Safety and Biomarker Assessment of ST316, a Novel Peptide Antagonist of β-catenin, in Patients with Advanced Solid Tumors

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Abstract# 286

Background

Aberrant Wnt pathway activation, resulting in β-catenin nuclear accumulation and deregulated transcriptional activity, is a key event in colorectal cancer (CRC). 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 (**Figure 1, Top**). Additionally, Wnt/β-catenin pathway mutations correlate with immune exclusion across different tumor types[1]. Soluble factors released from Wnt/β-catenin-driven tumors activate maturation of tumor-associated macrophages (TAMs) towards an immunosuppressive M2-like phenotype[1,2] and drive accumulation of immunosuppressive myeloid-derived suppressor cell (MDSC) populations, contributing to tumor growth[3].

Disruption of the interaction of β-catenin and BCL9 is sufficient to suppress oncogenic β-catenin transcriptional activity and immune exclusion[4-7] (**Figure 1, Bottom**), without impacting its homeostatic functions in normal tissue. ST316 is a first-in-class, cell-penetrating peptide antagonist of the β-catenin and BCL9 interaction. Preclinical evaluation revealed significant antagonism of Wnt/β-catenin signaling, bioavailability and potent activity in CRC models with no toxicity or impact on β-catenin regulation of intestinal stem cell survival or bone morphology.

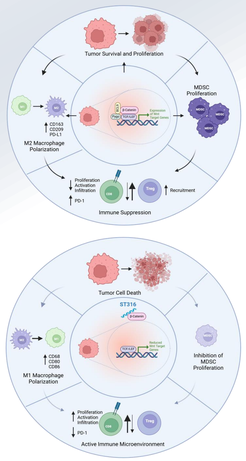


Figure 1: The Wnt/β-catenin pathway is a master regulator of both oncogenesis and immune evasion in the TME. (Top) Depiction of the impact of deregulated β-catenin in tumor cells and the tumor microenvironment. **(Bottom)** ST316 antagonism of the interaction of β-catenin and BCL9 results in tumor cell death, reprogramming of TAMs to a pro-inflammatory M1-like phenotype and reduction in MDSC expansion and maturation.

References: [1]Pai et al., J of Hemat and Onc 2017; [2]Sarode et al., Sci Adv 2020; [3]Wang et al., Cancer Letters 2024; [4]Feng et al., Sci. Adv. 2021; [5]Ruiz de Galarreta et al., Cancer Discov 2019; [6]Sieminska et al., Trans Oncol 2022; [7]Salah et al., Front Oncol 2020.

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

In non-clinical studies, the effects of ST316 on the Wnt/β-catenin signaling pathway, tumor growth, and immune modulation were investigated. ST316 antagonizes the Wnt/β-catenin pathway in CRC cells, leading to a reduction in the expression of key β-catenin target genes. As a result, ST316 demonstrates dose-dependent anti-tumor activity in a CRC syngeneic tumor model, while concomitantly modulating the immune landscape by depleting immunosuppressive MDSC populations and reprogramming TAMs to adopt a pro-inflammatory M1-like phenotype.

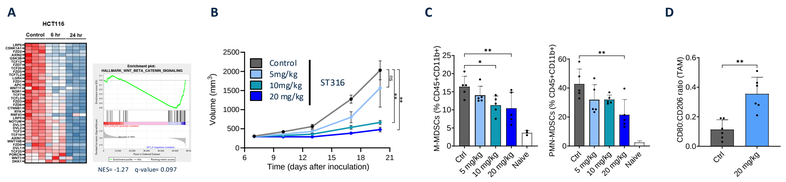


Figure 2: ST316 impacts the Wnt/β-catenin pathway. (A) Exposure of HCT116 CRC cells to ST316 results in antagonism of the Wnt/β-catenin genetic signature. Heat map indicates decreased expression of β-catenin target genes, confirmed by GSEA. **(B)** ST316 results in dose-dependent anti-tumor activity and **(C)** reduction of immunosuppressive M- and PMN-MDSC populations from splenocytes in the CT26 CRC syngeneic tumor model. **(D)** ST316 reprograms TAMs to a pro-inflammatory M1-like phenotype, as indicated by flow cytometry using antibodies against CD80 (M1 marker) and CD206 (M2 marker). * indicates p<0.05 vs. control; ** indicates p<0.01 vs. control.

