Highly Potent Fully Human Anti-VISTA Antibodies Efficiently Abrogate the Interaction of VISTA to Its Putative Receptors at Different pH.

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Background

- VISTA (V-domain Ig Suppressor of T cell Activation) is a unique CD28/B7 family member with poorly defined receptors. However, VISTA itself, PSGL-1, VSIG3, VSIG8 and LRIG1 have been suggested as putative receptors.
- VISTA is highly expressed on circulating and intratumoral myeloid cells as well as NK cells and Treg.
- VISTA is a negative regulator that directly suppresses T cell activation and proliferation.
- High VISTA expression correlates with poor survival in cancer patients.
- VISTA is a unique immune checkpoint inhibitor for tumor immunotherapy.

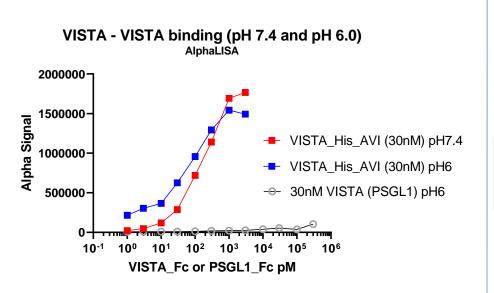
Objectives

- Various research groups have demonstrated that VISTA can interact with at least 5 receptors: VISTA itself, PSGL-1 in acidic conditions, VSIG3, VSIG8 and LRIG1.
 - Identify and/or confirm the putative receptors for VISTA.
 - Describe the expression of these potential receptors on the different blood cell populations.

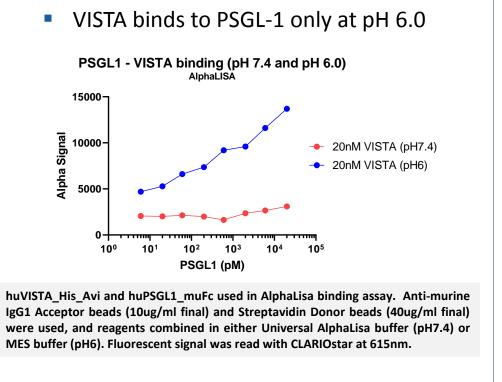
Results VISTA and VISTA Receptors Expression on Human Whole Blood Leukocyte Populations Results representative of 3 healthy donors: Donor # 4903 shown Eosinophils Whole peripheral blood from three donors was stained with against granulocytic, myeloid, a series of antibodies against VISTA, VISTA ligands, or isotype controls. Samples were fixed and RBC lysed, acquired on an Attune flow cytometer, and analyzed with FlowJo software. Histogram overlays of VISTA or Ligand staining vs. isotype control are shown. Fold Over Background = Anti-VISTA MFI or Anti-Ligand MFI ÷ Isotype Control **Granulocytic Lineage** Myeloid Lineage T Cell Lineage NK Lineage CD56 bright CD4/CD69 CD8/CD69 Comp-RL1-A:: AF647-Alexa Fluor 647-A

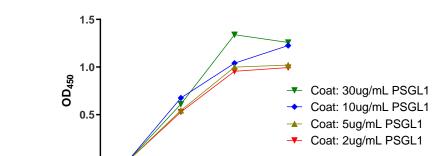
VISTA interacts with its putative receptors at different pH

 VISTA binds strongly to VISTA at pH 7.4 and pH 6.0



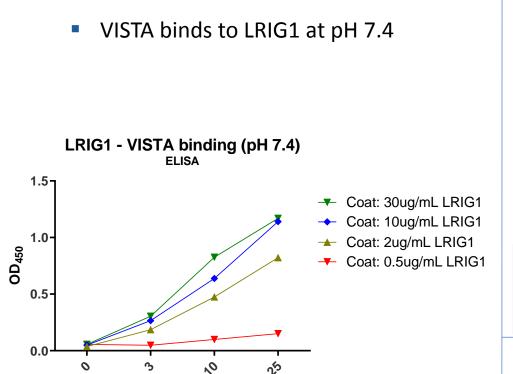
huVISTA_His_Avi and huVISTA_huFc or huPSGL1_muFc used in AlphaLisa binding assay. Anti-human IgG1 or anti-murine IgG1 Acceptor beads (10ug/ml final) and Streptavidin Donor beads (40ug/ml final) were used, and reagents were combined in either Universal AlphaLisa buffer (pH7.4) or MES buffer (pH6). Fluorescent signal was read with CLARIOstar at 615nm.





