VISTA checkpoint targeting by SNS-101, a pH-selective antibody with enhanced safety and pharmacokinetic profiles, alters the tumor microenvironment and overcomes immune checkpoint inhibitor resistance

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INTRODUCTION

VISTA (V-domain immunoglobulin suppressor of T-cell activation) is an inhibitory myeloid-T-cell checkpoint that is upregulated upon treatment with anti-PD-1 and anti-CTLA-4 immunotherapies.[1] VISTA promotes T-cell and myeloid quiescence through pH-dependent interaction with its T-cell receptor, P-selectin glycoprotein ligand-1 (PSGL-1) in the acidic tumor microenvironment (TME).[2] Our hypothesis is that targeting this inhibitory checkpoint may relieve suppressive myeloid signaling in the TME and support potent anti-tumor immunity, especially in combination with anti-PD-1/PD-L1 treatment.

Prior efforts at clinical development of anti-VISTA antibodies were stifled by cellular activation and dose-limiting on-target cytokine release syndrome (CRS) at sub-therapeutic doses, and target-mediated drug disposition (TMDD)-based drug clearance by VISTA$^+$ neutrophils and monocytes at physiologic pH.[3]

We developed SNS-101, a fully-human monoclonal antibody specific for the protonated/active form of VISTA. SNS-101 blocks VISTA's interaction with PSGL-1, as well as other putative binding partners, at low pH.

Here we present structural analysis, cytokine release assays, *in vivo* pharmacology including safety and pharmacokinetic (PK) studies in mice and non-human primates, anti-tumor activity in pre-clinical syngeneic models, and exploratory proteomic analysis of VISTA signaling in T-cells. Our preclinical data show that SNS-101's selectivity for active VISTA significantly reduces CRS risk and that SNS-101 maintains dose-proportional exposure and linear elimination kinetics, with no evidence for TMDD. Our data collectively support the notion that coordinated targeting of T-cell checkpoints and myeloid suppressive signaling is an effective approach to combat resistance to first-line checkpoint inhibition therapy. SNS-101 has therefore entered a Phase I clinical trial in advanced solid tumors (NCT05864144), displaying favorable PK and safety profiles through initial monotherapy cohorts.

RESULTS

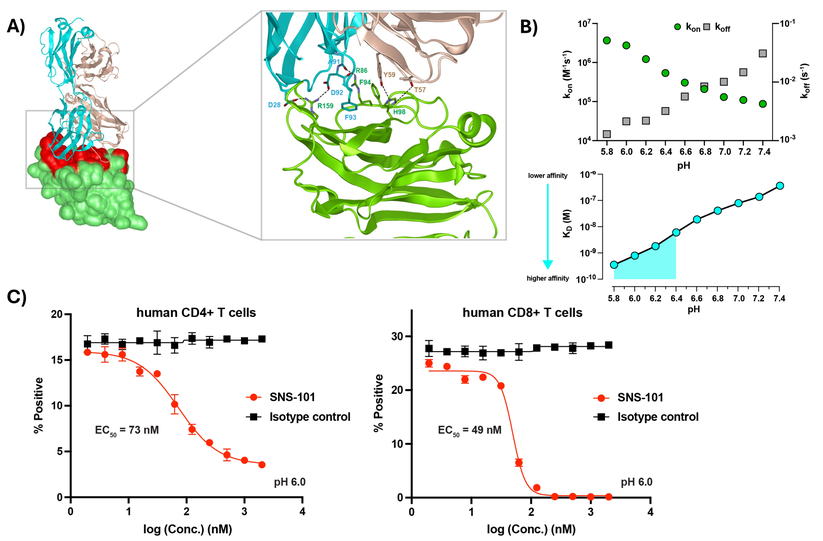

Figure 1. Structural and functional characterization of interaction between SNS-101 and VISTA. A) The crystal structure of the VISTA:SNS-101-Fab complex is shown with VISTA and SNS-101-Fab depicted in molecular surface (green) and ribbon cartoon (cyan–LC; Irish cream–HC), respectively (PDB: 8TBQ). The surface of VISTA blocked by SNS-101 (including all VISTA residues within 4Å of SNS-101) is marked in red. The zoomed inset highlights the interaction between critical residues essential for high affinity binding between VISTA and SNS-101. **B)** The pH-dependent association (k_{on}) and dissociation (k_{off}) rate constants (top panel) and equilibrium dissociation (K_D) constants (bottom panel) for the VISTA:SNS-101 interaction were determined by SPR. **C)** Competition assay for inhibition of VISTA binding to PSGL-1$^+$ human CD4$^+$ (left) or CD8$^+$ T-cells (right) by SNS-101 at pH 6.0. EC_{50} values from curve fits are indicated.

