ST316, a Clinical Peptide Antagonist of β-catenin, Induces Anti-Tumor Immune Responses by Multiple Mechanisms of Action

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SAPIENCE THERAPEUTICS

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Abstract

Wnt/β-catenin plays several important roles in cancer, including driving oncogenesis via cell proliferation, survival and metabolic reprogramming of cancer cells, and enhancing immune-desertification of the tumor immune microenvironment (TIME). ST316 is a clinical stage cell-penetrating peptide antagonist of the interaction of β-catenin with its co-activator BCL9, selectively impairing a subset of Wnt target genes and demonstrating potent anti-tumor activity against Wnt-driven tumors in vivo. ST316 is currently being evaluated in a Phase 1-2 study (NCT05848739) enrolling patients with selected advanced solid tumors likely to harbor abnormalities of the Wnt/β-catenin signaling pathway.

Here we explore the potential of ST316 to activate the TIME and provide data to support combination treatment with anti-PD-1 and anti-TIGIT therapies. Macrophages derived from human peripheral blood mononuclear cells (hPBMCs) were activated by LPS and IFNγ (M1) or IL-4 (M2) in the presence of ST316 or control and were immunophenotyped by expression of CD80 and CD163 to assess M1-like (M1) and M2-like (M2) macrophages, respectively. ST316 induced marked repolarization of hPBMC-derived M2 macrophages to the M1 identity in vitro, as shown by increased CD80 and decreased CD163 staining. Increasing concentration of ST316 in co-cultures of M2 cells with CD8+ T cells induced up to a three-fold increase in IFN-γ expressing cells triple negative breast cancer (TNBC) cells, but not MV411 TNBC that are not Wnt-dependent. When combined with anti-TIGIT treatment, ST316 led to increased T cell activation in an ex vivo assay where 4T1 cells were co-incubated with syngeneic splenic CD8+ cells and frequency of IFN-γ producing cells was assessed. In in vivo experiments, Balb/C mice bearing syngeneic 4T1 TNBC orthotopic tumors were treated with vehicle, ST316, anti-PD-1 or combination. Sub-pharmacologic ST316 enhanced anti-PD-1 suppression of 4T1 tumor growth and induced a substantial decrease of the M2 marker CD209 (DC-SIGN) in the Tumor Associated Macrophage (TAMs). These data support two novel mechanisms of action for the β-catenin antagonist peptide ST316. First, ST316 exposure results in macrophage repolarization towards an immune-active M1 program, both *in vitro* and *in vivo,* and increases T-cell activation in co-culture assays. Second, ST316 induced CD155/PVR upregulation and T-cell activation in the presence of anti-TIGIT antibody. Collectively these results suggest a novel immune-modulatory role for ST316 in the TIME and provide rational for combination therapy with checkpoint inhibitors.

Wnt/APC/β-catenin in Cancer and TIME

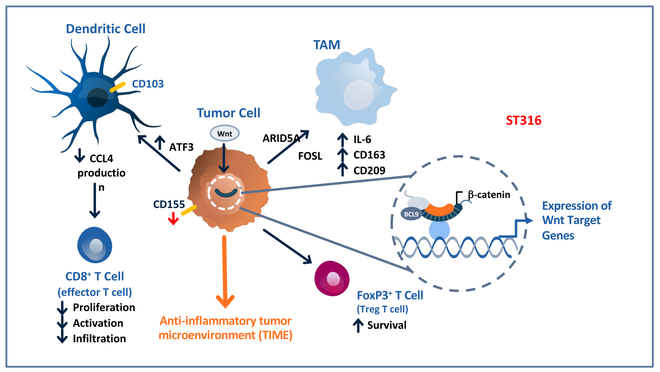

Figure 1: The Wnt/APC/β-catenin axis is a master regulator of both oncogenesis and the TIME. In cancer cells, this pathway is frequently activated by inactivating APC mutations or β-catenin mutations leading to its stabilization. In the TIME, Wnt signaling promotes an immunosuppressive program in TAM 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