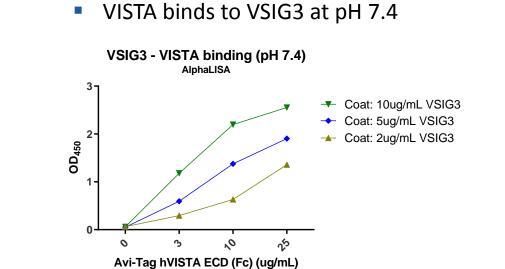
PSGL1 - VISTA binding (pH 6.0)

Wells were coated with 2, 5, 10, or 30 ug/ml of PSGL1 ECD (Fc). After washing, washed and TMB substrate added for 10 minutes, and the reaction stopped with 2N sulfuric acid. Plates were read with the CLARIOstar at 450nm

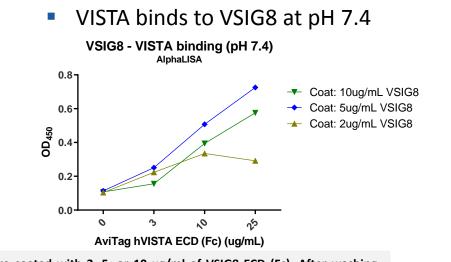


Wells were coated with 0.5, 2, 10, or 30 ug/ml of LRIG1 ECD (His). After washing hVISTA ECD (biotinylated) was diluted to 3, 10, and 25 ug/ml and added to the wells. After incubation at room temperature for 2 hours, plates were washed. Streptavidin-HRP diluted to 1:200 was added for 30 minutes. Plates were then washed and TMB substrate added for 10 minutes, and the reaction was stopped with 2N sulfuric acid. Plates were read with the CLARIOstar at 450nm

hVISTA ECD (Fc) (ug/mL)



for 30 minutes. Plates were then washed and TMB substrate added for 10 minutes, and the reaction was stopped with 2N sulfuric acid. Plates were read with the CLARIOstar at 450nm.



Ab EC50 (nM)

3.0

1.5

N/A

~ 6.1

2.6

Pos. Cont 2.9

KVA 1.1

KVA 5.1

KVA 17.1

KVA 12.1

KVA 23.1

VA 5.1

(VA 12.1

(VA 23.1

(VA 27.1

CVA 17.1 ~ 30.23

~ 31.23

stopped with 2N sulfuric acid. Plates were read with the CLARIOstar at 450nm.

