SNS-101, a clinical-stage pH-dependent and TME-selective anti-VISTA antibody with favorable pharmacokinetic and safety characteristics, promotes anti tumoral M1 macrophage polarization in PD-1 refractory settings

#P020

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INTRODUCTION

VISTA (V-domain Ig suppressor of T-cell activation) is an immune checkpoint expressed on abundant myeloid-lineage cells, including monocytes and neutrophils, suppressing T-cell activation when engaged with its target receptor PSGL-1[1, 2]. Importantly, VISTA is only active at low pH (~pH 6) such as in the tumor microenvironment (TME) due to protonation of surface exposed histidine residues[2]. VISTA inhibition demonstrated excellent therapeutic combinability with CTLA-4 or PD-1/PD-L1 T-cell checkpoint inhibitors in preclinical studies[3]. However, clinical development of anti-VISTA antibodies has been challenging due to (1) high clearance via target-mediated drug disposition (TMDD) by VISTA-positive neutrophils and monocytes at physiologic pH and (2) immune activation and cytokine release syndrome (CRS) at sub-therapeutic doses upon engagement of VISTA in the blood. Moreover, the molecular mechanism by which VISTA mediates immune suppression in the TME remains elusive.

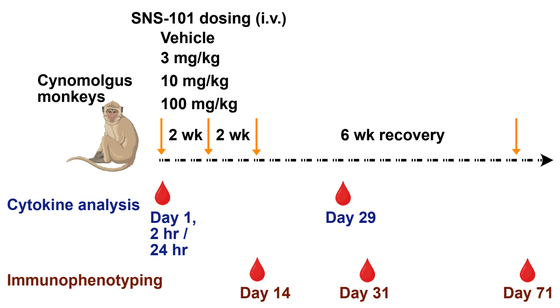
OBJECTIVE

- To prevent TMDD and mitigate potential CRS, we developed SNS-101, a human monoclonal IgG1 antibody specific for the protonated, active form of VISTA
- Pharmacokinetic (PK) and toxicity profile of SNS-101 was assessed in cynomolgus monkeys
- Serum from MC38 tumor-bearing animals treated with either SNS-101 alone or in combination with anti-PD-1 was analyzed to test the hypothesis that VISTA inhibition repolarizes myeloid cells to a proinflammatory/immune activating state

MATERIALS & METHODS

- A multi-dose GLP toxicology study in cynomolgus monkeys was conducted, dosing SNS-101 at 3, 10, and 100 mg/kg by 30-min intravenous infusion every 2 weeks (total of 3 doses), followed by a 6-week treatment-free recovery period
- Blood samples for cytokine analysis were collected on days 1 and 29, and at 2 and 24 hours after the end of each infusion
- Blood samples for leukocyte count determination and immunophenotyping were collected from animals in all groups at days 14, 31 and 71
- Anti-tumor efficacy was assessed in VISTA-KI mice implanted with syngeneic MC38 tumors, and serum of SNS-101-treated animals was analyzed using a 49-plex cytokine/chemokine panel

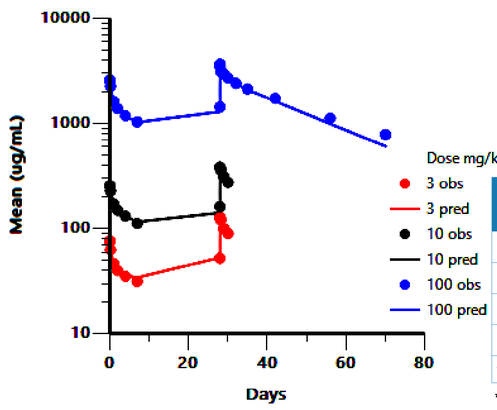
RESULTS



Group Number	Test Material	Dose Level (mg/kg/dose)	Number of Animals			
			Main Study		Recovery Study	
			Males	Females	Males	Females
1	Vehicle	0	3	3	2	2
2	SNS-101	3	3	3	–	–
3	SNS-101	10	3	3	–	–
4	SNS-101	100	3	3	2	2

Figure 1. Design of multi-dose toxicology study in NHPs. SNS-101 and vehicle were administered as indicated with 6 weeks recovery at the highest dose. Blood samples (red) for cytokine analysis or for immunophenotyping are indicated in blue and brown, respectively.

