Anti-tumor and Immunostimulatory Properties of ST316, a Peptide Antagonist of β-Catenin for Treatment of Cancers with Aberrant Wnt Pathway Activity

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Introduction

Sapience Therapeutics, Inc. is a clinical-stage biotechnology company focused on the discovery and development of peptide therapeutics to address oncogenic and immune dysregulation that drive cancer. Our first-in-class β-catenin antagonist, ST316, targets the interaction between β-catenin and its co-activator BCL9, a complex that drives oncogene expression in cancers where aberrant Wnt/β-catenin pathway signaling is observed. ST316 is currently being evaluated in a Phase I-II clinical study (NCT05848739) in patients with selected advanced solid tumors.

Abstract

β-catenin represents a significant challenge as a therapeutic target due to its multifaceted role in tumor development as well as normal cell homeostasis. Dysregulation of the Wnt/β-catenin signaling pathway is implicated in various cancers, including colorectal, hepatocellular, breast, ovarian, and pancreatic cancers. We developed ST316 as a peptide antagonist of the interaction of β-catenin with BCL9, a co-activator implicated in oncogenic β-catenin signaling. ST316 is currently being evaluated in a Phase 1-2 study (NCT05848739) that is currently enrolling patients with selected advanced solid tumors likely to harbor abnormalities of the Wnt/β-catenin signaling pathway. *In vitro* studies indicate that ST316 selectively attenuates Wnt transcriptional activity in β-catenin-dependent but not -independent cell lines. Gene expression profiling using RNA sequencing in β-catenin mutant HCT116 cells and APC mutant COLO320DM cells demonstrates a significant downregulation of Wnt pathway target genes, oncogenic signature genes and pro-tumor immunologic gene sets within 24 hours of ST316 treatment. Consequently, ST316 induced a dose-dependent decrease in viability of β-catenin dependent cells while beta-catenin-independent cells were resistant, indicating that the anticancer effects of ST316 are mediated by inhibition of the Wnt/β-catenin pathway. To evaluate the impact of ST316 *in vivo*, a 4T1 triple negative breast cancer orthotopic tumor model was employed. Once weekly injection of ST316 (5mg/kg SC) over eight weeks suppressed expression of Wnt target genes Cdk4 and cMyc and resulted in a significant 84.3% inhibition of tumor growth compared to the control group. In addition to direct anti-tumor activity, ST316 triggers a pro-inflammatory immune microenvironment. ST316 induces polarization of human macrophages derived from peripheral blood mononuclear cells, resulting in >100-fold shift from immunosuppressive M2 macrophages towards the anti-tumor M1 phenotype. Co-cultures of M2 macrophages with T cells treated with ST316 demonstrate a significant three-fold increase in T-cell activation, as indicated by intracellular IFN-γ staining. Finally, ST316 enhances the anti-tumor activity of anti-PD-1 therapy in a syngeneic orthotopic 4T1 mouse model, where combination ST316 and anti-PD-1 antibody results in enhanced tumor growth inhibition compared to either single agent alone ($p<0.01$). These findings demonstrate the antitumor and immunostimulatory effects of ST316 and highlight its potential as a therapeutic agent for targeting cancers with aberrantly activated Wnt signaling pathways.

