SNS-101, a conditionally active anti-VISTA antibody, potentiates anti-tumor effects of PD-1 blockade and displays favorable pharmacokinetic and cytokine release characteristics

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INTRODUCTION

VISTA (V-domain Ig suppressor of T-cell activation) is an immune checkpoint, which suppresses T-cell activation and is highly expressed on myeloid cells, including macrophages and neutrophils[1]. 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, which enables its binding to the T-cell receptor PSGL-1[2]. VISTA inhibition demonstrates 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 historically been challenging due to three major factors: 1) lack of clarity on the identity of the critical counter-receptor responsible for T-cell suppression; 2) high clearance via target-mediated drug disposition (TMDD) by VISTA$^+$ neutrophils and monocytes at physiologic pH and 3) cellular activation and cytokine release syndrome (CRS) at sub-therapeutic doses by engagement of VISTA in the blood[4]. We developed SNS-101, a conditionally active human monoclonal IgG1 antibody specific for the protonated, active form of VISTA, which is designed to disrupt the immunosuppressive VISTA:PSGL-1 interaction, avoid TMDD and mitigate potential CRS.

MATERIALS & METHODS

- A hVISTA:SNS-101 Fab complex was crystallized and structure determined at 2.59 Å resolution to assess the epitope of SNS-101.
- Circulating whole blood assay (ID.Flow) with six healthy donors was performed to predict CRS[5]. Plasma samples were collected at baseline and after 4h.
- Effect of SNS-101 on human monocytes and T-cells was evaluated *in vivo* in human CD34$^+$ cord blood cell reconstituted BRGSF-HIS mice (reconstituted functional **h**uman **i**mmune **s**ystem), which develop both human lymphoid and myeloid compartments.
- Pharmacokinetic (PK) profile was assessed in NHPs. SNS-101 (1, 10, and 100 mg/kg) and h26A (10 mg/kg) were administered to monkeys (n=4; 2/sex) once via intravenous infusion for 1hr.
- Anti-tumor efficacy was assessed in VISTA-KI mice implanted with the syngeneic tumor models, MC38 and 1956.
- Tumor-infiltrating T-cells were analyzed by flow cytometry.
- SNS-101 was compared to two clinical stage, non-pH-selective anti-VISTA antibodies by grafting variable regions onto a human IgG1 framework: 1) JNJ (JNJ-61610588[6] (now CI-8993)) or 2) h26A[7]

RESULTS

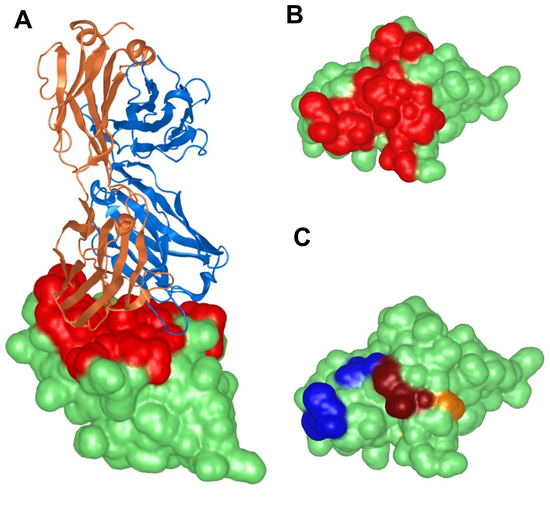


Figure 1. Crystal structure of hVISTA:SNS-101 Fab complex. (**A**) Orange and blue ribbon indicate light and heavy chain of SNS-101, respectively. The SNS-101 epitope, including amino acids within 6Å, is shown in red (**A & B**), (**C**) Residues on VISTA involved in interaction with PSGL-1[2] (blue), VSIG-3[8] (brown) and LRIG-1[8] (orange) highlighted on the VISTA structure.