RESULTS



Dose (mg/kg)	$T_{1/2}\lambda$ (hours)		CL (mL/h/kg)		V_{ss} (mL/kg)	
	Mean	Range	Mean	Range	Mean	Range
*3	521	388-658	0.11	0.07-0.16	73.6	66.0-81.9
*10	566	433-728	0.09	0.08-0.11	71.1	57.3-77.3
*100	530	292-921	0.12	0.07-0.18	76.9	69.0-87.7
100	582	473-691	0.07	0.06-0.08	56.6	40.0-73.2

$T_{1/2}$=terminal half-life, CL=clearance, V_{SS}=Volume of distribution

* PK Parameters from single dose PK study in NHPs

Figure 2. PK profile of SNS-101 shows linear elimination kinetics in NHPs demonstrating absence of TMDD. PK data in NHPs demonstrate that serum concentrations of SNS-101 are dose-proportional over the range of 3 to 100 mg/kg. NHP blood samples were collected on Days 1 and 29 at the following time points processed to serum: pre-dose and at approximately 0.083, 1, 4, 24, 48, 96 (Day 1 only) and 168 (Day 1 only) hours post end of infusion (EOI) from all animals and on Day 29 at approximately 96, 168, 336, 672, and 1008 hours post EOI from recovery animals only. Points and lines represent observed concentrations and concentrations predicted by pharmacokinetic modeling, respectively.

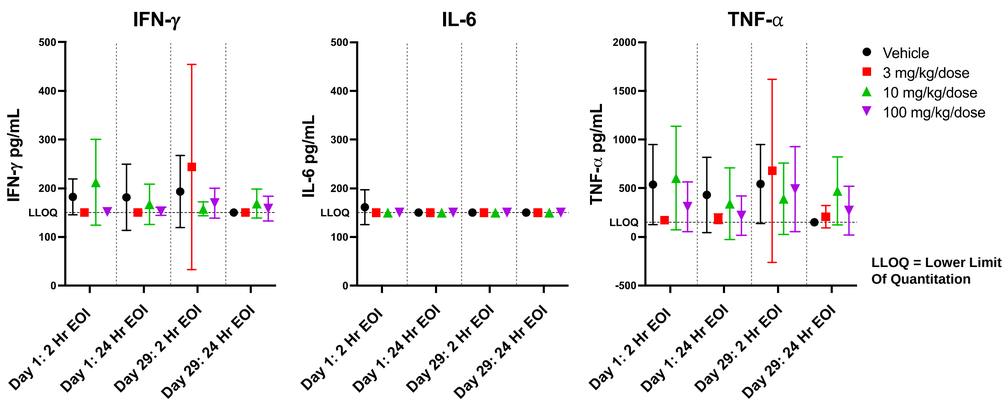


Figure 3. SNS-101 does not induce IFN-γ, IL-6, TNF-α in NHPs. There were no changes in the plasma IFN-γ, TNF-α, or IL-6 concentrations attributed to SNS-101 administration at any time point or dose level compared with the control group. NHP blood was collected by venipuncture and processed to plasma (K_2EDTA) for cytokine analysis. The basic design of this bead-based multiplex sandwich immunoassay was a solid phase protein assay, which used spectrally encoded magnetic beads conjugated to analyte specific capture antibodies as the solid support.