Wnt/ β-Catenin in Cancer and TME

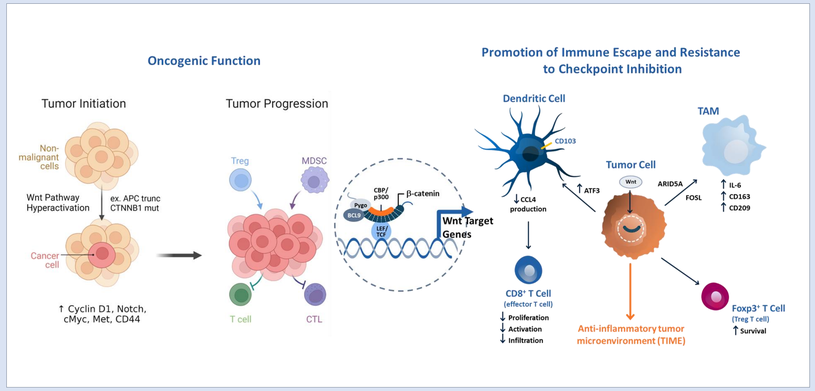

Figure 1. Wnt/β-catenin pathway signaling impacts both oncogenesis and the tumor immune microenvironment (TIME). In cancer cells, this pathway is frequently activated by inactivating APC mutations or β-catenin mutations leading to its stabilization. In the TIME, Wnt signaling promotes an immunosuppressive program in Tumor Associated Macrophages (TAM) and Dendritic Cells (DC) leading to upregulation of M2-markers (CD163, DC-SIGN CD209, IL-6) and decrease of immunogenic chemokines (CCL4). This program suppresses cytotoxic CD8+ T cell activity and supports survival of immunosuppressive T_{reg} populations. Additionally, Wnt can modulate surface expression of checkpoint molecules such as CD155.
References: Pai et al., J of Hemat and Onc 2017; Sarode et al., Sci Adv 2020; Ruiz de Galarreta et al., Cancer Discov 2019; Feng et al., Sci. Adv. 2021).

Results

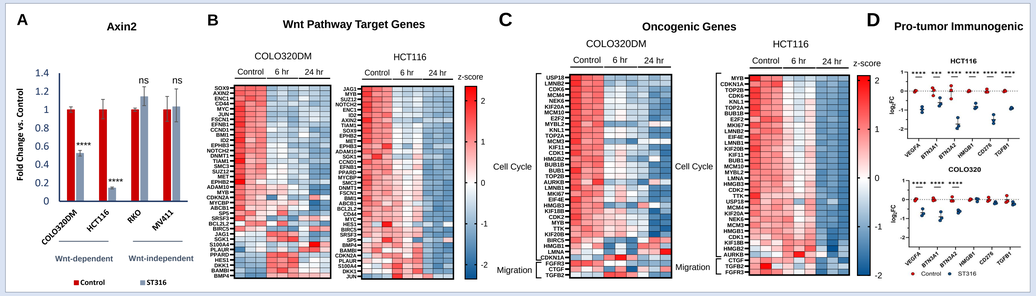

Figure 2. (A) ST316 inhibits the Wnt/ β-catenin pathway, as evidenced by the downregulation of Axin2 in Wnt-dependent cell lines COLO320DM and HCT116. No impact on Axin2 expression was observed in Wnt-independent cell lines RKO and MV4-11. Cells were treated with 5μM ST316 for 24 hrs and Axin2 expression quantified by qPCR (****p<0.001, ns: not significant, t-test). RNASeq analysis of Wnt/ β-catenin target genes in Wnt-dependent cell lines demonstrates a significant downregulation of **(B)** β-catenin target genes, **(C)** oncogenic gene signature, and **(D)** pro-tumor immunogenic genes.

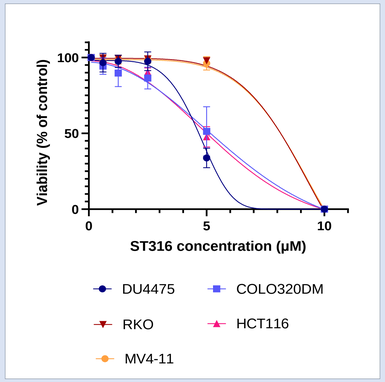

Figure 3. Wnt/ β-catenin-dependent cell lines (DU4475, COLO320DM and HCT116) exhibit greater sensitivity to the cytotoxic effect of ST316 when compared to Wnt–independent cell lines (RKO and MV4-11). Viability was assessed using Annexin V/PI staining following 48h ST316 exposures.

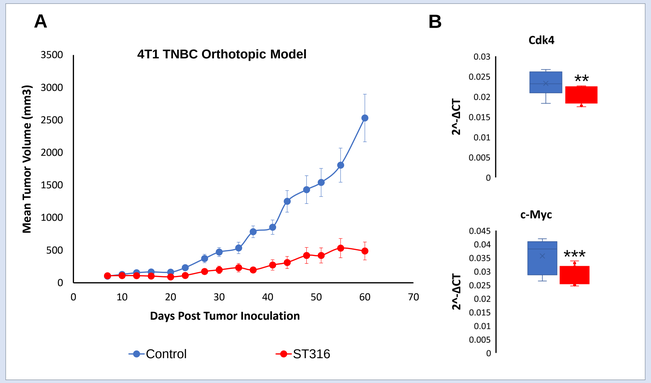

Figure 4. (A) ST316 displays significant anti-tumor activity in mouse 4T1-luc TNBC orthotopic tumor model. Tumor-bearing mice were administered vehicle or 5 mg/kg ST316 once weekly by subcutaneous injection for 8 weeks. An 84.3% tumor growth inhibition (TGI) was observed following ST316 exposure (n= 5/group; p<0.01). **(B)** ST316 resulted in a significant downregulation of Wnt target genes in tumor tissues (**p<0.05; ***p<0.001 measured by t-test).