Methods

A phase 1-2 dose escalation-expansion study has enrolled patients with advanced solid tumors likely to harbor abnormalities in the Wnt/β-catenin pathway, to assess the safety, pharmacokinetics (PK), biomarker and preliminary activity of ST316, and to recommend a P2 dose (RP2D). Paired biopsies were collected when feasible and assessed for drug penetration and pharmacodynamic biomarkers. Peripheral blood (PB) collected pre- and post-treatment was assessed by flow cytometry for expression of myeloid and lymphocyte populations.

Study Design

Phase 1 Escalation - Completed

Phase 1 Dose Escalation Overview

- **POPULATION** • Relapsed/refractory solid tumor types that are known to harbor abnormalities of the Wnt/β-catenin signaling pathway
- **OBJECTIVES** • Understand safety, PK, PD • Refine dosing
- **DESIGN** • 3 + 3 • Monotherapy • 1x/weekly IV

12 mg/kg QW IV N=4
8 mg/kg QW IV N=4
4 mg/kg QW IV N=4
2 mg/kg QW IV N=4
1 mg/kg QW IV N=4
0.5 mg/kg QW IV N=3
Predicted Minimal Effective Dose
8 mg/kg RPTD for combinations

Phase 2 Expansion in CRC - Ongoing

Phase 2 Dose Expansion Overview

- **POPULATION** • Colorectal Cancer
- **OBJECTIVES** • Confirm safety • Explore PD • Assess anti-tumor activity
- **DESIGN**

≥1 RESPONSE And /or ≥20% DCR

2nd Line: ST316 + FOLFIRI + beva (N=15) → ST316 + FOLFIRI + beva (N=30)
3rd Line: ST316 + fruquintinib (N=15) → ST316 + fruquintinib (N=30)
3rd Line: ST316 + Lonsurf + beva (N=15) → ST316 + Lonsurf + beva (N=30)

Schedule of Assessments

Clinical Results

Baseline Characteristics

Variable	Phase 1 escalation N=23
Gender	
Male	9 (39%)
Female	14 (60%)
Age (mean)	62
ECOG	
0	3 (13%)
1	18 (78.3%)
2	2 (8.7%)
Disease Under Treatment	
Colorectal Cancer	15 (65.2%)
Non-Small Cell Lung Cancer	4 (17.4%)
Pancreatic	2 (8.7%)
Breast Cancer	1 (4.3%)
Ovarian cancer	1 (4.3%)

Efficacy

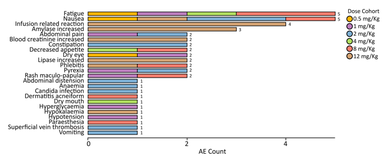
ST316 was shown to demonstrate clinical proof-of-concept activity as a single agent. Four out of 23 patients with advanced solid tumors presented with SD, including two patients with NSCLC and two patients with CRC. Among the CRC patients, one SD lasted 12 months and the second remains ongoing for more than 14 months.

Safety

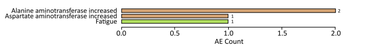
Overall ST316 was safe and well tolerated with the majority of AEs being mild to moderate (G1-G2) and without dose response. No dose limiting toxicity (DLT) was identified, however increased incidence of G1/2 and reversible infusion-related reactions were reported in the 12mg/kg QW cohort.

Grade 3 Adverse events of ALT and AST elevation were reported in one patient with liver metastasis and PD

Treatment related AEs ≤ G2



Treatment related AEs ≥ G3



Best Response	All patients N=23	CRC patients N=15
PD	19	13
SD	4	2

Biomarker Results

Biopsy Analysis

ST316 penetrates into tumor tissue (Figure 3) and demonstrates target engagement (Figure 4), as shown by a shift in the localization of β-catenin from the nucleus to the cytoplasm/membrane post treatment. Images reveal a treatment-induced shift of β-catenin from nuclear (pretreatment) to cytoplasm/membrane (post treatment) as indicated by loss of brown nuclear stain and appearance of blue nuclear counterstain. Decreased nuclear β-catenin was observed in 4 of 7 patients where pre- and post-treatment biopsies were available.

