ST316, a Peptide Antagonist of β-Catenin, Reprograms Immunosuppressive Myeloid Cell Populations in a Phase 1 Clinical Study to Enhance Anti-Cancer Immune Responses

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SAPIENCE THERAPEUTICS

Abstract# 1451

Background

Wnt/β-catenin pathway mutations correlate with immune exclusion across different tumor types[1], however the mechanism of β-catenin-mediated immune suppression is not fully understood. Soluble factors secreted from Wnt/β-catenin-driven tumors activate maturation of tumor-associated macrophages (TAMs) towards an immunosuppressive M2-like phenotype[1,2], an event that induces recruitment of FOXP3+ regulatory cells and impairs recruitment and activation of CD8+ T cells. Nonclinical studies confirm that inhibition of Wnt/β-catenin results in increased CD8+ T cell infiltration into syngeneic colorectal (CRC) and triple negative breast cancer (TNBC) tumors[3]. Additionally, Wnt/β-catenin pathway activation increases expression of checkpoint molecules on the surface of tumor and myeloid cells, and disruption of the pathway enhances the anti-tumor effects of checkpoint inhibition in colon cancer models[3,4]. Thus, pharmacologic antagonism of Wnt/β-catenin signaling has the potential to enhance anti-tumor immune responses in addition to exerting a direct anti-tumor effect.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid cells that originate from the bone marrow and display potent immunosuppressive activity. Tumor-derived factors including CCL2 and TFGβ drive the expansion and differentiation of MDSCs into either monocytic (M-MDSCs) or polymorphonuclear (PMN-MDSCs) subtypes that contribute to tumor immune evasion and progression[5]. PMN-MDSCs are elevated in colorectal cancer[5] and inversely correlate with clinical outcomes[6]. Despite the prevalence of PMN-MDSCs in CRC that is >90% driven by mutations in the Wnt/β-catenin pathway, a clear role for Wnt/β-catenin signaling in MDSC activation and proliferation has not yet been established.

ST316 is a peptide antagonist of the interaction between β-catenin and BCL9 that is currently in a Phase 2 clinical study (NCT05848739). ST316 demonstrates potent anti-tumor activity in preclinical Wnt/β-catenin-driven colorectal (CRC) and triple-negative breast cancer (TNBC) models. Here we assessed the impact of ST316 on immunosuppressive myeloid cell populations in nonclinical and clinical settings.

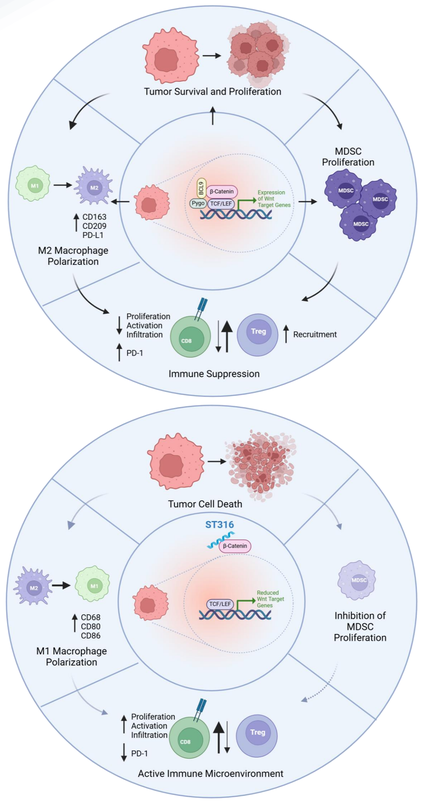

Figure 1: The Wnt/β-catenin pathway is a master regulator of both oncogenesis and immune evasion in the TME. (Top) In cancer cells, interaction of β-catenin with its co-activator BCL9 results in hyperactivation of a genetic signature that supports tumor cell survival and proliferation. Further, factors from Wnt/β-catenin-driven tumors promote immunosuppression by driving M2 polarization of TAMs and accumulation of immunosuppressive MDSC populations. Additionally, programs within the M2 TAMs and PMN-MDSC require Wnt/β-catenin, as disruption of β-catenin results in depletion of these cell populations. (Bottom) ST316 antagonism of the interaction of β-catenin and BCL9 results in tumor cell death. Additionally, ST316 directly induces reprogramming of TAMs to a pro-inflammatory M1-like phenotype and presumptively converts MDSC to an immature NK-like phenotype. Collectively, depletion of immunosuppressive TAMs and MDSC populations drives a decrease in tumor infiltration of immunosuppressive T-regs and increased cytotoxic CD8 T cells, resulting in reversal of Wnt-driven immune exclusion.