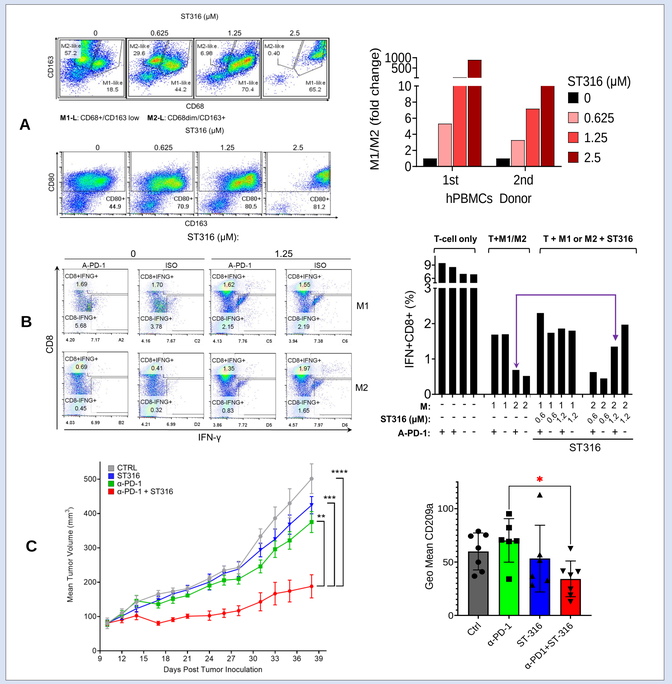

Figure 5. (A) ST316 treatment of PBMC-derived M1 and M2 cultures for 10 days results in M2 to M1 conversion as evidenced by a reduction of M2 subtype ($CD163^{high}CD68^{low}$) and parallel increase of the M1 marker CD80. **(B)** ST316 increases CD8+ T cell activation in 3 days co-culture of CD8+ cells and macrophage. A three-fold increase in IFN-γ(+) CD8+ T cells co-incubated with immune-suppressive M2 macrophage was observed after ST316 exposure. Cells were treated with ST316 (0.6 or 1.2 μM) and anti-PD-1 (20 ng/mL) or isotype IgG4 control (ISO). **(C)** 4T1 orthotopic tumors were administered subpharmacologic ST316 (2 mg/kg/wk) and/or anti-PD-1 (12.5 mg/kg/wk) (n=5/group). An 85% TGI observed in the combination group vs. 51% with anti-PD1 alone vs. 9% with subtherapeutic ST316 alone). Error bars represent SEM. P-value statistics are one-way ANOVA for the indicated comparisons at Day 38 (n=5/group; ****p<0.001; ***<p<0.01; **p<0.05). Expression of the M2 marker CD209a in TAMs was measured (gated as $CD45^+;Gr1^{int};F4/80^+$) in the indicated cohorts.

Conclusions

- Sapience Therapeutics is developing the β-catenin antagonist peptide ST316 for Wnt pathway-dependent tumors such as colorectal cancers.
- ST316 effectively inhibits the transcriptional activity of oncogenic β-catenin, resulting in reduction of Wnt target genes involved in carcinogenesis, cell division, cell migration and immunoinhibitory processes. Consequently, ST316 treatment results in reduced cell viability and tumor growth inhibition, marked by the reduced expression of Wnt target genes within tumor tissue.
- ST316 induces polarization of hPBMC-derived M2 macrophages toward the M1 phenotype and enhances CD8+ T cell activation in mixed cultures of macrophages and T cells. Furthermore, subpharmacologic ST316 augments the efficacy of anti-PD-1 antibody in an orthotopic 4T1 TNBC tumor model.
- These findings underscore ST316's potential as a therapeutic agent for targeting cancers characterized by aberrantly activated Wnt signaling pathways, showcasing its antitumor and immunostimulatory effects.