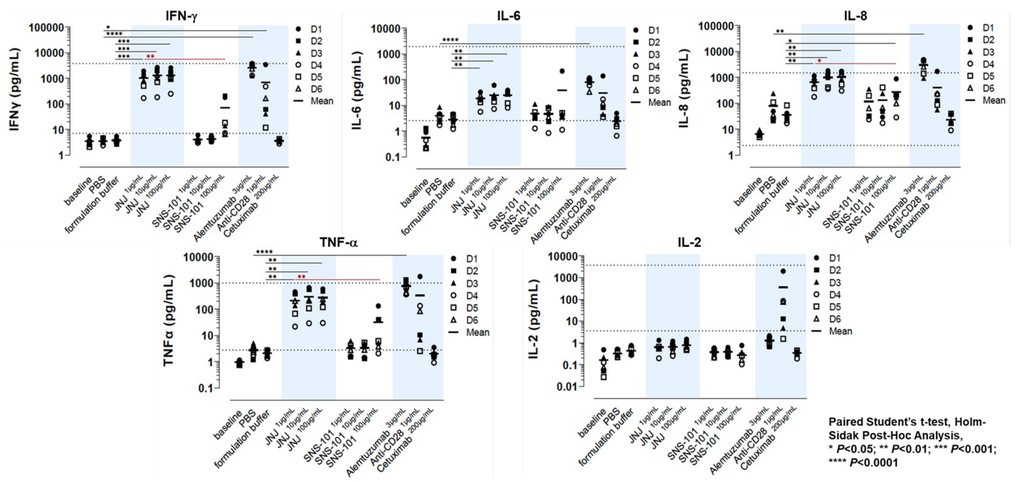


Figure 2. SNS-101 does not induce significant cytokine induction (IL-2, IL-6, IFN-γ, TNF-α) compared to JNJ. Positive control antibodies alemtuzumab & anti-CD28, and cetuximab (neg. isotype control) were compared to PBS, while JNJ and SNS-101-treated samples were compared to formulation buffer, and selected JNJ- and SNS-101-treated samples were compared to each other (red).

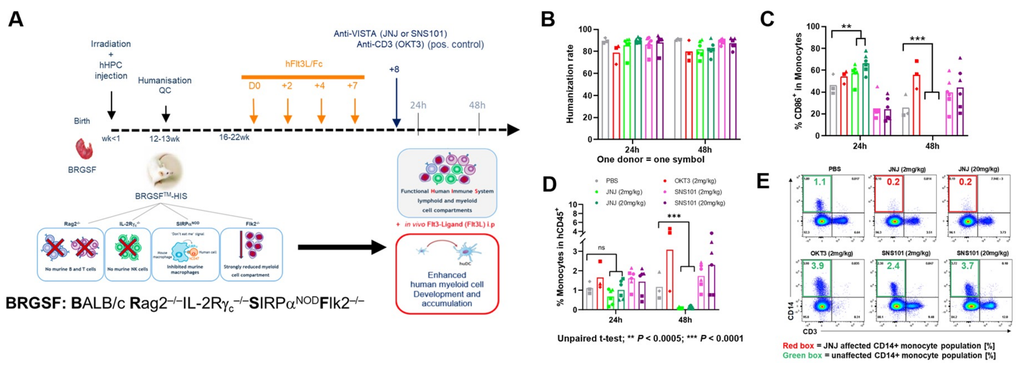


Figure 3. SNS-101 has no significant impact on monocyte activation. (**A**) BRGSF-HIS mice were generated as previously described[9]. (**B**) BRGSF-HIS mice were quality controlled by assessing human CD45$^+$ cells in spleen. Mice were sacrificed at 24h and 48h and immune cell proportions in spleen were evaluated by flow cytometry. Non-pH-sensitive antibody JNJ induces (**C**) monocyte activation (CD86$^+$) followed by (**D**) a decrease in monocyte proportions (CD45$^+$). (**E**) Representative dot plot at 48h post-treatment shows JNJ-induced decrease in CD14$^+$ monocytes.

