Antagonistic pH-selective VISTA antibody SNS-101 potentiates anti-PD-1/PD-L1-induced anti-tumor immunity

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BACKGROUND

Immunotherapies, especially immune checkpoint inhibitors, are a cornerstone of cancer treatment. Remarkable clinical responses have been observed blocking the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis across a spectrum of indications. However, innate and/or acquired resistance to anti-PD-1 blockade remains a major challenge. V-domain Ig suppressor of T-cell activation (VISTA) is a B7-family member, which promotes T-cell and myeloid quiescence and represents a promising target, particularly in combination with anti-PD-1/PD-L1 treatment[1,2]. Recently, the interaction of VISTA with its receptor PSGL-1 was demonstrated to be significantly enhanced by the acidic tumor microenvironment (TME)[3]. As VISTA is highly expressed on myeloid cells, including those in the blood, antibodies binding **VISTA** at **p**hysiological **pH** 7.4 (**VISTA^{pph}**) could result in rapid elimination from circulation through target-mediated drug disposition (TMDD), making efficacious drug occupancy levels difficult to reach and potentially narrowing the therapeutic window. A pH-non-selective clinical molecule (JNJ-61610588) exhibited TMDD and induced dose-limiting on-target cytokine release syndrome (CRS) at subtherapeutic dose levels. An antibody engineered to selectively bind and block **VISTA** at **l**ow **pH** in the TME (**VISTA^{lph}**) may therefore be required as an anti-VISTA drug candidate.

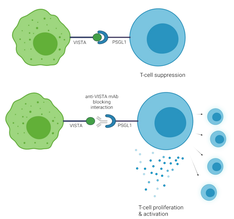

Figure 1. VISTA is a negative regulator of T-cell function. Antibody-mediated blockade of VISTA:PSGL-1 interaction in the tumor microenvironment results in T-cell activation.

METHODS

- Fully human anti-VISTA antibodies were generated through pH-selective enrichment strategies of a yeast-based platform library comprising highly diverse synthetic immune repertoires
- 'Parental' antibodies were extensively characterized using flow-cytometry, surface-plasmon resonance (SPR) and PSGL-1/VISTA inhibition assays in primary human CD4 and CD8 T-cells at pH 6.0 and pH 7.4
- 8 parental antibodies were identified and tested for combinatorial efficacy with anti-mouse PD-1 (rat mAb clone RMP1-14) *in vivo* in human VISTA knock-in mice inoculated with syngeneic MC-38 tumors
- Further optimization of top 8 parental antibodies for enhanced binding affinity and selectivity at pH 6.0 over pH 7.4 was performed
- 'Progeny' antibody ranking was based on the same *in vitro* and *in vivo* characterization techniques as parental antibodies

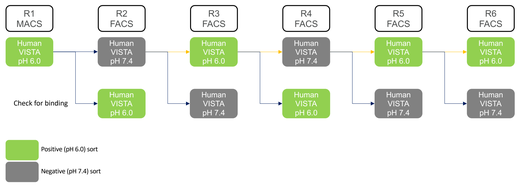

Figure 2. Strategy implemented for primary selection of pH-dependent anti-VISTA antibodies. Selection of yeast-based platform libraries alternated between positive enrichment rounds at pH 6.0 and negative selection rounds at pH 7.4. Antibody-expressing yeast populations were incubated with the antigen at the specified pH, which was maintained during secondary labeling and sorting. Round of selection is represented by "R". MACS = Magnetic Activated Cell Sorting. FACS = Fluorescence Activated Cell Sorting.

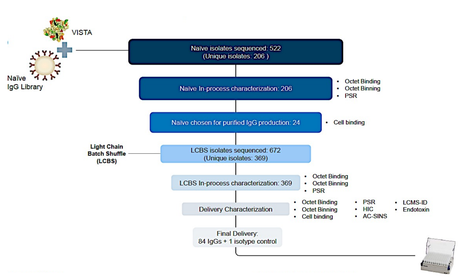

Figure 3§. Discovery of parental anti-VISTA antibodies.

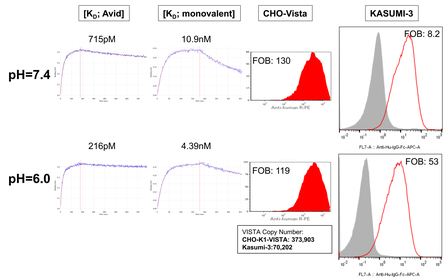

Figure 4. Binding characteristics of exemplary antibody. Avidity and monovalent affinity measurements at pH 7.4 and pH 6.0 by Octet. Binding profile of anti-VISTA mAb on CHO-VISTA (VISTA overexpressed) and KASUMI-3 (endogenous VISTA) cells. FOB = Fold over background.

RESULTS

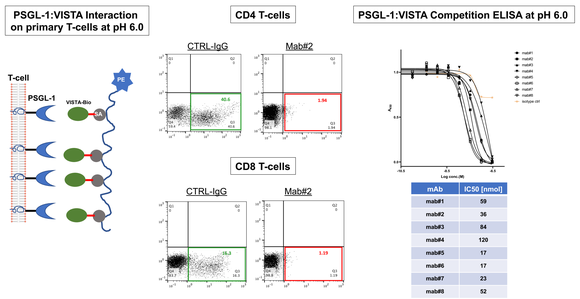

Figure 5. Inhibitory characteristics of anti-VISTA antibodies. VISTA-Dextramer-PE interacts with PSGL-1 on primary T-cells at pH 6.0 (left panel). Anti-VISTA mAb#2 inhibits VISTA:PSGL-1 interaction on CD4 and CD8 T-cells (middle panel). Top 8 anti-VISTA mAbs ranked by PSGL-1:VISTA competition ELISA (right panel).