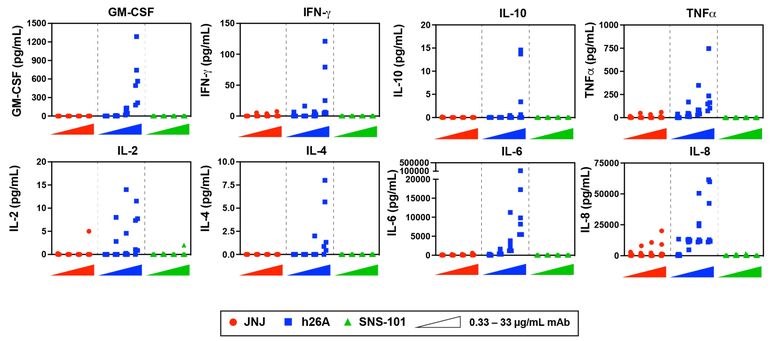

Figure 2. *In vitro* cytokine release. HUVEC:PBMC co-cultures were incubated with soluble JNJ (red), h26A (blue) or SNS-101 (green) mAbs. Eight cytokines in the culture supernatants from mAb-treated co-cultures (n=6 donors) were quantified by Multiplex Luminex assays (Bio-Rad). Each point represents the result from one donor. Antibody concentrations of 0.33, 3.33, 10 and 33.3 µg/ml were tested.

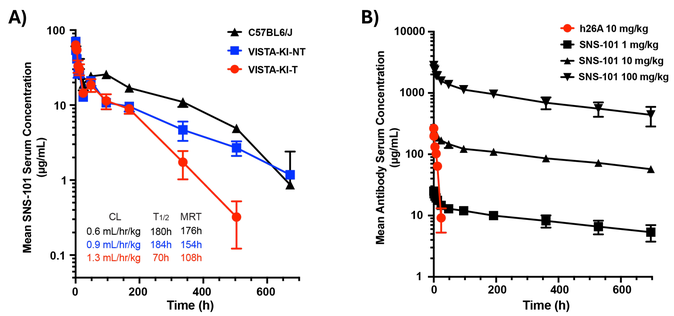

Figure 3. PK profile of SNS-101 in mice and cynomolgus monkeys. A) WT C57BL6/J and human VISTA knock-in (hVISTA-KI) mice with (T) or without (NT) MB49 tumors (n=4 per group) were given a single intravenous injection of 5 mg/kg SNS-101. Serum samples were collected over a 28-day observation period and tested for the presence of SNS-101 by ELISA. **B)** Non-human primates (n=4 per group) were administered a single intravenous infusion of SNS-101 or the comparator anti-VISTA antibody h26A over 1 hour, followed by a 28-day observation period. Circulating mAb was measured by ELISA.

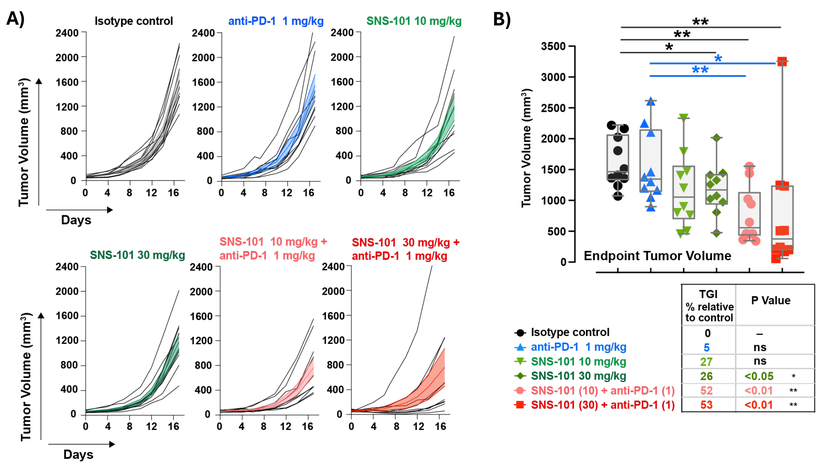

Figure 4. In vivo efficacy of SNS-101 and anti-PD-1 combination in syngeneic mouse models. A) Spider plots of MC38 tumor volume measurements for each mouse (n=12/group). **B)** Endpoint tumor volumes of MC38 tumors; statistical significance was evaluated using the Mann-Whitney unpaired t-test (*P<0.05; **P<0.01; *** P<0.01; **** P<0.0001). Box plots display the 25th to 75th percentiles with a median line. Whiskers represent the min-max range from smallest value to largest value. Table shows percent tumor growth inhibition (TGI).