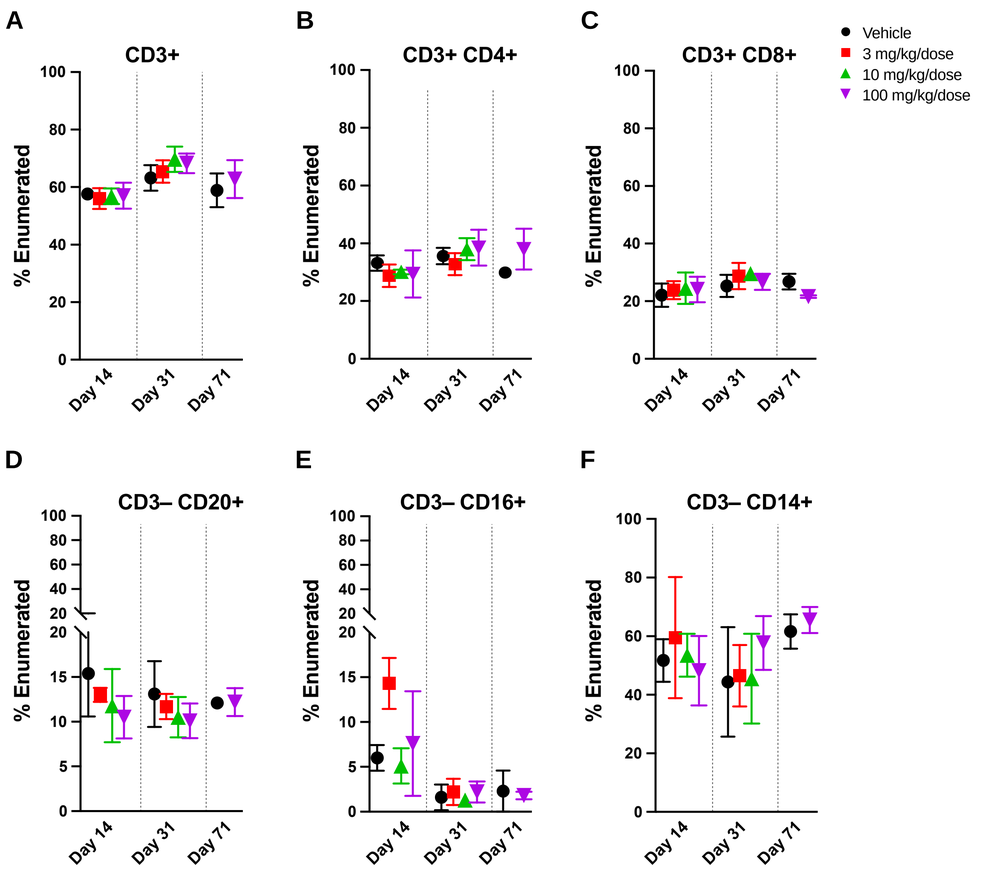


Figure 4. SNS-101 does not elicit peripheral blood immunophenotyping changes. No SNS-101-related peripheral blood immunophenotyping changes were noted at any time point or dose level compared with the control group. NHP blood samples were processed according to Standard Operating Procedures for immunophenotyping and results acquired using BD FACSCanto™ II Flow Cytometer with FACS Diva Software. White blood cells, which are positive for CD45 (pan leukocyte marker), were utilized as a marker for lymphocyte and monocyte gating. Flow cytometry analysis of the subsets shown was conducted using FlowJo X Software. (**A**) Total T-cells, (**B**) Helper T-cells, (**C**) Cytotoxic T-cells, (**D**) B-cells, (**E**) NK-cells, (**F**) Monocytes.

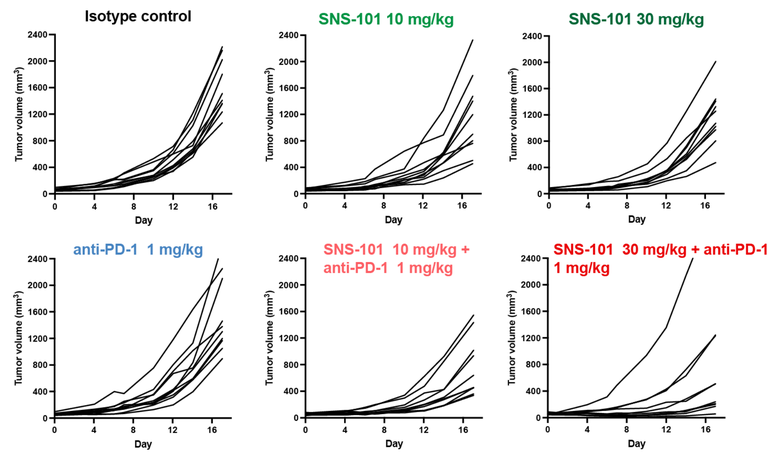


Figure 5. SNS-101 dose-dependently enhances anti-PD-1 response. Spider plots are shown. 1 x 10^6 MC-38 were implanted into female VISTA-KI mice. Mice were randomized (n=12/cohort) once tumor volumes reached ~100 mm^3 and received IP injections every 3 days for 2 weeks as indicated.

