Regulating the Regulators for Cancer Immunotherapy: LAG-3 Finally Catches Up

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The hostile immune microenvironment within a tumor

- Tumor
  - Stat3
  - B7-H1
  - B7-H4
  - MΦ/MSC/iMC
  - NO
  - A2aR
  - B7-H4
  - adenosine
  - VEGF, IL6, IL10, IL23

- NK cell
  - Stat3
  - Lytic activity
  - degranulation
  - granulocyte

- CD4 T cell
  - FoxP3
  - Treg
  - TGFβ
  - IL10
  - LAG-3

- DC
  - Stat3
  - maturation
  - IL-23

- Tumor-specific CTL
  - Stat3
  - maturation
  - IL-23

- MSC/iMC
  - B7-H1
  - IL-23

- PDC/VEGF, IL6, IL10, IL23

- TGFβ
Combinatorial immune therapies to hit distinct steps in the evolution of antitumor immunity

- Vaccines
  - TLR agonists
  - Costimulators

- Blockade of Immunologic Check Points
  - CTLA-4 blockade
  - B7-H1/4, PD1 blockade
  - STAT-3 blockade

- Blockade of Regulatory T cells

- Manipulation of the Tumor Microenvironment
  - Combinatorial immune therapies to hit distinct steps in the evolution of antitumor immunity

↑Ag Presentation By Appropriately Activated DCs + ↑Costimulation
Peripheral T cell tolerance

**Activation**

(γ-IFN, CTL activity)

**Tolerance**

(Anergy)

**Regulatory T cell**

FoxP3
LAG-3
GITR
A2aR

**Naïve T cell**

TCR

**APC**

MHC-Ag

**signal 1**

**signal 2**

**B7**

**CD28**

Activation

(Anergy)
The Probasin – Hemaglutinin (ProHA) Transgenic Mouse

HA wt

HAs

HAF
The ProHA x TRAMP Mouse (ProTRAMP)
A tumor tolerance model

- ProSV40 – Oncogenic
- ProHA – A Tumor / and Tissue Specific Antigen
- Disease grossly identical to TRAMP
- 12’th Generation Intercross onto B10.D2
- HA-specific CD4 and CD8 TCR transgenic T cells transferred into ProTRAMP are TOLERIZED. HA-specific CD4s become Treg
Mouse model → Immunogenomics → Therapeutic Ab production

Back to the mouse model to test therapeutic Ab
Genes Involved in Expression of the Tolerant Phenotype in T Cells

**In VIVO**
- Upregulated Day 4 Anergy vs. Day 4 Activation (n=20,000)
- Upregulated Day 4 Anergy vs. Day 7 Activation (n=18,000)

**In VITRO**
- Upregulated in Signal 1 Alone vs. Unstimulated (n=17,000)
- Upregulated in Signal 1 Alone vs. CSA Blocked (n=18,000)

N=13,000

N=12,000

N=4,500

HAM Analysis
N = 151

<table>
<thead>
<tr>
<th>Symbol</th>
<th>IN VIVO Ratio Anergy / Memory</th>
<th>IN VITRO Ratio Anergy / Resting</th>
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LAG-3 Real Time PCR

Days in vivo

Relative Expression

- Anergy/Treg
- Effector/Memory

Days in vivo
LAG-3 is highly expressed on the surface of induced Treg
LAG-3

- Cloned in early 1990s
- CD4 homologue
- Does not substitute for CD4 in T cell development or helper T cell function
- Binds MHC II with higher affinity than CD4 but at a distinct site from CD4
- Function unclear - reported to play a role in modulating NK function, T cell function, APC function but no clear conclusions
- Cytoplasmic tail completely different from CD4. Signaling pathways unclear
Transduction of CD4+CD25- T cells with wild type LAG-3 confers regulatory capacity.
Can LAG-3 blockade alter endogenous T cell function?

Day 0: α-LAG-3 (0.2mg)
Day 1: VAC-HA
Day 3: α-LAG-3 (0.2mg)
Day 6: Adoptive transfer targets

Mix 1:1; inject i.v.

Unstimulated
CFSE<sup>lo</sup>
B10.D2 splenocytes

HA peptide-pulsed
CFSE<sup>hi</sup>

Day 7: Harvest spleens; Flow

ProTramp

Unstimulated

ProTRAMP+vaccine

ProTRAMP+vaccine+anti-LAG3
Tumor-tolerized endogenous CTL regain effector function in vivo after LAG-3 blockade

ProTramp (14-16 wk old)
α-LAG-3 leads to endogenous CD8 migration and TNF-α production within prostates of ProTRAMP mice.

ProTramp

ProTramp + α-LAG-3
Prostate tissue from Pro-Tramp mice 7 days post-AT

Clone 4 Adoptive Transfer

Clone 4 Adoptive Transfer + anti-LAG-3
Histology of prostate cancers treated with vaccine + anti-LAG-3 antibodies
LAG-3 and FoxP3 expression are concordant on antigen-specific CD4 T cells only when antigen is present as self or tumor.
CD4+25+ Treg from tumor bearing mice are highly suppressive in *in vitro* assays
Membrane protein encoding genes upregulated among CD4+ cells infiltrating human prostate cancer

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<th>Probe ID</th>
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LAG3 expression on tumor specific CD8 T cells restrains their accumulation and function of in prostate cancers.
CD4-independent role of LAG-3 for tumor-specific CD8 T cells
LAG-3 downregulates TCR dependent signaling

A

LAG-3+  
LAG-3-/-  

Min. post P/I
P-PLCγ1  
total PLCγ1

B

Ca++

LAG-3 KO  
LAG-3 WT

Time (ms)

Cross-Link
ProTRAMP model
Charles Drake
Adam Adler

LAG-3
Charles Drake
Ching-Tai Huang
Joe Grosso
Tulia Bruno
Ed Hipkiss
Christin Kelleher
Dario Vignale
Craig Workman