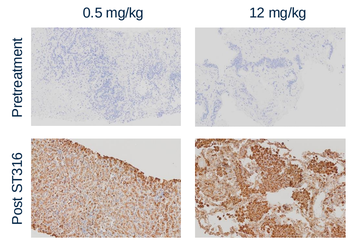


Figure 3: ST316 uptake into tumor biopsies. Tumor biopsies collected from patients in Phase 1 study ST316-101 were analyzed pre-treatment (C1D1; top panels) or Cycle 3 (C3D1; bottom panels) of ST316 treatment for ST316 uptake by IHC using a polyclonal anti-ST316 antibody. Representative data shown from a patient receiving 0.5 or 12 mg/kg ST316.

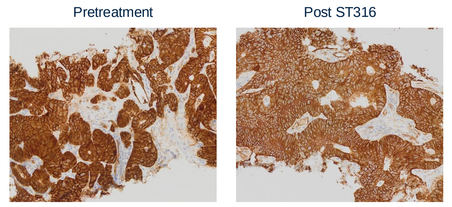


Figure 4: ST316 results in redistribution of β-catenin subcellular localization. Tumor biopsies collected as in Fig. 3 were analyzed for β-catenin localization by IHC using mouse monoclonal antibody targeting β-catenin (Biocare CM406A). Stained sections were assessed by a pathologist.

Peripheral Blood Analysis

Peripheral blood analysis highlights a decrease in the highly immunosuppressive polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) population after ST316 treatment. PMN-MDSCs play a critical role in CRC by promoting immune evasion, suppressing anti-tumor immune responses, and supporting tumor progression. Higher levels are prognostic of negative outcomes in CRC. The observed decrease in PMN-MDSCs is consistent across all patients that displayed elevated baseline levels.

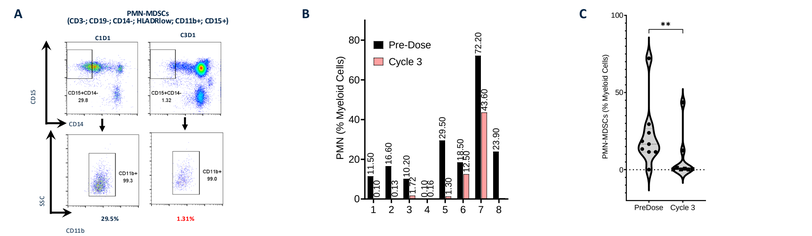


Figure 5: ST316 reduces PMN-MDSCs in phase I patients. A) PB collected from study ST316-101 were analyzed pre-treatment (C1D1) or following Cycle 3 (C3D1). PMN-MDSCs were gated according to the indicated strategy. Representative data shown from a patient receiving 4 mg/kg ST316. PMN-MDSCs are calculated as percentage of total myeloid cells. **B)** Individual PMN-MDSC levels from 8 patients evaluated at C1D1 and C3D1. **C)** Violin plot for aggregate statistics of PMN-MDSCs. Statistics, Paired Student's-t test, n=8. **p<0.01.

Conclusions

- ST316 disrupts the interaction of β-catenin with BCL9 to result in Wnt/β-catenin pathway inhibition
- ST316 was shown to be safe and well tolerated at all doses tested
- The prolonged stable disease noted with ST316 is consistent with early signals of anti-tumor activity
- ST316 demonstrates tumor uptake and target engagement, as shown by a shift in the localization of β-catenin from nucleus to cytoplasm/membrane following treatment in 4 of 7 patients
- ST316 dramatically reduced the expression of immunosuppressive PMN-MDSCs in all phase I patients that displayed elevated baseline levels
- Based on safety and pharmacodynamic effects that are consistent with the mechanism of action, ST316 is now being tested in combination with chemotherapy in advanced colorectal across different lines of treatment

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