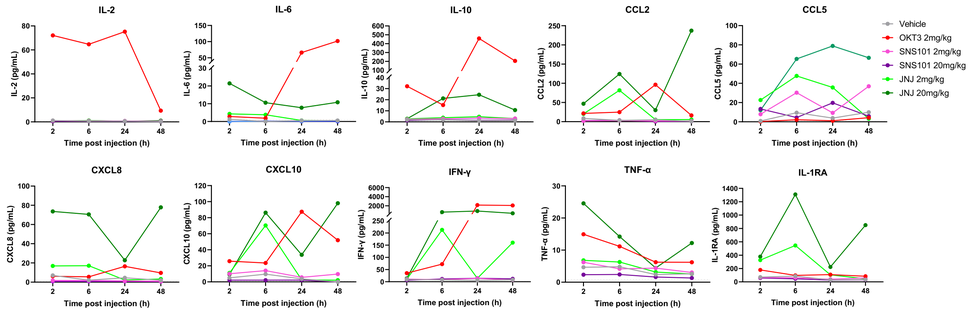
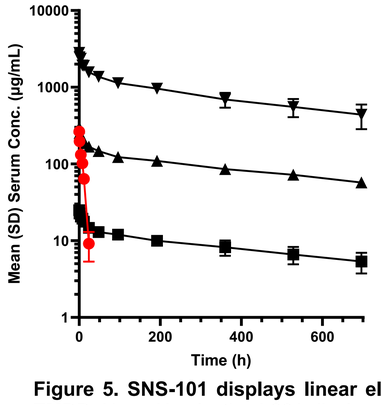


Figure 4. SNS-101 only mildly induces CCL-5 vs. dose-dependent induction of IL-6, IL-10, CCL-2 CCL-5, CXCL-8 CXCL-10, IFN-γ, TNF-α, and IL-1RA by JNJ. Serum of 6 mice per time point was collected and cytokines were quantified by a Multiplex bead-based assay. Positive control anti-CD3 (OKT3) efficiently induced CRS. Dotted line = detection limit.



Dose (mg/kg)	$T_{1/2}\lambda$ (hours)		CL (mL/h/kg)		V_{ss} (mL/kg)	
	Mean	Range	Mean	Range	Mean	Range
1	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

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

Figure 5. SNS-101 displays linear elimination kinetics in NHPs consistent with the absence of TMDD. Non-pH-selective h26A displayed pronounced TMDD and was below limit of quantitation at 48h.

