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# A Fully Human anti-VISTA Antibody as a Promising Therapy Against Poorly Immunogenic Tumors

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## Background

- VISTA (V-domain Ig Suppressor of T cell Activation) is a unique B7 family member, mainly expressed on myeloid cells, neutrophils, NK cells and Treg.
- VISTA is highly expressed on MDSC and Treg in the TME and play an important role in immune modulation.
- 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.

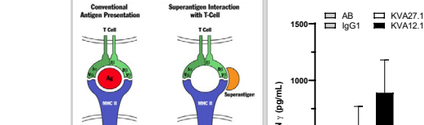
### Increasing Patient Survival Remains a Significant Unmet Need for Cancer Patients

**Hot Tumors**

- Current Checkpoint therapies are effective and improve patient survival: Only for 20% of the Patient population.

↑ Anti-cancer T cells

Solution = T cell targeted therapies




**Cold Tumors**

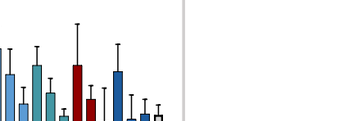
- Not responsive to current and T cell centered checkpoint therapies
- Poor patient survival: 80% of the Patient population

↓ Anti-cancer T cells : Anergy, MHCneg, No T cells

↑ Immunosuppressive TME

↑ VISTA expression high and driver of Immunosuppression

Solution = TME targeted therapies

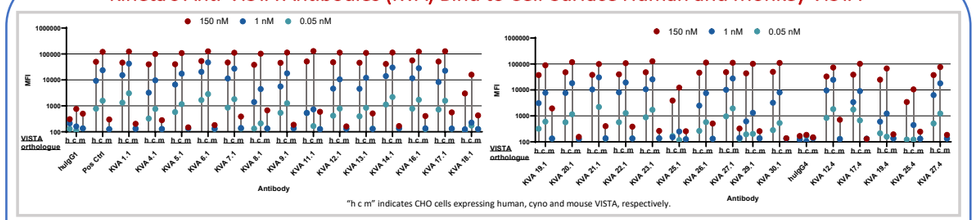



## Objectives

- Screen for specific fully human anti-VISTA antibodies.
- Evaluate and select an antagonist anti-VISTA antibody (KVA) as clinical candidate from *in vitro* and *in vivo* studies.
- Characterize KVA12.1, our lead candidate for IND submission.

## Results

### Kineta's Anti-VISTA Antibodies (KVA) Bind to Cell-Surface Human and Monkey VISTA



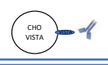




**Figure 1. Anti-human VISTA antibodies titrate against CHO-K1 cell lines stably expressing human and cynomolgus, but not mouse VISTA.** 1.5e5 VISTA CHO-K1 cells were indirectly labeled with titrating amounts of human IgG1 or human IgG4 KVA series anti-VISTA antibodies and Gt anti-Human IgG (H+L-specific)-PE. PE Mean Fluorescence Intensity was determined on viable cells by flow cytometry. h=human, c=cynomolgus, m=mouse.

### KVA Antibodies Bind Only To VISTA and Not Other B7 Family Proteins

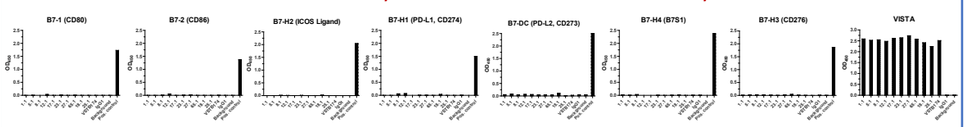



**Figure 2.** Plot of $OD_{450}$ values of anti-VISTA antibodies (10 µg/mL) on a plate coated with 2 µg/mL B7 family proteins (his tag) or 2 µg/mL VISTA protein (his tag).

### KVA Antibodies Enhance T cell Activation in SEB activation Assay




**Figure 3.** Human PBMCs were negatively selected for CD56+ NK cells using EasySep kits. 2 x $10^5$ cells per well were stimulated with 5 ng/mL SEB super-antigen in the presence of 0.03, 0.3 or 3.0 µg/mL (0.2 – 20 nM) of the indicated anti-VISTA antibody for 3 or 4 days at 37°C. IFNγ production was measured using a ProQuantum kit. Data are represented as the mean fold-induction of IFNγ over background (isotype antibody treatment at 3 µg/mL). N=5 data points per sample. Mean ± SD.

