Immunotherapeutic potential of ST316, a peptide antagonist of β-catenin

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Abstract LB016

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. Sapience has received clearance from U.S. Food and Drug Administration (FDA) to proceed with a ST316 Phase 1-2 clinical trial for the treatment of solid tumors.

Abstract

The transcription factor β-catenin is a key player in many cellular processes, including stem cell renewal, cellular homeostasis and inflammation. Deregulation of β-catenin occurs frequently in several cancer types by mutation or overexpression of the β-catenin gene or mutation of negative regulators such as Adenomatous Polyposis Coli (APC). ST316 is a novel peptide antagonist that specifically interferes with the association of β-catenin with BCL9, disrupting β-catenin nuclear localization and attenuating target gene expression. In an orthotopic triple-negative breast cancer (TNBC) model, ST316 demonstrates potent anti-tumor activity, resulting in 84% tumor growth inhibition (TGI) (p<0.001 vs. vehicle control). In addition to its cell-autonomous mechanisms, deregulated Wnt/β-catenin signaling can promote tumorigenesis by impacting the tumor immune microenvironment (TIME). Reprogramming of immunosuppressive M2-like Tumor Associated Macrophages (TAMs) toward an immune-promoting program (M1-like) is an attractive cancer immunotherapeutic strategy. Here we explored ST316 potential for macrophage repolarization toward the M1-like phenotype, activation of cytotoxic T-cells in macrophage/T-cell co-culture assays and cooperation of ST316 with anti-PD-1 to enhance anti-tumor activity in vivo. Initial studies demonstrate that ST316 reprograms human macrophages derived from Peripheral Blood Mononuclear Cells (hPBMCs) and subsequently committed to the M2-like identity, toward an M1-like phenotype. ST316 treatment dose-dependently suppressed expression of the M2 marker CD163 by flow cytometry and quantitative PCR, resulting in 100-fold increase in the M1/M2 ratio without substantial impact on cell viability. Importantly, activation markers associated with M1-state (CD80,CD86), and T-cell viability were not affected by ST316 at the concentrations used in these studies. Further, in co-cultures of M2 macrophages with T cells, ST316 exposure resulted in a three-fold increase in T-cell activation compared to untreated M2/T cell co-cultures, as measured by intracellular IFN-γ staining. Finally, in an orthotopic TNBC model in vivo, subtherapeutic ST316 enhanced the anti-tumor activity of anti-PD-1 by increasing the M1/M2 ratio, increasing the TGI (observed 85% vs. 51% with anti-PD1 alone, p<0.01; vs. -9% with subtherapeutic ST316 alone, p<0.001). Overall, these results support the immuno-therapeutic potential of ST316 and extend the application range of ST316 to include Wnt-driven cancers with poor clinical response to immune checkpoint blockade and other immunotherapeutic agents.

Wnt/APC/β-catenin in cancer and TIME

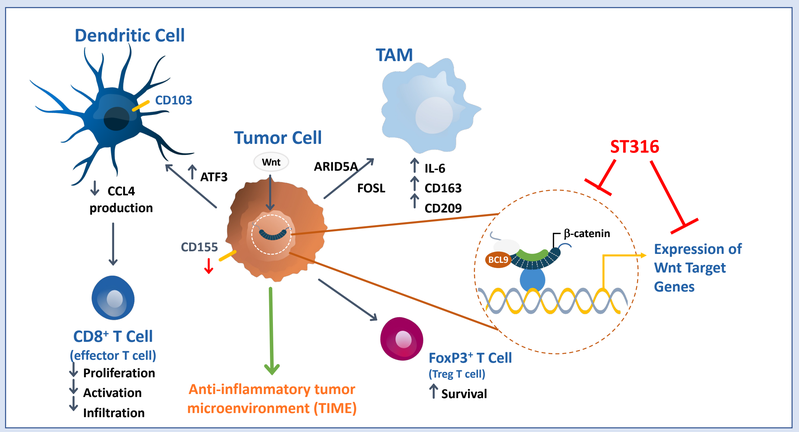

Figure 1: The Wnt/APC/β-catenin axis is a master regulator of both oncogenesis and the tumor immune microenvironment (TIME). In cancer cells, this pathway is frequently activated by inactivating APC mutations or β-catenin mutations leading to its stabilization. In the TIME, Wnt signaling promotes an 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 results in suppression of cytotoxic CD8+ T cell activity and supports survival of immunosuppressive Treg 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