RESULTS

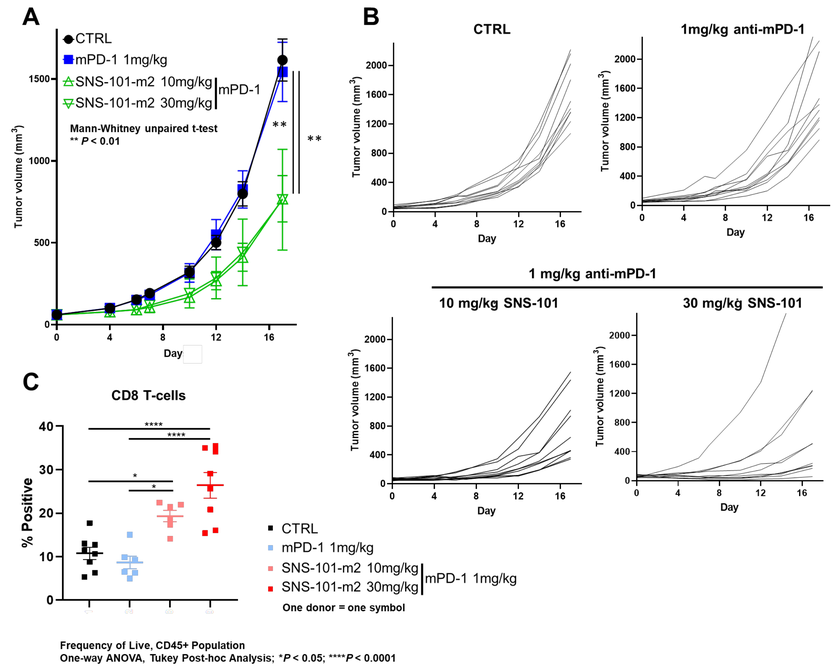


Figure 6. SNS-101 enhances anti-PD-1 response and dose-dependently increases tumor-infiltrating CD8 T-cells. (**A**) Mean tumor volumes, (**B**) spider plots and (**C**) tumor-infiltrating CD8 T-cells are shown. 1 x 10^6 MC-38 were implanted into female VISTA-KI mice. Mice were randomized (n=10/cohort) once tumor volumes reached ~60-80 mm^3 and received IP injections every 3 days for 2 weeks as indicated. At Day 17 post-treatment, single cell suspensions were generated from tumor extracts through physical and enzymatic dissociation. Frequency of CD8+ cells was determined in the singlet, live, CD45$^+$ population by analytical flow cytometry analysis.

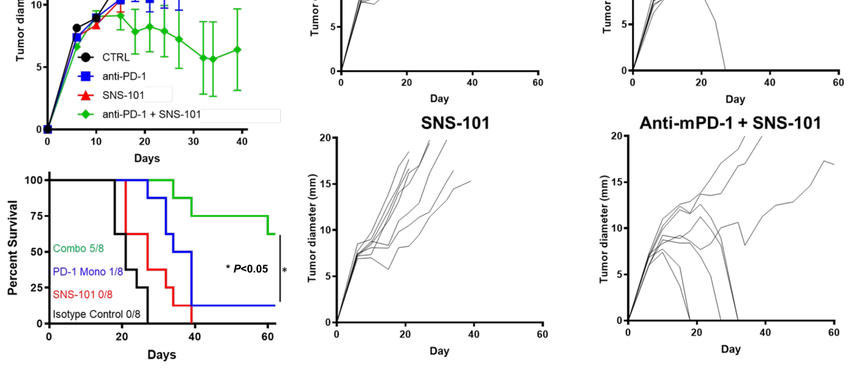


Figure 7. SNS-101 re-sensitizes anti-PD-1 insensitive 1956 sarcomas. Mean tumor diameters (top left), spiderplots (right) and survival curves (lower left) are shown. 1.5 x 10^6 1956 cells were implanted into female VISTA-KI mice. After 10 days, mice (n=8/cohort) received IP injections every 3 days (anti-mPD-1 and SNS-101 were dosed at 10 mg/kg and 20 mg/kg, respectively).

CONCLUSIONS

- Crystal structure analysis at 2.59 Å resolution suggests SNS-101 directly blocks the pH-dependent interaction between VISTA and PSGL-1, as well as interactions with other putative receptors
- *In vitro* and *in vivo* CRS assays suggest that SNS-101 has a significantly lower risk of inducing CRS than a non-pH-dependent VISTA antibody
- SNS-101 exhibited linear elimination kinetics in NHPs, overcoming TMDD-induced PK limitations observed with other anti-VISTA antibodies
- SNS-101 induced expansion of naïve and memory T-cell phenotypes *in vivo* without activation or depletion of monocytes, differentiating it from non-pH-selective VISTA antibodies
- SNS-101 demonstrated significant enhancement of anti-tumor effects in combination with anti-PD-1 antibodies in syngeneic tumor models. In the MC-38 model this was association with an increase in CD8$^+$ T-cells
- Together, these data demonstrate that SNS-101's exquisite selectivity for active, protonated VISTA can abrogate TMDD and lower CRS risk, while significantly enhancing the anti-tumor effects of PD-1 blockade

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; 4. Curis Corporate Presentation Jan 2022 [http://investors.curis.com/events-and-presentations?item=100]; 5. Fletcher *et al. Int Immunopharmacol.* 54, (2018); 6. Patent WO2017175058; 7. Patent WO2016094837; 8 Thakkar *et al. J. Immunother Cancer* 10, (2022); 9. Lopez-Lastra *et al.* Blood Adv 2017; 10:601-614.