Molecular mechanisms of the T cell-inflamed tumor microenvironment: Implications for cancer immunotherapy

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Working model of the immunobiology of T cell-inflamed and non-T cell-inflamed tumors

T cell-inflamed

- Chemokines
- CD8⁺ T cells
- Type I IFN signature
- Immune escape: Inhibitory pathways
- Most immunotherapy responders have this phenotype (vaccines, anti-CTLA-4, anti-PD-1)

Non- T cell-inflamed

- Low inflammatory signature
- Absent intratumoral CD8⁺ T cells
- Immune escape: T cell exclusion

Nature Immunol. 2013
Presence of Tregs and expression of PD-L1 and IDO are associated with a CD8$^+$ T cell infiltrate

Lag3 and 4-1BB further define CD8\(^+\) TIL phenotype beyond PD-1 (Egr2 transcriptional targets)

Gate: CD8

![Flow cytometry plots for Spleen and Tumor showing the distribution of Lag3 and PD1 expressions.](image-url)
Lag3^{+}41BB^{hi}PD1^{+} TIL exhibit the most severe reduction in IL-2 production

- Also show defective proliferation
- Blunted Ras pathway activation c/w in vitro anergy
- Express majority of anergy-associated transcripts
- Still make IFN-γ and chemokines (CCL1, CCL22)
- Tumor Ag-specific T cells present in this subset

*Fields et al, Science 1996*
*Zha et al, Nature Immunol. 2006*
*Zha et al, EMBO Reports. 2008*
*Zheng et al, JEM 2012*

*Williams et al., Manuscript in preparation*
Interventions aimed at uncoupling negative regulatory mechanisms in T cell-infiltrated melanomas

- **Anergy reversal:** Anti-41BB? Anti-LAG3?
- **Inhibitory receptor blockade:** PD-1/PD-L1 CTLA-4
- **Treg depletion:** Targeting CD25
- **IDO inhibition:** Small molecule inhibitors
- **Anergy reversal:** Anti-41BB? Anti-LAG3?
Combinatorial targeting of CTLA4 ± PDL1 ± IDO results in improved tumor control.
Synergistic permutations markedly increase the number of proliferating IL-2-producing CD8\(^+\) T cells in tumor microenvironment.
Increase in functional T cells within the tumor microenvironment does not require migration of new T cells (FTY720 blockade)

Spranger et al., JITC 2014
Combination immunotherapy clinical trials in metastatic melanoma: anti-CTLA-4 + anti-PD-1 and anti-CTLA-4 + IDOi

Anti-CTLA-4 + anti-PD-1

Anti-CTLA-4 + IDOi

Wolchok et al. NEJM. 2013

ASCO 2014

Numerous additional logical combinations also being pursued.
What approaches can be developed to gain therapeutic efficacy in non-T cell-inflamed tumors?
Model for innate immune sensing of tumors through activation of the host STING pathway by tumor-derived DNA

Woo et al, Immunity. 2014
Intratumoral STING agonists trigger durable rejection in multiple tumors models

**CT26**
- Control
- ML RR-S2 CDA

**4T1**

**Corrales et al, Manuscript submitted**
The host STING pathway is necessary for the therapeutic effect of radiation in vivo.
What are the molecular mechanisms that explain the T cell-inflamed versus non-inflamed tumor microenvironments?

Three major hypotheses

1. **Germline genetic differences at the level of the host**
   - Polymorphisms in immune regulatory genes

2. **Somatic differences at the level of tumor cells**
   - Distinct oncogene pathways activated in different patients
   - Mutational landscape and antigenic repertoire

3. **Environmental differences**
   - Commensal microbiota
   - Immunologic/pathogen exposure history of patients

Currently evaluating these possibilities in melanoma patients and multiple genomics platforms
Melanoma samples segregated according to T cell-inflamed gene expression signature and analyzed for molecular aberrations

T cell/chemokine Signature ABSENT

T cell/chemokine Signature PRESENT
Molecular analysis to identify oncogene pathways mutated/active in non-T cell-inflamed melanoma metastases

- Exome sequencing and pathway analysis collated based on immune phenotype (T cell-inflamed versus non-T cell-inflamed)
- Pathways being identified that are preferentially mutated/activated in non-T cell-inflamed tumors
- Engineering into inducible Tg mouse models for mechanistic experiments, in permutations with active B-Raf and PTEN deletion
- Long-term goal: drugging these pathways could improve immunotherapies in addition to direct effect on tumor cells
Model of how melanoma-intrinsic β-catenin activation prevents host anti-tumor immune response

Spranger et al, Manuscript submitted
Immunotherapy with anti-CTLA-4 + anti-PD-L1 is effective in Braf\textsuperscript{V600E}/PTEN\textsuperscript{-/-} but not Braf\textsuperscript{V600E}/PTEN\textsuperscript{-/-}/CAT-STA mice

Analysis of tumor growth and T cell infiltration

Combination therapy of αCTLA-4 and αPD-L1

4 weeks

every other day

<table>
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<th>Days of therapy</th>
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- αCTLA-4 + αPD-L1
- no therapy

n.s.
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Major points

- T cell-inflamed tumor microenvironment may serve as a predictive biomarker for response to immunotherapies
- Mechanism of spontaneous immune response appears to be driven by host STING/IFN-β pathway activated by tumor-derived DNA acquisition by DCs
- STING agonists may provide means to deliberately initiate innate immune inflammation to promote an endogenous T cell response in non-T cell-inflamed tumors
  - Developing human STING agonists for clinical translation
- Molecular mechanisms for T cell exclusion being uncovered
  - Somatic alterations: tumor-intrinsic β-catenin results in loss of DC-mediated T cell priming and recruitment
  - Intestinal microbiome as environmental variable
  - Germline polymorphisms: first SNP identified with minor allele associated with presence of T cell infiltrate
  - Each of these should lead to new therapeutic opportunities
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- Michael Leung

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