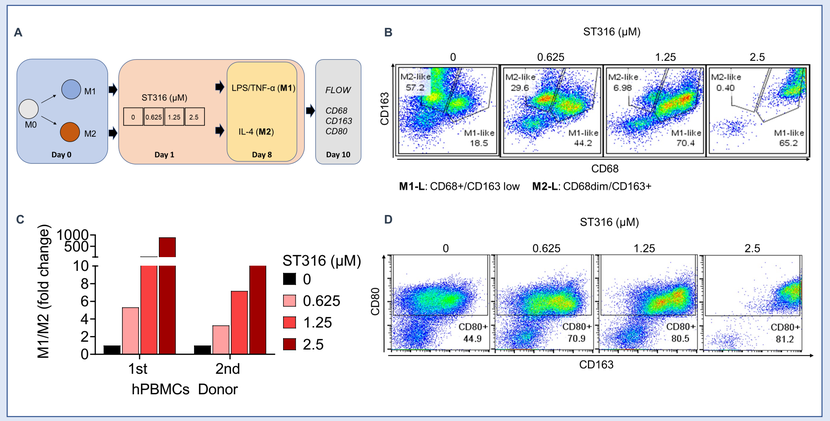

Figure 2: ST316 converts M2-like macrophages to M1-like phenotype. **A**) Experimental outline of M1 and M2 establishment from hPBMCs. **B**) Flow cytometry plots at the indicated ST316 concentrations of M1-like (CD68+CD163low) and M2–like (CD68dimCD163+) populations in M2 conditions at Day 10. **C**) Normalized hPBMCs M1/M2 population ratios for two unrelated donors at the indicated ST316 concentrations. **D**) Flow Cytometry plots for the M1 Marker CD80 and CD163 in hPBMC-derived M2 Macrophages treated with the indicated ST316 concentrations

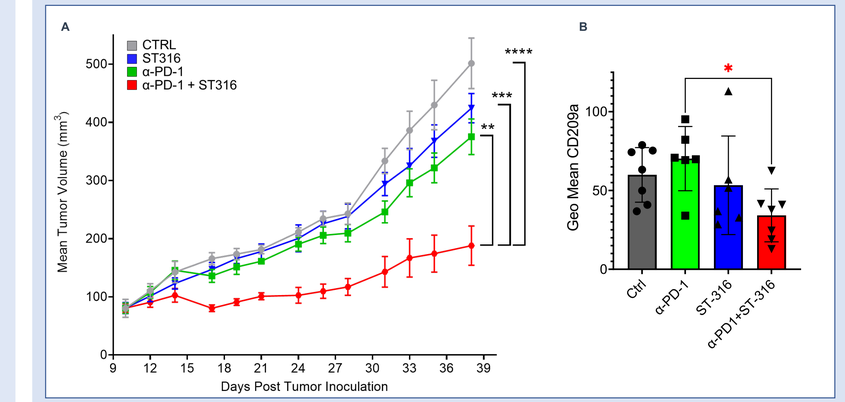

Figure 4. Subtherapeutic ST316 potentiates response to anti-PD-1 therapy in vivo in the orthotopic 4T1 Triple Negative Breast Cancer (TNBC) model. **A**) Balb/c mice harboring 4T1 tumors implanted into the 4th mammary fatpad were administered subtherapeutic ST316 (2mg/kg once weekly) and/or anti-PD-1 (BioXCell #BE0146, clone 29F.1A12; 12.5 mg/kg/wk). Mean Tumor volumes are shown for the indicated treatment cohorts (n=5/group). 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). **B**) Geometric means for expression of the M2 marker CD209a in TAMs (gated as CD45⁺;Gr1^{int};F4/80⁺) for the indicated cohorts (n=5/group; *,p<0.05;one-way ANOVA).

