Considerations to overcome downstream resistance to melanoma antigen-specific effector T cells

Thomas F. Gajewski, M.D., Ph.D.
University of Chicago
Recognition of class I MHC-restricted tumor antigen peptides by CD8\(^+\) CTL

**Antigen discovery:**
- Quickly led to vaccine clinical trials
- Based on notion that fundamental defect in patients is failed T cell priming
- Results: Vaccines often increase specific CD8\(^+\) T cells in blood
- Nonetheless, tumor regressions are rare

**Present conundrum:**
- Was that the right hypothesis?
- Spontaneously activated melanoma antigen-specific T cells can be found in patients
- Detected in blood and within tumors
- e.g. this is starting point for TIL therapy
- **Points to downstream resistance as dominant defect in many patients**
Tumor escape from the effector phase of an anti-tumor immune response may be a major obstacle.
Melanoma patients can exhibit very high frequencies of circulating Melan-A-specific IFN-γ-producing CD8+ T cells.

HIV control subtracted.
Some melanoma metastases are replete with lymphocytes
Focus on defect in effector phase of immune response in tumor microenvironment
Understanding mechanisms of negative regulation of T cell function in tumor microenvironment

- Candidate processes
  - Inhibitory receptors (e.g. PD-L1/PD-1)
  - Inhibitory cell populations (e.g. Tregs)
  - T cell intrinsic dysfunction (e.g. anergy)

- Analyze tumor microenvironment from metastatic melanoma tumors
  - TIL function, phenotype, and molecular profile
  - Real-time RT-PCR candidates and validation
  - Gene array analysis of stromal elements

Are there drugable targets?
1. PD-1/PD-L1

- PD-1: receptor induced on activated T cells
- Contains ITIM and ITSM domains that can recruit SHP2
- PD-1-deficient mice develop autoimmune syndromes => dominant role is negative
- Two defined ligands: PD-L1/B7-H1 and PD-L2/B7-DC
- PD-L1 can be expressed in non-hematopoietic tissues, including tumor cells
IFN-$\gamma$-treated B16.SIY-GFP melanoma stimulates PD-1$^{-/-}$ but not PD1$^{+/+}$ 2C TCR Tg T cells in vitro
PD-1\(^{-/-}\) 2C T cells reject tumors in vivo under conditions in which CTLA-4\(^{-/-}\) 2C cells do not.
IFN-γ upregulates PD-L1 on all human melanoma cell lines tested
PD-L1 mRNA is expressed in fresh melanoma tumor biopsies. Tumor cells also positive by IHC. Therefore, the PD-1/PD-L1 interaction is an important candidate negative regulator of anti-tumor immunity in human melanoma.
2. Regulatory T cells

• Defined by CD4^+/CD25^+ phenotype
• Selectively express the transcription factor FoxP3, and preferentially express the TNFR family member GITR
• Functionally suppress activation of CD4^+ and CD8^+ effector T cells in vitro and in vivo
• Observed to be present in increased numbers in cancer patients and within tumors
B16 melanoma cells expressing the model antigen SIY-GFP grow progressively in vivo.
Spontaneous induction of anti-SIY CD8+ T cells on day 6 in vivo despite lack of tumor rejection
Involvement of CD25+ Tregs in preventing spontaneous rejection of B16.SIY melanoma

TIL FACS analysis

CD25

Tumor day +28

Tumor rejection

Mean tumor diameter (mm)

Time (days)

Whole T cells

CD25− T cells
Human metastatic melanoma biopsies contain FoxP3 and GITR transcripts
CD4^+CD25^+ cells are present among human melanoma TILs

Therefore, regulatory T cells represent an important candidate negative regulator of anti-tumor immunity in human melanoma
3. T cell anergy

- Can result from TCR ligation in the absence of CD28 costimulation by B7-1/B7-2
- Characterized by defective TCR-induced cytokine production and proliferation
- Hypothesized to represent one mechanism of tolerance to tumor antigens
- Reversible by proliferation via cytokines (IL-2, IL-7, IL-15)
Hypo responsiveness of 2C TCR Tg T cells isolated from P1.HTR tumor-bearing P14/RAG2^{-/-} mice (day 28)

- Restimulated 16 hrs with antigen in vitro
- Cytokine production to PMA+Ionomycin intact
Malignant melanoma ascites fluid contains melanoma antigen-specific CD8⁺ T cells bearing an activated phenotype.