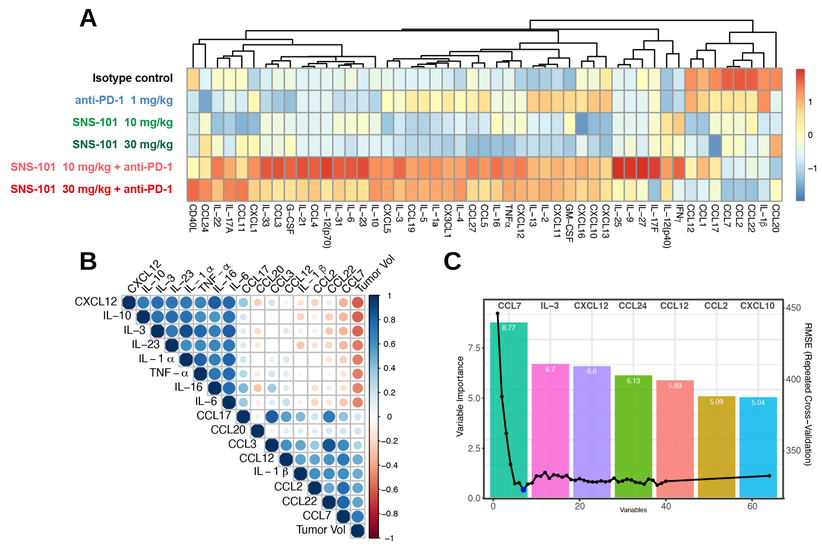


Figure 6. In vivo efficacy SNS-101 and anti-PD-1 correlates with changes in key cytokine and chemokine networks. Recursive feature selection by machine learning identified significant changes in key cytokines/chemokines related to tumor growth inhibition. Levels of CXCL-12, CCL-24 and IL-3 inversely correlated, and CCL-2, -7, -12 and CXCL-10 directly correlated with tumor volume reduction, respectively. Cytokines/chemokines were measured by 49-plex cytokine/chemokine Luminex panel. (**A**) Log-transformed data was analyzed by ANOVA with follow-up Tukey HSD testing. Heatmap was plotted using the "Pheatmap" R package and shows scaled cytokine/chemokine levels within each cytokine across different treatment groups. Hierarchical clustering was performed by calculating the pairwise Euclidean distance between scaled cytokine levels of individual (group) samples and merging into larger groups with the complete linkage algorithm until all samples merged into one final group. (**B**) Correlation between cytokines and tumor volume were generated using Pearson correlation coefficients. Top positively and negatively correlated cytokines are shown in the correlation matrix. (**C**) Fit of a linear regression model between tumor volume and all cytokine/chemokine levels showed a strong relationship (R^2=82%). Recursive feature selection was performed with the Random Forest algorithm. Selected cytokines were used to build a linear model to predict the tumor volume. The bar graph (left y-axis, top x-axis) shows the "importance" for model performance of the selected features. The line plot overlay (right y-axis, bottom x-axis) shows Root mean square error (RMSE) as a function of number of variables incorporated. Blue dot indicates the minimized RMSE of CCL7, IL-3, CXCL12, CCL24, CCL12, CCL2 and CXCL10.

CONCLUSIONS

- SNS-101 was well tolerated and no SNS-101-related adverse effects were observed up to doses of 100 mg/kg/dose
- SNS-101's exquisite selectivity for active, protonated VISTA significantly reduced CRS risk and previously observed rapid clearance with non-pH-sensitive VISTA antibodies was eliminated
- Cytokine/chemokine data supports a model in which SNS-101 targets suppressive signaling in the myeloid compartment, and in combination with anti-PD-1, helps shift the balance of macrophage polarization toward an anti-tumor M1 phenotype
- SNS-101 has entered clinical trials either as monotherapy or in combination therapy with a PD-1 inhibitor in patients with advanced solid tumors (NCT05864144)

1. Yuan L, *et al.* Cancer Immunotherapy. Trends Immunol. 2021; 42:209-227; 2. Johnston RJ, *et al.* Nature 2019; 574:565–570; 3. Gao J, *et al*. Nat. Med. 2017; 23:551–555