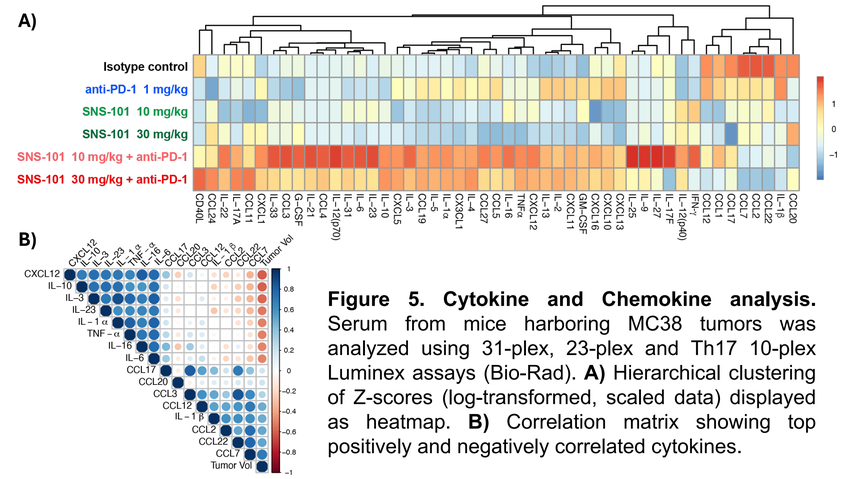

Figure 5. Cytokine and Chemokine analysis. Serum from mice harboring MC38 tumors was analyzed using 31-plex, 23-plex and Th17 10-plex Luminex assays (Bio-Rad). **A)** Hierarchical clustering of Z-scores (log-transformed, scaled data) displayed as heatmap. **B)** Correlation matrix showing top positively and negatively correlated cytokines.

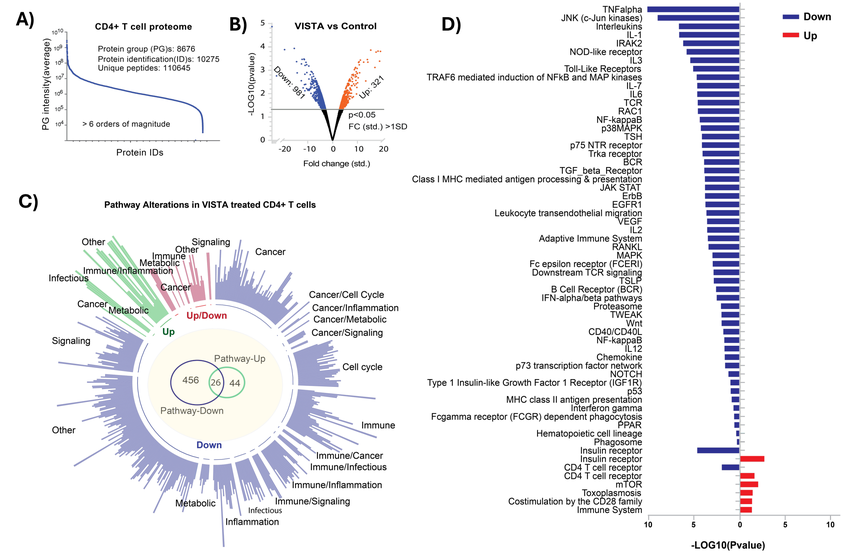

Figure 6. Proteomics and pathway analysis of VISTA signaling. PSGL-1 positive CD4$^+$ T-cells isolated from human PBMCs were treated with VISTA-Fc at 37℃ for 30 minutes, followed by LC-DIA-MS based proteomics. **A)** Qualitative assessment of protein identification. **B)** Volcano plot showing proteome changes upon VISTA binding. **C)** Overview of VISTA-dependent signaling pathway alterations detected. Line length corresponds to p-value for each enriched pathway. **D)** Pathway analysis demonstrates that VISTA binding impacts diverse CD4$^+$ T-cell functions.

CONCLUSIONS

- SNS-101's selectivity for active VISTA significantly reduces inflammatory cytokine release and CRS risk
- SNS-101 exhibits favorable pharmacokinetic properties including dose-proportional exposure and linear elimination kinetics, with no evidence for TMDD
- SNS-101 anti-tumor activity was demonstrated in multiple syngeneic mouse models, particularly in combination with anti-PD-1
- Mechanistic studies support the hypothesis that targeting the VISTA-PSGL-1 checkpoint alters macrophage function toward an M1-like pro-inflammatory, anti-tumor phenotype
- Coordinated targeting of T-cell checkpoints and myeloid suppressive signaling is likely an effective approach to combat resistance to first-line checkpoint inhibition
- SNS-101 has entered a Phase I clinical trial in advanced solid tumors (NCT05864144)

1. Gao J, *et al.* Nat. Med (2017) 23:551–555;4; **2.** Johnston RJ, *et al.* Nature (2019) 574:565–570; **3.** Curis Corporate Presentation Jan 2022 [http://investors.curis.com/events-and-presentations?item=100]