87	28.29	14.45	56.95	25.85	13.15	57.65	38.03	38.04
0.128	62.85	36.54	16.50	55.91	23.22	13.83	50.62	39.06	45.69
0.064	60.58	37.89	4.53	51.55	1.71	-4.66	51.61	36.26	21.00
0.032	71.81	33.71	13.32	50.93	24.22	2.13	50.27	32.53	30.94
0.016	51.25	12.12	-5.86	16.93	-7.33	-18.92	30.38	15.47	-5.90
0.008	67.03	20.80	-2.78	46.42	17.89	-10.29	36.27	13.30	17.81
0.004	59.46	6.20	-13.10	33.32	-0.13	-37.94	28.75	11.80	7.15
0.002	65.69	19.04	-2.90	32.06	11.86	-16.39	21.65	4.67	7.73
0	60.67	16.23	17.96	40.63	3.07	16.48	13.98	11.83	22.45

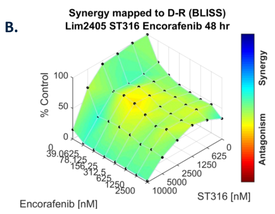


Fig 3. ST316 – Encorafenib combinations. (A) Table showing percent cell death of BRAFV600E-mutant, CRC patient-derived tumor organoids treated with combinations of ST316 and encorafenib. Study conducted in collaboration with Crown Biosciences. **(B)** Bliss synergy analysis of ST316 and encorafenib combination treatment in vitro in the BRAFV600E mutant CRC LIM2405 cell line. "% Control" indicates cells viability relative to vehicle.

Combination Studies – In Vivo

β-Catenin activation is recognized as a relevant resistance mechanism to BRAF inhibition in colorectal cancer (CRC), enabling tumor cell survival despite targeted therapy. ST316 and encorafenib combination strategy holds clinical significance, as the WNT pathway is active in most CRC patients with BRAF mutations. These patients are typically treated with an encorafenib and cetuximab regimen, a substitute for the more conventional BRAF–MEK dual inhibition, where β-catenin-driven resistance may compromise treatment effectiveness.

Here we show potent anti-tumor activity of single-agent ST316 (Fig. 4A) and enhanced anti-tumor efficacy with dual inhibition of BRAF and β-catenin in vivo (Fig. 4B).

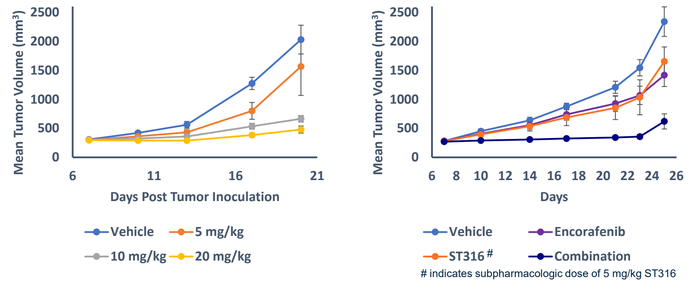
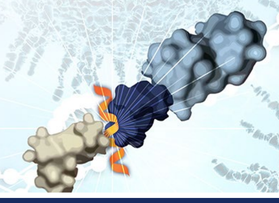


Fig 4. ST316 displays potent anti-tumor activity as a monotherapy and in combination settings. (A) ST316 displays dose-dependent anti-tumor activity in the syngeneic CT26 tumor model. CT26 cells were inoculated subcutaneously and dosing started when tumor volumes reached ~300 mm^3. ST316 was administered S.C. 1x/week at the indicated dose levels. **(B)** Sub-pharmacologic ST316 (5mg/kg), and encorafenib (20mg/kg) have minimal impact on LIM2405 BRAFV600E CRC tumor growth as monotherapy. Combination subpharmacologic ST316 and encorafenib effectively reduced tumor growth in vivo. LIM2405 tumors were inoculated as a subcutaneous xenograft and dosing started when tumors reached ~250 mm^3. ST316 was administered as described in A. Encorafenib was dosed daily at 20 mg/kg.

Conclusions

1. ST316 disrupts the interaction of β-catenin with BCL9, resulting in WNT/β-catenin pathway inhibition.
2. ST316 inhibits tumor growth in vitro and in vivo in tumor models and is currently being evaluated in clinical trials.
3. TP53 WT status and MAPK-related mutations were enriched in highly ST316-sensitive patient-derived tumor organoids, suggesting a potential co-dependency between the MAPK and the WNT pathway.
4. ST316 displays potent anti-tumor activity as a monotherapy and in combination settings in organoids and CRC models.
5. ST316 and encorafenib (BRAF inhibitor) exhibit additive to synergistic anti-tumor effects in BRAFV600-mutant CRC models.
6. Co-inhibiting BRAF and β-catenin enhances in vivo anti-tumor efficacy and may overcome resistance.



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