VISTA, a novel immune checkpoint protein ligand that suppresses anti-tumor T cell responses

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The B7 Immunoglobulin Super-Family immune regulators

APC

MHC

TCR

Co-stimulatory:
B7.1/2 - CD28
B7-H2 - ICOS

Co-inhibitory

B7 - CTLA-4
PD-L1/2 - PD-1
B7-H3 – TLT2
B7-H4 – ?

etc……
Immune checkpoint-mediated T cell suppression during tumorigenesis

Tumor

PDL-1

PD-1

CTLA-4

B71/2

T effector cells

Myeloid Suppressor cells

PD-1

PD-1

PDL-1

CD28

PDL-1

PD-1

Foxp3+ Tregs

CTLA-4
Antibody-mediated CTLA-4 blockade in combination with a cellular vaccine (Gvax) induced regression of established poorly immunogenic B16 melanoma.  

Ipilimumab, the human aCTLA-4 mab, was approved by the FDA in March 2011 to treat patients with late-stage melanoma.

Ipilimumab has also undergone early phase trials for other cancers, such as lung cancer and prostate cancer.  
● Calabro et.al. 2010, *Semin Oncol* 37:460-467
Blocking the PD-L1:PD-1 pathway, in conjunction with other immune therapies, inhibits tumor progression.

- Geng et.al. 2006, *Int J Cancer* 118:2657-2664
- Pilon-Thomas et.al. 2010 *J Immunol*. 184: 3442-9

MDX-1106, the human anti-PD-1 mab has entered clinical trials.

  Phase I study of anti-programmed death-1 (MDX-1106) as single-agent in refractory solid tumors is well tolerated and demonstrates clinical anti-tumor activity.
The B7 Immunoglobulin Super-Family immune regulators

**Co-stimulatory:**
- B7.1/2 - CD28
- B7-H2 - ICOS

**Co-inhibitory Checkpoint Pathways:**
- B7 – CTLA-4
- PD-L1/2 – PD-1
- B7-H3 – TLT2
- B7-H4 – ?
- VISTA
VISTA: a new checkpoint protein, and a V-domain Immunoglobulin Suppressor of T cell Activation

Ig-v domain structural model of VISTA, using PD-L1 as template

Sequence of the Ig-v domain
VISTA is highly expressed on CD11b^{hi} myeloid cells

Monocytes/macrophages

DCs
VISTA is expressed on T cells

Peripheral LN  Spleen

CD8+ cells  CD4+ cells  isotype

Foxp3+ nTregs  CD4+ naive  CD4+ memory

VISTA
VISTA expression on human PBMC cells

Data contributed by Janet L. Lines
Immobilized VISTA-Ig fusion protein inhibits T cell activation

T cell proliferation

Plate-bound \( \alpha \text{CD3} + \text{VISTA-Ig or control Ig} \)

\( \alpha \text{CD3} : \text{VISTA or control Ig} \)

1:1

1:4

CD4\(^+\) T cells

CD8\(^+\) T cells

Control Ig

VISTA-Ig
Plate-bound αCD3 + VISTA-Ig or control Ig

► Inhibit proliferation, but do not enhance apoptosis.
► Inhibit activation markers: CD69, CD44, CD62L.
► Inhibit T cell cytokine production (IL2, IFNγ etc).
► Suppression can be partially rescued by exogenous IL-2.
► PD-1 KO T cells are also inhibited.
VISTA promotes the induction of adaptive Tregs

Plate-bound αCD3 + VISTA-Ig or control-Ig +/- TGFβ

**human**

<table>
<thead>
<tr>
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<th>VISTA-Ig</th>
<th>TGFβ</th>
<th>Foxp3GFP+%</th>
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<tr>
<td>untreated</td>
<td>-</td>
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<td>0</td>
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<tr>
<td>2.5 ug/mL</td>
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<tr>
<td>5 ug/mL</td>
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**mouse**