### Kineta's Anti-VISTA Antibodies Induce Monocyte Activation

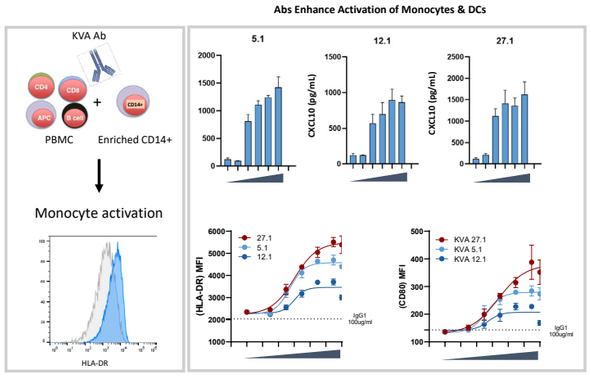



**Figure 4.** 0.5E5 CD14⁺ enriched human PBMCs were co-cultured with 2E5 human PBMCs in the presence of KVA Abs or control Abs tested at 100, 10, 1, 0.1, 0.01, 0.001 and 0.0001 µg/ml for 24 hrs. Upregulation of activation markers on CD14⁺ cells at 24 hrs was quantified by Flow Cytometry on gated CD14⁺ live cells (MFI). CXCL10 production was measured by ELISA. Data are mean ± SD of n=3.

### KVA Mediated Monocyte Activation is Dependent on NK Cells

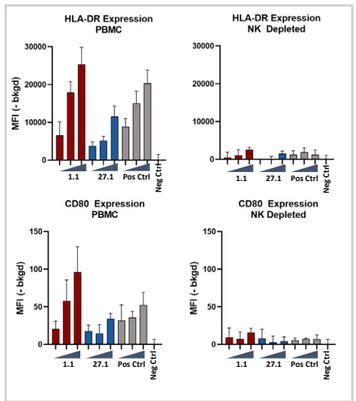



**Figure 5.** CD14⁺ enriched human PBMCs were co-cultured with either human PBMCs, or NK-depleted PBMCs from the same donor with KVA Abs or control Abs tested at 3, 0.3, 0.03 µg/ml for 24 hrs. Upregulation of activation markers on CD14⁺ cells at 24 hrs was quantified by Flow Cytometry on gated CD14⁺ live cells.

### KVA Antibodies Enhance NK Cell Activation

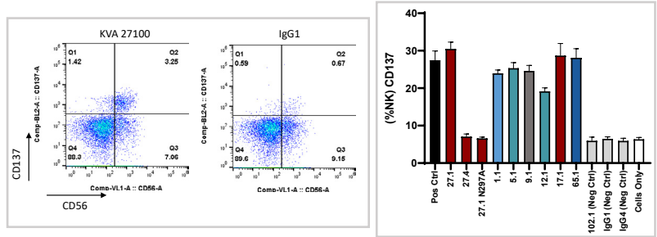



**Figure 6.** Monocyte activation assay performed with total PBMCs as described previously with 10 µg/mL Ab. At 24 hrs, sample was measured for the number of CD137+ NK (CD56+) cells by Flow Cytometry. Data are mean ± SD of n=4.

