Understanding and overcoming immunoregulatory barriers within the tumor microenvironment

Thomas F. Gajewski, M.D., Ph.D.

Professor, Departments of Pathology and Medicine
Program Leader, Immunology and Cancer Program of the University of Chicago Comprehensive Cancer Center
President, Society for the Immunotherapy of Cancer (SITC)

I have the following financial relationships to disclose:

GSK Bio (consultant)
BMS (consultant)
Incyte (consultant)
Eisai (consultant)
Genentech/Roche (consultant)
Immunotherapy is effective in only a subset of melanoma patients

- HD IL-2 generates response rates of around 15%
- The most potent vaccines to date have yielded a 10% ORR + 20% SD rate
- Anti-CTLA-4 mAb ipilimumab also gives rise to tumor regression in 10-15% of patients, with around 40% of patients alive at 1 year
- While the same phenomenon is seen with all cancer therapeutics, the clear mechanism of action of immunotherapies may make it particularly amenable to dissection of the reasons for this inter-patient heterogeneity

Candidate sources of interpatient heterogeneity that could explain efficacy of immunotherapies in only a subset of patients

- Somatic differences at the level of the tumor cell
  - Permutations of oncogenic pathways
  - Epigenetic changes
- Genetic differences at the level of the host
  - Polymorphisms in immunoregulatory genes that affect the host response
- Environmental exposure differences
  - Immunologic experience with prior pathogens
  - Intestinal microbiome
Single alteration in gut flora can have profound influence on host immunity

- In arthritis-prone mouse model driven by Th17 cells, germ-free mice do not get disease
- Reintroduction of a single commensal organism, segmental filamentous bacteria, restored disease development and autoimmunity

Wu et al. Immunity 2010

CCR5 gene polymorphisms may be associated with clinical response to immunotherapy

- 782 melanoma patients analyzed; 90 (11.5%) were heterozygous and 12 (1.5%) were homozygous for CCR5Delta32
- CCR5Delta32 associated with a decreased survival compared to patients not carrying the deletion (median 12.5 vs. 20.3 months, P = 0.029).
- No association seen for patients who did not receive immunotherapy

Candidate sources of interpatient heterogeneity that could explain efficacy of immunotherapies in only a subset of patients

- Somatic differences at the level of the tumor cell
  - Permutations of oncogenic pathways
  - Epigenetic changes
- Genetic differences at the level of the host
  - Polymorphisms in immunoregulatory genes that affect the host response
- Environmental exposure differences
  - Immunologic experience with prior pathogens
  - Intestinal microbiome

Expression of a subset of chemokine genes is associated with presence of CD8 transcripts:
Clinical responders to peptide/IL-12 vaccine fall into this subset

Harlin et al.
“Inflamed” gene expression pattern of tumor microenvironment is associated with favorable clinical outcome to dendritic cell vaccine

Survival based on clinical response

No correlation with immune response in blood

Schüler collaboration, ASCO 2009

“Inflamed” gene expression signature is associated with survival following GSK MAGE3 protein vaccine

Louahed et al., EORTC-NCI-AACR 2009
Ipilimumab clinical responders also appear to show an “inflamed” tumor gene expression profile

- CXCL9, 10, 11
- CCL4, CCL5
- Granzyme B
- Perforin
- CD8α

Two broad categories of melanoma metastases defined by gene expression profiling and confirmatory assays

- Non-inflamed
  - Lack chemokines for recruitment
  - Low indicators of inflammation

- Inflamed
  - Chemokines for T cell recruitment
  - CD8+ T cells in tumor microenvironment
  - Broad inflammatory signature
  - Apparently predictive of clinical benefit to vaccines

Why are tumors that contain activated CD8+ T cells all not rejected?

Gajewski, Brichard; Cancer J. 2010
Inflamed melanomas containing CD8+ T cells have highest expression of immune inhibitory pathways

- IDO (indoleamine-2,3-dioxygenase)
- PD-L1 (engages PD-1)
- CD4+CD25+FoxP3+Tregs
- T cell anergy (B7-poor)

Clin. Can. Res. 2007*

Expression of IDO and PD-L1 in B16 melanoma depends on host CD8+ T cells and IFN-γ

A: IDO

B: PD-L1

![Graphs showing expression levels of IDO and PD-L1](image)
…but IFN-γ is necessary for tumor control by adoptively transferred T cells

Strategies to block immune inhibitory mechanisms tested in mouse models and being translated to the clinic