References: 1. Pai et al., J of Hemat and Onc 2017; 2. Sarode et al., Sci Adv 2020; 3. Feng et al., Sci. Adv. 2021; 4. Ruiz de Galarreta et al., Cancer Discov 2019; 5. Sieminska et al., Trans Oncol 2022; 6. Salah et al., Front Oncol 2020.

Clinical Impact on MDSCs

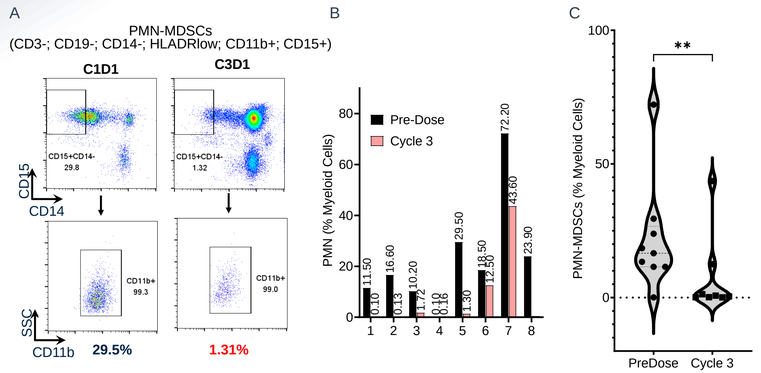

Figure 2: ST316 reduces PMN-MDSCs in Peripheral Blood (PB) pf ST316-treated phase I patients. A) PB collected from patients in Phase 1 study ST316-101 were analyzed pre-treatment (C1D1) or Cycle 3 (C3D1) of ST316 treatment. PMN-MDSCs were gated according to the indicated strategy. PMN-MDSCs fractions were calculated as percentages of total myeloid cells (bottom). B) PMN-MDSCs from 8 patients evaluated C1D1 and C3D1 in phase I ST316 trial. C) Violin plot for aggregate statistics of PMN-MDSCs percentages pre- and post-ST316 exposure. Statistics, Paired Student's-t test, n=8. **p<0.01.

Human Macrophage Polarization Ex Vivo

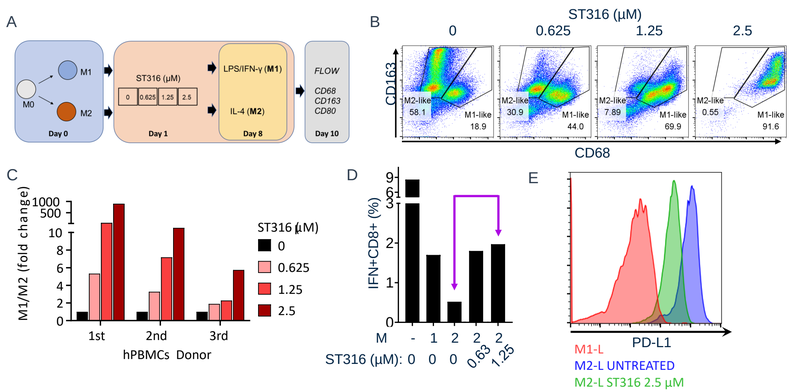

Figure 3: ST316 increases M1/M2 ratio and CD8+ T cell activation in vitro. A) Experimental outline establishing M1 and M2 macrophages from hPBMCs. B) ST316 increases M1 macrophage in culture conditions that promote M2 differentiation at Day 10. Plots indicate M1-like (CD68+CD163low) and M2–like (CD68dimCD163+) populations. C) Normalized M1/M2 macrophage ratios from culture conditions described in A and B. D) Frequency of IFN- γ producing CD8+ cells following 3-day co-culture with M1 or M2 macrophages in presence or absence of ST316. Purple arrows compare the ST316 impact on T cells incubated with M2 macrophages (three-fold increase in IFN-γ(+) cells). E) PD-L1 expression of hPBMC-derived M1 and M2 cells indicates decreased expression on M2 in presence of ST316.