### Kineta's Anti-VISTA Antibodies Reverse MDSC Suppression of T Cells

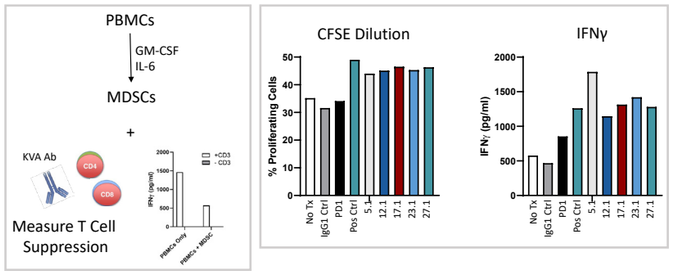
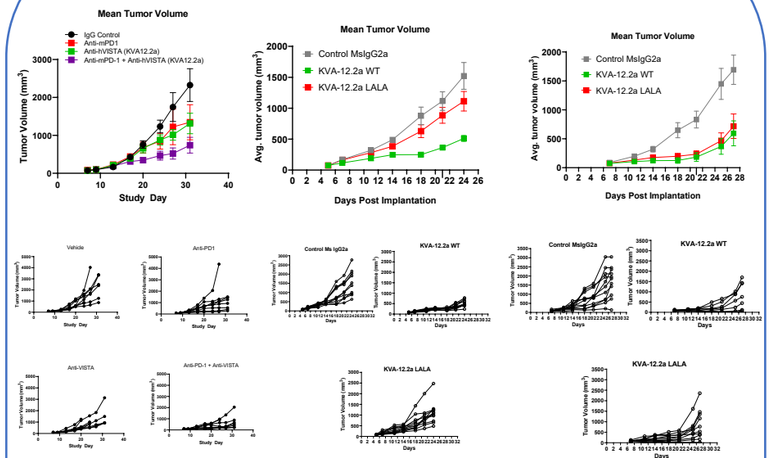



**Figure 7.** 6E7 PBMCs were cultures for 7 days with GM-CSF and IL6 (10 ng/mL ea.) after which cells were collected and CD11b+ or CD33+ cells were isolated and evaluated for VISTA expression by flow cytometry. 1E5 MDSCs were added to 1E5 PBMCs labeled with Cell Trace in the presence or absence of 4 µg/mL anti-CD3 antibody and 10 µg/mL anti-VISTA antibody and incubated for 4 days. Cell proliferation of CD3+ T cells was measured by flow cytometry and IFNγ secretion was measured using ProQuantum.

### KVA12 Antibody Induces Strong Anti-tumor Response as a Single Agent or in Combo-therapies




**MC38 Tumor Model:** 1E6 MC38 tumor cells were inoculated subcutaneously in the right rear flank region subcutaneously to female hVISTA HuGEMM KI mice (Genoway). Mice were randomized to study groups when tumors reached 70 – 100 $mm^3$. Day 8 was the first dosing day with mouse anti-PD1 (5 mg/kg Q2W), Kineta anti-hVISTA (10 mg/kg Q3W), isotype control (vehicle) or combination of anti-PD-1 and anti-hVISTA. Treatments were dosed IP. Tumor volumes (L x $W^2$ x 0.5) were measured twice per week.

**MB49 Tumor Model:** 5E5 MB49 tumor cells were inoculated subcutaneously to female hVISTA HuGEMM KI mice (Genoway). Mice were randomized to study groups when tumors reached 70 – 100 $mm^3$. Day 5 was the first dosing day with Kineta anti-hVISTA (WT or LALA on mIgG2a; 30 mg/kg Q2W), or isotype control (vehicle). Treatments were dosed IP. Tumor volumes (L x $W^2$ x 0.5) were measured twice per week.

**EG-7 Tumor Model:** 1E6 EG-7 tumor cells were inoculated subcutaneously to female hVISTA HuGEMM KI mice (Genoway). Mice were randomized to study groups when tumors reached 70 – 100 $mm^3$. Day 7 was the first dosing day with Kineta anti-hVISTA (WT or LALA on mIgG2a; 30 mg/kg Q2W), or isotype control (vehicle). Treatments were dosed IP. Tumor volumes (L x $W^2$ x 0.5) were measured twice per week.

## Conclusions

- **107 fully human ScFv anti-VISTA antibodies were generated and analyzed.**
- **Kineta's anti-VISTA lead antibody is highly specific.**
- **Kineta's anti-VISTA lead antibody activates monocytes, and this activation is NK dependent.**
- **Kineta's anti-VISTA lead antibody reverse MDSC suppression of T cells.**
- **Kineta's anti-human VISTA lead antibody induces strong anti-tumor response as a single agent or in combo-therapies with anti-PDL1 or anti-CTLA4 in different hard to treat tumor models.**
- **KVA 12.1 is our lead antibody for IND enabling studies.**