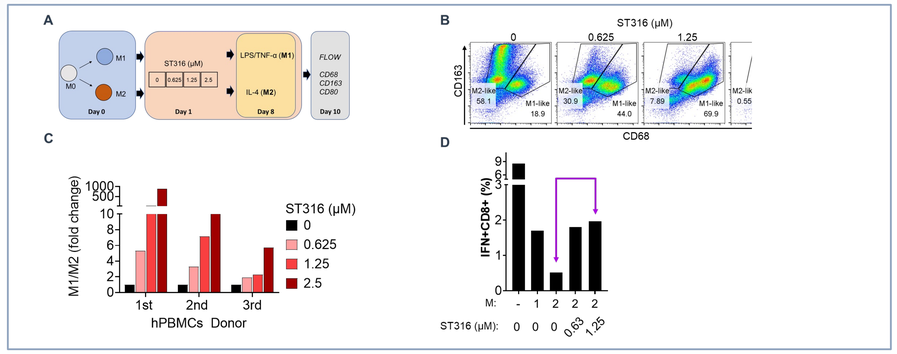

Figure 2: ST316 converts M2 macrophages to M1 phenotype and increases macrophage-induced CD8+ T cell activation in M2 suppressive conditions. A) Experimental outline of M1 and M2 macrophage establishment from hPBMCs. **B**) Flow cytometry plots of macrophages in culture conditions that promote M2 differentiation at Day 10 at the indicated ST316 concentrations. M1 (CD68+CD163low) and M2 (CD68dimCD163+) populations indicated. **C**) Normalized hPBMCs M1/M2 macrophage ratios from three unrelated donors at the indicated ST316 concentrations. **D**) CD8+ T cell were co-cultured for 3 days with M1 or M2 macrophages and left untreated (0) or treated with 0.625 or 1.25 μM ST316. Frequency of IFN-gamma producing CD8+ cells at the indicated conditions. Purple Arrows point to the comparison between M2 conditions in the absence or presence of 1.25 μM ST316, indicating a three-fold increase in IFN-γ(+) cells.

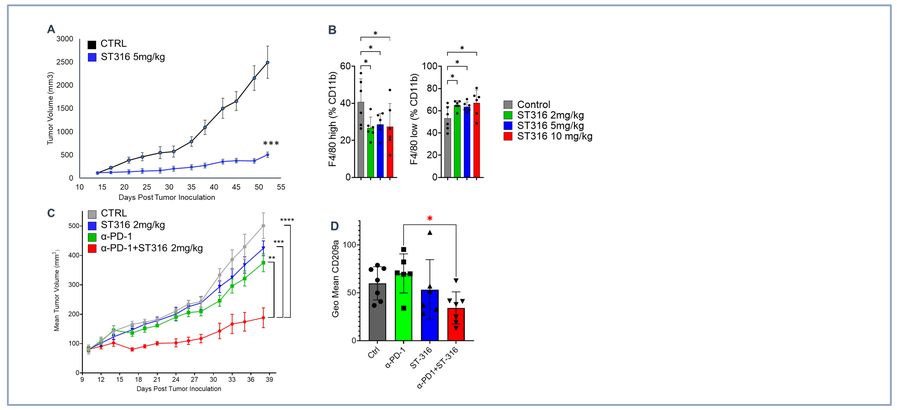

Figure 3. ST316 shifts macrophage identity to suppress tumor growth and potentiate anti-PD-1 responses in immunocompetent models in vivo. A) Anti-tumor activity of ST316 in the orthotopic 4T1 TNBC model. Balb/c mice harboring 4T1 tumors implanted into the 4th mammary fatpad were administered ST316 (5mg/kg once weekly). Mean tumor volumes are shown for the indicated treatment cohorts (n=6/group). Error bars represent SEM. Statistics: one-way ANOVA for the indicated comparisons at Day 52, ***p<0.01. **B)** ST316 induces a shift in the myeloid F4/80^{high} and F4/80^{low} CD11b^{+} cells from CT-26 tumors at Day 15 (n=5/group). Statistics: one-way ANOVA; *p<0.05. **C)** Subtherapeutic ST316 potentiates response to anti-PD-1 therapy in vivo in the orthotopic 4T1 TNBC model. Balb/c mice 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. Statistics: one-way ANOVA for the indicated comparisons at Day 38, ****p<0.001; ***p<0.01; *p<0.05). **D)** 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).