KVA Abs Disrupt VISTA-LRIG1 Interaction

── KVA 1.1

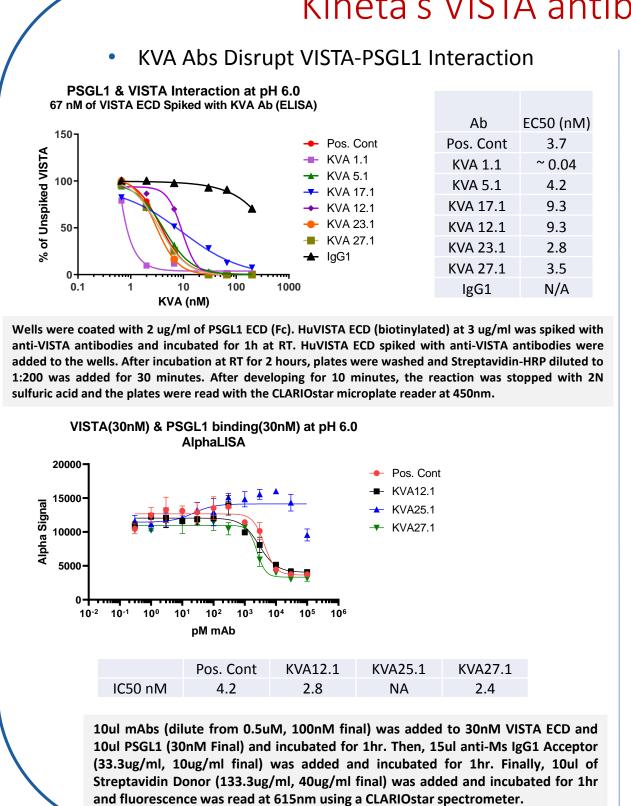
★ KVA 5.1

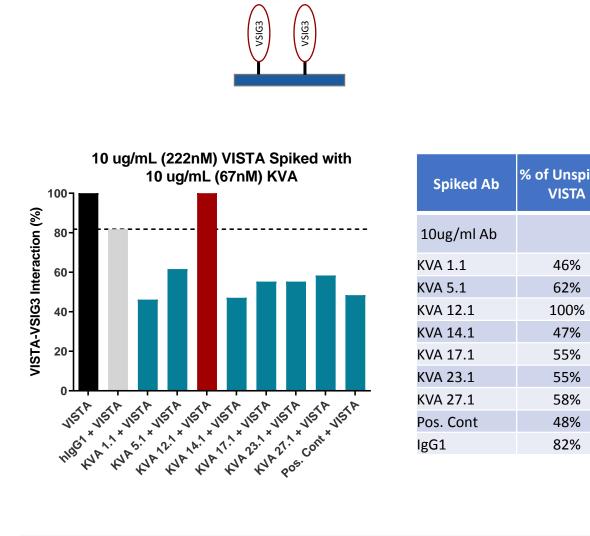
→ KVA 17.1

→ KVA 12.1

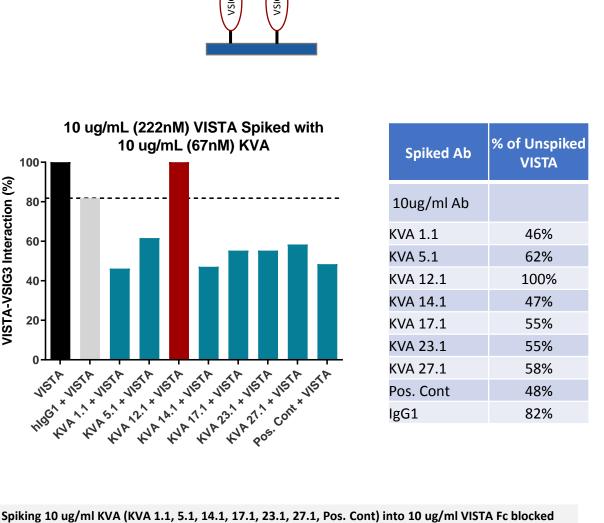
◆ KVA 23.1

Kineta's VISTA antibodies block VISTA interaction with PSGL-1, VSIG3, VSIG8 and LRIG1

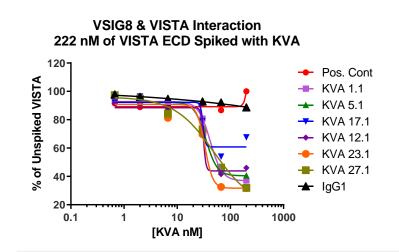




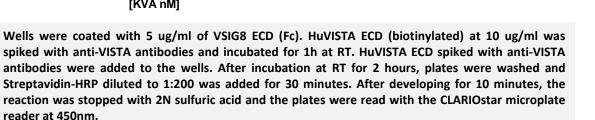
KVA Abs Disrupt VISTA-VSIG3 Interaction



KVA 27.1 2.5 N/A lgG1 [KVA nM] Wells were coated with 10 ug/ml of LRIG1 ECD (His). HuVISTA ECD (biotinylated) at 3 ug/ml was spiked with anti-VISTA antibodies and incubated for 1h at RT. HuVISTA ECD spiked with anti-VISTA antibodies were added to the wells. After incubation at RT for 2 hours, plates were washed and Streptavidin-HRP diluted to 1:200 was added for 30 minutes. After developing for 10 minutes, the reaction was stopped with 2N sulfuric acid and the plates were read with the CLARIOstar microplate reader at 450nm. KVA Abs Disrupt VISTA-VSIG8 Interaction **VSIG8 & VISTA Interaction** EC50 (nM



67 nM of VISTA ECD Spiked with KVA Ab



Conclusion

- VISTA is highly expressed on Myeloid cells as well as Granulocytes, NK and NKT cells.
- VISTA is also expressed on Treg and modestly on CD4 and CD8 cells. CD4/CD69 activated T cells seems to be high expresser.
- PSGL-1 is highly expressed on all immune cells.
- LRIG-1 has low level expression on Myeloid cells as well as CD4+ T cells.
- VISTA binds to the 5 putative receptors already identified including itself at neutral or acidic pH.
- Kineta's anti-VISTA antibodies selectively inhibit these interactions with different potencies.