Non-clinical Impact on MDSCs and TAMs

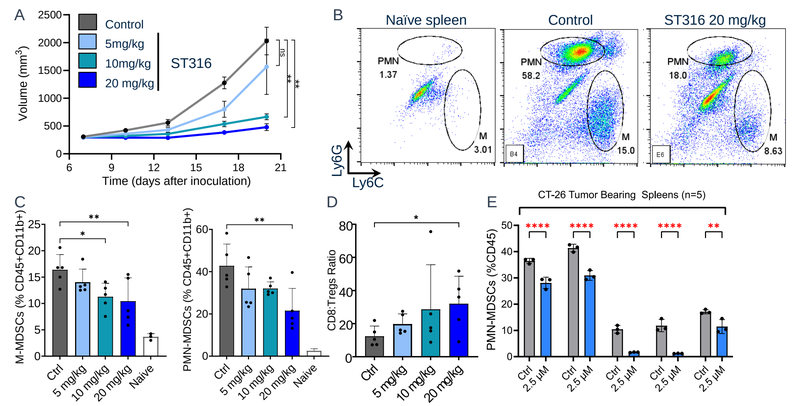

Figure 4: ST316 reduces PMN- and M-MDSCs and increases CD8:Treg ratio in the CT-26 syngeneic CRC tumor model. A) Mean tumor volumes of CT-26 tumors in Balb/c hosts dosed once weekly (SC) with ST316 (n=6/group). Statistics, 1-way ANOVA,**p<0.01, ns, not significant, n=5/group. B) Plots indicate PMN- and M-MDSC populations from spleens of BALB/C mice not transduced with tumors (Naïve), or transplanted with CT-26 cells and untreated (Ctrl) or treated with ST316. C) Frequency of PMN- and M-MDSCs as fraction of Cd11b+CD45+ splenic cells collected on day 20 from the experiment described in A. D) CD8 to Treg ratio in tumors for the indicated groups. E) Ex vivo impact of ST316 on PMN-MDSC from spleen of CT-26 bearing mice. No impact on PMN-MDSCs from naïve mice observed. Statistics, 1-way ANOVA, *p<0.05, **p<0.01, ns, not significant, n=5/group.

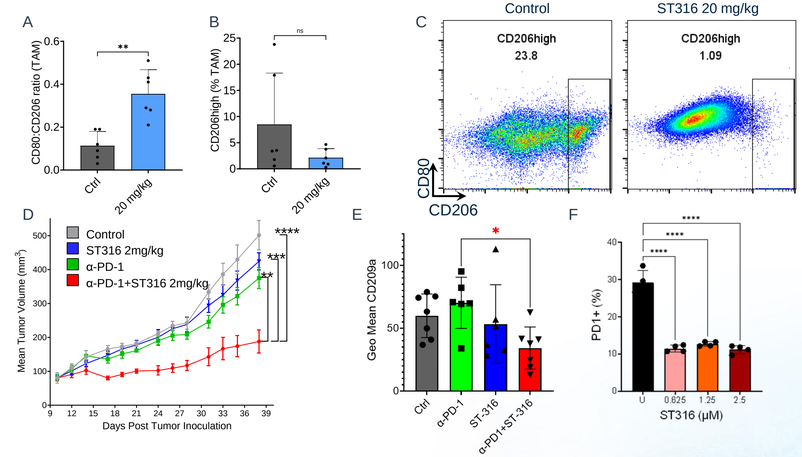

Figure 5. ST316 enhances anti-PD-1 responses in syngeneic tumor models in vivo. A) CD80 (M1 marker) to CD206 (M2 marker) ratio in CT-26 tumors. B) $CD206^{high}$ population was depleted in ST316-treated mice. Statistics, Student T-test ,**p<0.01, ns, not significant, n=6/group. C) Representative plots for CD80 and CD206 in TAM (gated as $F4/80^{low}$;$CD11b^{low}$). D) Subtherapeutic ST316 (2mg/kg 1x/wk) enhances anti-tumor activity in anti-PD-1 (BioXCell #BE0146, clone 29F.1A12; 12.5 mg/kg/wk) treated Balb/c mice harboring 4T1 TNBC tumors. Mean tumor volumes ± SEM are indicated (n=5/group). Statistics, 1-way ANOVA at Day 38 (**** p<0.001; *** p<0.01; * p<0.05). . E) Geometric means for CD209a expression (M2 marker) in TAMs (gated as $CD45+;Gr1^{int};F4/80+$) (n=5/group; *,p<0.05; one-way ANOVA). F) PD-1+ frequency (% CD8+ T cells) from hPBMCs cultured in the presence of ST316 for 24 hrs. Statistics: ****p<0.001, 1-way Anova, n=3/group.

Conclusions

- ST316 disrupts β-catenin-driven immune-exclusion and promotes a shift to a pro-inflammatory TME via depletion of immunosuppressive MDSCs and TAMs, resulting in enhanced cytotoxic T cell activity.
- In the clinic, ST316 reduces immunosuppressive PMN-MDSCs in phase I patients.
- In human PBMCs, ST316 induces a dose-dependent increase in macrophage polarization to the M1-like phenotype, which results in increased T cell activation.
- In murine tumor models, ST316 inhibition of tumor growth is accompanied by a reduction in M- and PMN-MDSC populations and repolarization of TAMs to an M1-like phenotype.
- In murine tumor models, anti-tumor activity of anti-PD-1 treatment is significantly enhanced by combination with ST316, an event accompanied by a reduction of M2-TAMs.
- These data identify ST316 as a candidate to enhance existing I/O approaches.

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