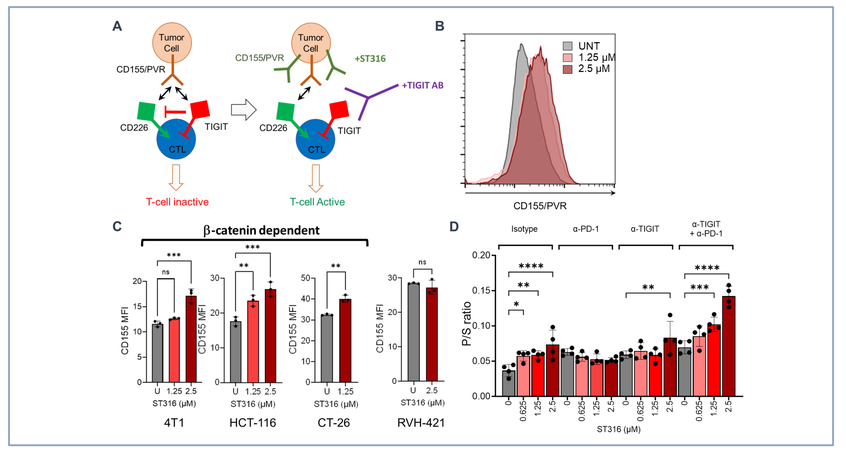

Figure 4. ST316 induces CD155 expression in β-catenin-dependent cell lines. A) Proposed Model for cooperation between ST316 and TIGIT-checkpoint blockade. Left, schematic of the CD155/TIGIT checkpoint. Right, ST316-driven CD155 upregulation leads to increased T cell activity in the presence of TIGIT inhibition. **B**) ST316 induces CD155 in β-catenin-dependent cell lines. Histograms for CD155 fluorescence for 4T1 cells untreated (gray) or treated with 1.25 μM (pink) or 2.5 μM (red) ST316. **C**) CD155 MFI for 4T1 (mouse TNBC), HCT116 (human CRC) and CT-26 (mouse CRC) at the indicated time points for the indicated lines and ST316 treatments. No induction is observed in the β-catenin-independent cell line RVH-421. Statistics (n=3/group): one-way ANOVA, ***p<0.001, **p<0.01, ns not significant. **D**) ST316 enhances CTL response in the presence of anti-TIGIT and anti-PD-1 Abs. 4T1 cells were exposed for 24hrs to ST316 and then incubated with CFSE-loaded CD8+ syngeneic T cells. After 4 days, T cells were recovered and the fraction of proliferative/static (P/S) culture was measured by comparing the proliferating T fraction (P) to the static fraction (S). Statistics (n=4/group): one-way ANOVA ****p<0.0001; ***p<0.001, **p<0.01, *p<0.05. Comparisons that are not significant are not indicated.

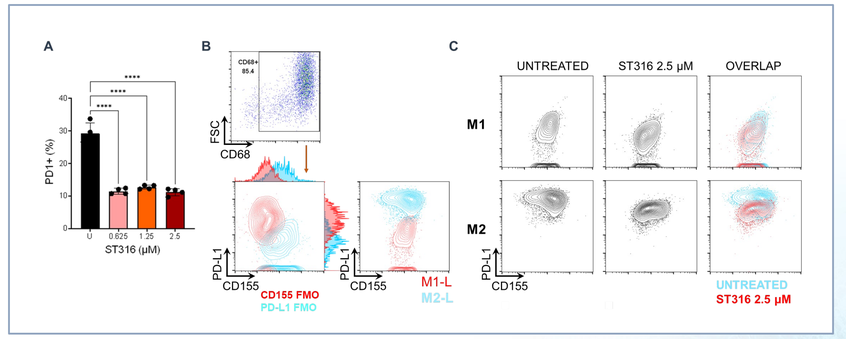

Figure 5. ST316 suppresses PD1 in CD8+ T cells and PD-L1 in M2 macrophages. A) CD8+ T cells from hPBMCs were cultured in the presence of ST316 for 24 hrs. PD1 expression was determined by flow cytometry (n=3/group). Statistics: one-way ANOVA, ****p<0.001. **B**) Gating strategy for hPBMC-derived M1 and M2 cultures stained for CD155 and PD-L1. M2 cultures show a substantial increase in the immunosuppressive PD-L1 marker. **C**) M1 (top) and M2 (bottom) cultures untreated or treated with ST316. Last column shows overlap (untreated- cyan; treated, red).

Conclusions

- ST316 induces a dose-dependent shift in hPBMC-derived M2 macrophages to the M1 identity in 3 independent donors and induces CD8+T cell activation in M2/T co-cultures.
- Single-agent ST316 reduces F4/80^{high} TAMs in CT-26 tumors; combination of subtherapeutic ST316 with anti-PD-1 results in significant anti-tumor activity in syngeneic orthotopic 4T1 TNBC tumors with significant reduction in M2 macrophages, as measured by DC-SIGN (CD209) expression.
- ST316 promotes cell surface expression of CD155/PVR, the ligand for TIGIT and CD226, in several β-catenin-dependent cell lines and induces an increased CTL response when combined with anti-PD-1 and anti-TIGIT Abs.
- ST316 decreases PD-L1 expression in immunosuppressive hPBMC-derived M2 macrophages.
- These data support a paradigm in which ST316 promotes a shift to an immune-active the tumor microenvironment via multiple mechanisms, including driving macrophage polarization toward an M1 immune-promoting phenotype, augmenting activity of cytotoxic T cells, and increasing expression of checkpoint activators like CD155/PVR on cancer cells.

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