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<th>TGFβ</th>
<th>Foxp3GFP+%</th>
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<tr>
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<td>-</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
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VISTA expression on APC suppress T cell proliferation

Similar results are obtained using BM-derived DCs that are transduced with VISTA-expressing retrovirus
VISTA expression on tumors impairs protective αtumor immunity

Day-14
Irradiated MCA105

Day 0
Live tumor
Challenge:
MCA105-RFP
MCA105-VISTA

Measure tumor growth (mm²)

- MCA105-control
- MCA105-VISTA

P = 0.0011

Tumor size (mm²)

Time (days)
VISTA expression on tumors impairs protective αtumor immunity

Day-14
Irradiated MCA105

Day 0
Live tumor
Challenge:
MCA105-RFP
MCA105-VISTA

Measure tumor growth (mm²)

Time (days)
Tumor size (mm²)

P = 0.0011

- MCA105-control
- MCA105-VISTA
- MCA105-control T-depletion
- MCA105-VISTA T-depletion
VISTA monoclonal antibody treatment enhances inflammatory disease

Adoptive transfer primed pathogenic CD4+ T cells

Day 0

treat with control-Ig or αVISTA

Monitor EAE progression

SJL

Disease score

Disease incidence (%)

13F3 (n=7)
control-Ig (n=8)
p<0.0001

13F3
control-Ig
p<0.0001
VISTA-specific antibody controls tumor growth

Bladder tumor MB49 (s.c.)

IFNγ ELISPOT

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<tr>
<th>spots/250k cells</th>
<th>control</th>
<th>13F3</th>
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<tr>
<td>25</td>
<td>⬆️</td>
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*p value: 0.001*
VISTA-specific antibody controls tumor growth

Fibrosarcoma
MCA105 (s.c)

Thymoma
EG7 (s.c)
VISTA-specific antibody controls tumor growth

Ovarian tumor ID8-luciferase (peritoneal)

Control-Ig | 13F3
---|---

Day30
- p value: 0.03
- (n=8)

Day55
- p = 0.0167
- (n=8)
VISTA-specific antibody controls tumor growth

Skin tumor B16F10-OVA

Vaccine: CD40 agonist + LPS + OVA

Days

control
13F3
control + vaccine
13F3+vaccine

Days

tumor size (mm^2)
VISTA-specific antibody controls tumor growth

B16F10
(day-2 prophylactic treatment)
Combinatorial blockade of VISTA and PD-L1/PD-1 results in better tumor control

**B16F10**
*(day+4 therapeutic treatment)*

The diagram shows the tumor size (mm²) over time in days for different treatments:
- **Control** (n=10)
- **MIH5** (n=10)
- **113F3** (n=16)
- **Combo** (n=16)

Significance is indicated by ***.
VISTA and PD-L1/PD-1 synergize to suppress T cell proliferation

VISTA-Ig fusion protein + PD-L1-Ig fusion protein + αCD3/CD28

CD4⁺ T cells

CD8⁺ T cells
The inducible melanoma model

**Tyr::Cre/ERT2:**
Tyrosinase promoter driven expression of Cre-ERT2, permitting tamoxifen-inducible, melanocyte-specific cre expression.

**Braf^CA:**
carrying a conditional Braf^{V600E} allele, permitting cre-mediated expression of Braf^{V600E}

**Pten^{1ox5}:**
carrying a conditional allele of Pten, permitting cre-mediated deletion of exon 5

*Dankort et al 2009 Nature Immunology*
High VISTA expression within the tumor microenvironment

- Gated CD11c^+
- Gated CD11b^+

Legend:
- TIL
- naïve LN
- dLN
- isotype
αVISTA blockade inhibited the growth of the inducible melanoma
Conclusions

VISTA functions as a novel immune checkpoint protein ligand:

🌟 controls inflammation and autoimmunity.

🌟 impairs the generation of anti-tumor immunity.

🌟 VISTA antibody-mediated blockade either alone, or in combination with other checkpoint blockade might provide a novel therapeutic strategy for cancer immunotherapy.

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