**Tetramer staining**

- Melan-A
- NA17

**Additional phenotyping**

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<th>EBV</th>
<th>Melan-A</th>
<th>NA17-A</th>
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<td>CD45RA⁺/CD62L⁻⁴</td>
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<td>84.6</td>
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Majority CD45RA⁻, CD62L⁻, CD28⁺
Ascites CD8+ T cells lack perforin and fail to respond to autologous tumor cell line.

Therefore, effector T cell dysfunction represents an important candidate negative regulatory process in human melanoma.
New interventions aiming to potentiate effector phase of anti-tumor T cells in clinical development

1. Interfere with PD-1/PD-L1 interactions
   - Neutralizing anti-human PD-1 mAbs

2. Remove regulatory T cells
   - Deplete in vivo, alone or prior to vaccination
   - Adoptively transfer CD25- T cells

3. Prevent/reverse T cell anergy
   - Transfer into lymphopenic recipients (IL-7-dependent homeostatic proliferation)
   - Intratumoral B7-1 (Fowlpox virus vector)
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Homeostasis-driven T cell proliferation

- Occurs when T cells are transferred into lymphopenic recipients
- Driven by excess available IL-7
- Results in partial activation and differentiation of transferred cells (pseudo-memory phenotype)
- We hypothesized that homeostatic proliferation would restore function and tumor rejection by anergic CD8+ T cells
Peptide-anergized 2C T cells undergo homeostatic proliferation in RAG2\(^{-/-}\) mice.
Anergic 2C T cells recover cytokine production following homeostatic proliferation in RAG2^−/− mice

Pre-transfer

Post-transfer
Anergic 2C T cells reject tumors after homeostatic proliferation in RAG2\(^{-/-}\) hosts.
Wildtype B6 CD8$^+$ T cells dilute CFSE on transfer to RAG$^{-/-}$ but not P14/RAG$^{-/-}$ recipients.
B7-1 transcripts are minimally expressed in metastatic melanoma tumors

**TCRβ**

**CD14**

**Ig kappa**

**B7-1**
B7-1 expression in tumor allows rejection with at least 10X fewer primed CD8$^+$ effector cells

![Graph showing mean tumor diameter over time for different cell types and B7-1 expression levels.](image-url)
Pilot clinical trial of intratumoral rfTRICOM in melanoma patients with detectable peptide-specific T cells

- HLA-A2+ patients with detectable circulating CD8+ T cells specific for defined melanoma epitopes
- Palpable lesions amenable to injection and biopsy
- Direct intratumoral injection of rfTRICOM (fowlpox virus encoding B7-1, ICAM-1, and LFA-3)
- Core biopsy pre- and post- to assess B7-1, ICAM-1, and LFA-3 expression by real-time RT-PCR
- Clinical response of injected and non-injected lesions assessed
- ELISPOT analysis pre- and post- to measure secondary changes in T cell frequency
rF-TRICOM efficiently transduces human melanoma cell lines in vitro
Additional insights gained by molecular analysis of metastatic melanoma tumors undergoing rejection or progressing

- Real-time RT-PCR for candidate genes and to follow effector phase dynamically
- Affymetrix gene array analysis
  - Aim to find stromal elements that correlate with regression versus progression
Real-time RT-PCR: Increased CD8 transcripts in tumors post-vaccination

Responder

Pre

Post

CD8α ↑ >100-fold

Non-responder

Pre

Post

CD8α ↑ >2000-fold
Affymetrix gene array: expression of IDO by non-responder and arginase by responder
IDO and Arginase

• Indoleamine 2,3-dioxygenase
  – Catabolizes tryptophan, an essential amino acid
  – Expressed in placenta, but also in cells in tumor microenvironment
  – Induced by IFN-γ
  – Leads to T cell hyporesponsiveness and apoptosis
  – Inhibitor, 1-methyl-L-tryptophan, can potentiate anti-tumor immunity in mice

• Arginase I
  – Catabolizes arginine
  – Induced by IL-4/IL-13
  – Expressed by myeloid cells in tumor microenvironment
  – Leads to diminished CD3-ζ expression in T cells, thus blunting TCR signaling
Conclusions

• Sufficient evidence exists to suggest that barriers to immune-mediated tumor regression downstream from T cell priming can be dominant
• New candidates for intervention: PD-1 blockade, depleting Tregs, reversing T cell anergy, and antagonism of IDO or arginase
• Ongoing studies analyzing gene expression profiles of tumor antigen-specific T cells and of cells in the tumor microenvironment from patients should identify major mechanisms that are clinically relevant
• Uncoupling the negative regulation of the effector phase of the anti-tumor immune response should allow an appropriately activated T cell population to mediate effective tumor regression
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**Metabolism**
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Vaccine patient #13 (non-responder): immune markers