- **Blockade of PD-L1/PD-1 interactions**
  - Anti-PD-1 and anti-PD-L1 mAbs (Medarex/BMS; Curetech)

- **Depletion of CD4⁺CD25⁺FoxP3⁺ Tregs**
  - Denileukin diftitox (IL-2/DT fusion)
  - Daclizumab or Basiliximab (anti-IL-2R mAb)
  - Ex vivo bead depletion of CD25⁺ cells from T cell product for adoptive transfer

- **IDO inhibition**
  - 1-methyltryptophan (RAID program)
  - New more potent IDO inhibitors (Incyte)

- **Anergy reversal**
  - Introduction of B7-1 into tumor sites
  - Homeostatic cytokine-driven proliferation
    - T cell adoptive transfer into lymphopoenic recipient
    - Exogenous IL-7 / IL-15
  - Decipher molecular mechanism and develop small molecule inhibitors to restore T cell function

- **Combinations of negative regulatory pathway blockade**
  - Synergy between blockade of 2 or more pathways
Strategies to block immune inhibitory mechanisms tested in mouse models and being translated to the clinic

• **Blockade of PD-L1/PD-1 interactions**
  - Anti-PD-1 and anti-PD-L1 mAbs (Medarex/BMS; Curetech)

• **Depletion of CD4\(^+\)CD25\(^+\)FoxP3\(^+\) Tregs**
  - Denileukin diftitox (IL-2/DT fusion)
  - Daclizumab or Basiliximab (anti-IL-2R mAb)
  - Ex vivo bead depletion of CD25\(^+\) cells from T cell product for adoptive transfer

• **IDO inhibition**
  - 1-methyltryptophan (RAID program)
  - New more potent IDO inhibitors (Incyte)

• **Anergy reversal**
  - Introduction of B7-1 into tumor sites
  - Homeostatic cytokine-driven proliferation
    - T cell adoptive transfer into lymphopenic recipient
    - Exogenous IL-7 / IL-15
  - Decipher molecular mechanism and develop small molecule inhibitors to restore T cell function

• **Combinations of negative regulatory pathway blockade**
  - Synergy between blockade of 2 or more pathways

---

Interference with PD-L1/PD-1 interactions can promote tumor rejection in mice

![Graph showing tumor rejection](image)

*Blank et al, Cancer Research, 2004*
Anti-PD-1 mAb phase I (MDX-1106; BMS 936558): Tumor response

Responses also seen in NSCLC and renal cell carcinoma

Sznol et al. ASCO 2010

Reduction of Treg number using Denileukin diftitox can have clinical activity in melanoma

Rasku et al

Multicenter phase II study currently ongoing
Uncoupling multiple immune suppressive mechanisms in combination: Treg depletion and anergy reversal synergize to promote rejection of B16 melanoma and vitiligo


Conclusions

• An inflammatory gene expression profile in metastatic melanoma might have utility as a predictive biomarker for response to vaccines and other immunotherapies
  – Being tested prospectively in GSK-Bio MAGE3 vaccine trials
• Underlying mechanism explaining “inflamed” versus “non-inflamed” tumor microenvironment not yet understood
  – Tumor somatic differences? Germline polymorphisms? Gut flora?
  – May be possible to develop simpler assay than tumor gene array based on better understanding of underlying mechanism
• One major barrier to effective immune-mediated tumor destruction is poor T cell migration
  – Strategies to promote local inflammation and T cell homing should be pursued
• “Inflamed” tumors likely are not rejected due to dominant immune suppressive mechanisms
  – IDO, PD-L1, Tregs, Anergy: We can target these!
• The near future of cancer immunotherapy will likely involve tumor profiling to phenotype microenvironment in individual patients, then strategies to overcome relevant immune inhibitory mechanisms combined with approaches to increase specific T cell frequencies
Acknowledgments

Melanoma gene array/
Chemokines/Tregs
Helena Harlin
Yuan-yuan Zha
Ruth Meng
Amy Peterson
Mark McKee
Craig Slingluff
Functional genomics core

Type I IFNs
Mercedes Fuertes
Robbert Spaapen
Aalok Kacha
Justin Kline
David Kranz
Hans Schreiber
Ken Murphy

Uncoupling negative
regulation
Robbert Spaapen
Justin Kline
Yuan-yuan Zha
Christian Blank
Amy Peterson
Ian Brown

Collaborative vaccine/gene
array data
Gerold Schuler (Erlangen group)
Vincent Brichard (GSK-Bio)

Perspectives 2011