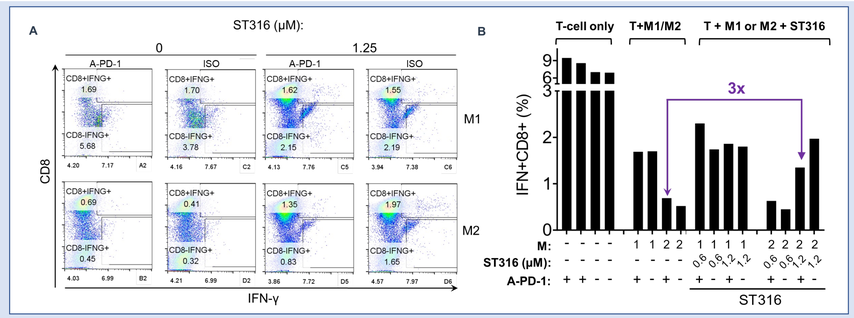

Figure 3: ST316 increases macrophage-induced CD8+ T cell activation in M2 suppressive conditions. A) CD8+ T cell were co-cultured for 3 days with M1 or M2 macrophage and left untreated (0) or treated with 0.625 or 1.25 μM ST316 and anti-PD-1 (A-PD-1, 20 ng/mL) or isotype IgG4 Control (ISO). Flow cytometry plots for CD8 and intracellular IFN-γ. **B**) Frequency of IFN-gamma producing CD8+ cells at the indicated conditions. Arrows point to the comparison between M2 condition in the absence or presence of 1.25 μM ST316, indicating a three-fold increase in IFN-γ(+) cells. T-cell only conditions were assessed in duplicate.

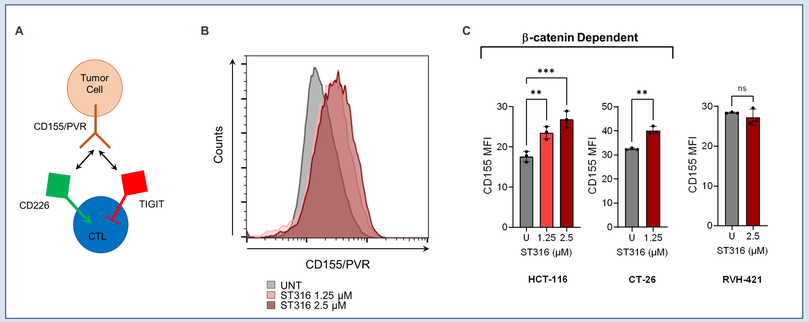

Figure 5. ST316 increases surface expression of checkpoint activator CD155/PVR in β-catenin-driven lines. A) Schematic representation of the CD155/TIGIT checkpoint. CD155/PVR mediates activation of cytotoxic T-cells (CTL) by binding CD226. TIGIT binds competitively CD155 and suppresses T-cell activation. **B**) Representative histograms (mode-normalized) for HCT116 cells untreated (dark gray) or treated with 1.25 μM ST316 (orange) or 2.5 μM ST316 (red) for 48hrs. **C**) Median Fluorescence Intensity of CD155 in the β-catenin-driven lines HCT116 and CT-26 untreated or treated at the indicated ST316 concentrations (Statistics represent t-test of the indicated pair; ***,p<0.001; **,p<0.01;ns, not significant). The melanoma cell line RVH-421 is shown as control.

Conclusions

- ST316 induces a dose-dependent shift in hPBMC-derived M2 macrophage to the M1 identity and induces the M1 markers CD80 in M2 conditions.
- Exposure to ST316 increases CD8 T-cell activation in macrophage/T cell mixed cultures as measured by IFN-γ production by CD8+ T cells.
- Subtherapeutic ST316 enhances the activity of anti-PD-1 antibody in the syngeneic (immunocompetent) orthotopic 4T1 TNBC tumor model; Isolated TAM expressed decreased M2 marker DC-SIGN (CD209a).
- ST316 promotes surface expression of CD155/PVR in β-catenin dependent cell lines.
- These data support a model in which ST316 promotes a shift in the tumor microenvironment via multiple mechanisms, including driving macrophage polarization toward an immune-promoting phenotype, augmenting activity of cytotoxic T cells and increasing expression of checkpoint activators like CD155/PVR in cancer cells.