Highly Potent Fully Human anti-VISTA Antibodies
A New Target Checkpoint Inhibitor against Immunosuppressive Myeloid Cells

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Kineta Inc. focuses on targets that integrate innate and adaptive immune response
VISTA a new Target Checkpoint Inhibitor in Immunotherapy

**VISTA a B7 family ligand**

- Membrane protein of the Ig SF member of the B7 family
- Strongest similarity with PD1-L1
- Express on Myeloid cells, Neutrophils, NK cells and Treg
- High express on MDSC and Treg in the TME

**Function**

- Inhibitory effect on T cell activation
- APC-expressed VISTA interacts as a ligand with an unknown receptor on activated T cells that results in co-inhibition
- Function as a co-inhibitory receptor expressed on T Cells

Ling Ni & Chen Dong Immunol. Reviews 2020
VISTA is predominantly expressed in the myeloid lineage within peripheral blood

Human PBMCs

VISTA is Highly expressed on Immune Cells

Human Blood

VISTA is predominantly expressed in the myeloid lineage within peripheral blood
VISTA Has a Profound Negative Regulatory Effect on T Cell Activation and Proliferation

Effect of VISTA-Fc on anti-CD3-induced T cell proliferation. Immobilized VISTA-Fc or IgG1-Fc was incubated on plates with immobilized anti-CD3 (OKT3), and the ability of VISTA to suppress anti-CD3-induced T cell proliferation and cytokine production was assessed. 96-well flat-bottomed plates were coated with 2 mcg/mL anti-CD3 +/- 0, 2, 6, 20 or 60 mcg/ml VISTA-Fc or IgG1-Fc. Human pan-T cells from a healthy donor were labeled with CellTrace Violet and plated at 2ES cells/well. After 4 days of incubation, T cell proliferation was analyzed via flow cytometry; IFNγ concentrations were measured by ELISA. Representative graphs are shown. Means of % proliferating cells or IFNγ production ± SEM are graphed.
Kineta Is Developing Human Anti-VISTA Antibodies

- We have screened 107 fully human ScFv antibodies directed against VISTA
- We have evaluated and selected highly potent VISTA antagonist antibodies in vitro and in vivo

**Immunization of humanized Trianni® mice**
- *In vivo* affinity maturation in mouse leads to high affinity antibodies
- VDJ recombination allows unique fully human variable domains

- Microfluidics-based sequencing of single B cell clones
- Native pairing of heavy and light chains
- Flow-based enrichment using yeast ScFv display libraries

- 15 V<sub>H</sub> diversity groups
- 15 V<sub>L</sub> diversity groups
- Highest diversity in CDR3H
Kineta’s anti-VISTA Antibodies are Highly Potent

### ELISA Binding

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<th>Concentration (ug/ml)</th>
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### Octet Binding

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### Flow Cytometry Binding

### Classical Monocytes CD14+/CD16-

### Kasumi-3 (AML)
Kineta’s anti-VISTA Antibodies Bind to Cynomolgus Monkey VISTA-ECD

Forty-two (42) fully human monoclonal antibodies were tested for cross-species binding to human and cynomolgus monkey VISTA. Scatter plot of log mean OD_{450} values of anti-VISTA antibodies (2 mcg/mL) with 1 mcg/mL plate bound human or cyto VISTA ECD.

- **ELISA Binding**

  - $R^2=0.99$
  - Slope=1.0

- **Octet Binding**

  - $R^2=0.87$
  - Slope=1.4

Lead monoclonal antibodies were tested for cross-species binding to human, mouse and cynomolgus monkey VISTA-ECD using Octet. Scatter plot of mean $K_D$ values of anti-VISTA antibodies immobilized on AHC biosensors and interrogated with human or cyto VISTA ECD (50nM).

- **Kineta’s Anti-VISTA Antibodies are Highly Specific to Human and Cynomolgus Monkey VISTA but do not recognize Mouse VISTA**
VISTA Antagonist Antibody Induces Strong Anti-Tumor Response as a Single Agent or in Combo-Therapies

Mean Tumor Volume
(Error Bars represent SEM)

Days Post Implantation
Avg. tumor volume (mm³)

Mean Tumor Volume
( Error Bars represent SEM)

Days Post Implantation
Avg. tumor volume (mm³)

Flow Analysis of Whole Blood

Increases in Dendritic Cells

Decreases in Myeloid Cells (MDSC)

CT26 mouse model

VISTA and/or PDL-1

VISTA and/or CTLA-4

Blood - CD11b+ DCs

Blood - CD11c+ DCs

Blood - CD11b+ Myeloid Cells

AARCR VIRTUAL SPECIAL CONFERENCE: TUMOR IMMUNOLOGY AND IMMUNOTHERAPY
Conclusions

- 107 fully human ScFv anti-VISTA antibodies were generated and analyzed
- Kineta’s anti-VISTA antibodies carry unique sequences and the selected lead candidates exhibit potencies in the subnanomolar range
- Kineta’s human anti-VISTA antibodies are highly specific and cross-react with Cyno-VISTA but not Mouse-VISTA
- VISTA antagonist antibody induces strong anti-tumor response as a single agent or in combo-therapies with anti-PDL1 or anti-CTLA-4 in CT26 tumor models
- VISTA antagonist antibody activates Dendritic Cells in the blood and reduces MDSCs