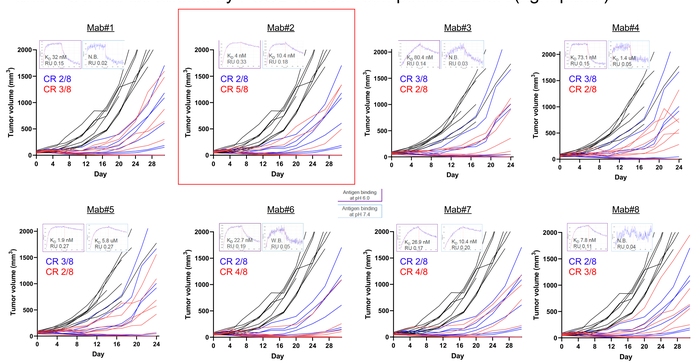

Figure 6. MC38 tumor growth inhibition in human VISTA knock-in mice. 1 x 10^6 MC-38 cells were implanted into female VISTA-KI mice. Mice were randomized (n=8/cohort) once tumor volumes reached ~60-80 mm^3. Antibodies were administered I.P. twice/wk for **2 weeks** at 40 mg/kg total (20 mg/kg each). **Black Line** (IgG CTRL human & rat), **Blue Line** (IgG CTRL human & rat anti-mPD-1; **Red Line** (rat anti-mPD-1 & anti-VISTA). CR= Complete response.

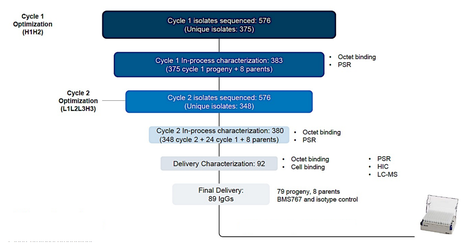

Figure 7§. Lead-optimization of top 8 parental anti-VISTA antibodies.

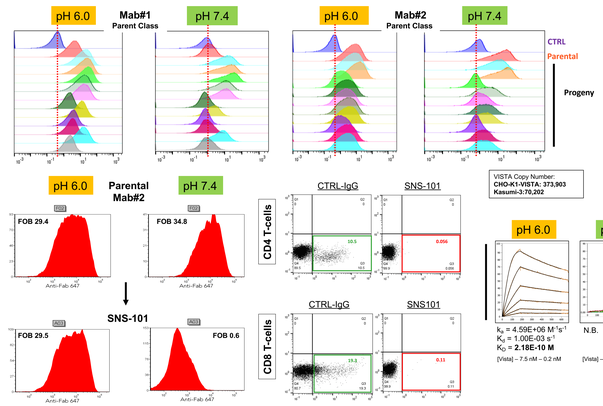

Figure 8. Characterization of optimized progeny antibodies. Binding profile of 2 representative anti-VISTA parental antibodies and their respective progeny at pH 6.0 and pH 7.4 to KASUMI-3 (top panel). Binding profile (F_{ab} on CHO-VISTA), inhibitory function and SPR characterization of SNS-101 (bottom panel). For SPR, monomeric VISTA at identical concentrations was passed over antibody immobilized on a Protein A surface at pH 6.0 or 7.4. RU = response units; N.B. = Non-binding; FOB = Fold over background.

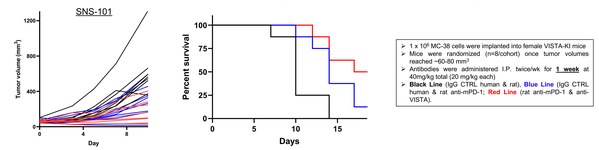

Figure 9. 'High-bar' *in vivo* test of SNS-101 activity.

SUMMARY

- 84 parental antibodies were initially discovered
- Flow-cytometry and SPR analysis revealed candidates displaying pH-dependent binding to endogenously expressed native VISTA on cells
- PSGL-1/VISTA inhibition assays at pH 6.0 identified potent antagonists
- 8 candidate antibodies were tested in an *in vivo* intervention study in combination with anti-murine PD-1 demonstrating varied combinatorial efficacy with a subset leading to superior tumor rejection
- Characterization of optimized progeny antibodies led to identification of anti-VISTA antibody SNS-101
- SNS-101 is a pH-selective, high-affinity, cynomolgus monkey cross-reactive IgG1 with excellent biophysical and biochemical properties

CONCLUSION

- Enrichment of highly diverse antibody libraries led to the identification of a pH-selective inhibitory anti-VISTA antibody SNS-101, which exerts excellent combinability with anti-PD-1 leading to superior anti-tumor activity with an anticipated reduced risk for CRS
- SNS-101 has entered IND-enabling studies

1. Gao *et al. Nature Medicine* 23, (2017); 2. Yuan *et al. Trends in Immunology* 42, (2021); 3. Johnston *et al. Nature* 574, (2019). § Created with BioRender.com.