Argument for combination immunotherapies in therapeutic cancer vaccine development

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Results of clinical studies of immunization with melanoma antigen peptides and IL-12

- Clinical response rate 10% (+ ~20% stable/mixed)
  - Better than zero
  - Not as high as hoped based on preclinical studies
- T cell responses (usually modest magnitude) induced in the majority of patients
- Some patients had a high T cell response to melanoma antigens even pre-treatment (up to 1% of CD8s) ➔ spontaneous immunity
- **Pivotal question: why does high T cell response not always lead to tumor regression?**
  - Quantitative deficiency (magnitude still not high enough)
  - Qualitative limitations (TCR avidity, phenotypic subtleties)
  - **Downstream resistance mechanisms at the level of tumor microenvironment**

*Int. J. Can. 1999*  
*J. Clin. Oncol. 2003*
Anti-tumor immune responses: Taking into account the effector phase

Lymph node (Priming phase)

- APC
- nCD8
- IL-2
- eCD8

Vaccine

Endogenous

Lymphatic

Blood

Tumor microenvironment (Effector phase)

- APC
- eCD8
- Chemokines
- IFN-γ
- Granzymes
- perforin

Inhibitory mechanisms
Can we profile tumor microenvironment and identify correlates with clinical outcome?

Phase II study with 4 peptide vaccine + IL-12

- 19 HLA-A2+ patients with metastatic melanoma
- All vaccinated with 4 peptides (MelanA, NA17, gp100, MAGE3) pulsed onto PBMC + rhIL12 q 3 weeks
- Patients had pre-treatment biopsy to prepare RNA for gene array analysis
- Clinically, 1 patient had a CR, 1 PR, and 4 had prolonged disease stabilization (>6 months)
- Affymetrix gene array on pretreatment samples:
  - U133A chips utilized, data were normalized
  - Supervised hierarchical clustering done comparing patients with SD or better versus patients with PD
  - Looking for genes differentially expressed 2-fold or greater
Affymetrix gene array analysis of pre-treatment biopsies from patients on melanoma vaccine sorted by clinical outcome

Represents only 7 genes:
- 4 upregulated
- 3 downregulated

6 mos SD or better
Tumors from favorable clinical outcome patients express higher levels of TCRα, CXCL9, and CCL21
Expression of a subset of chemokine genes is associated with presence of CD8 transcripts

CD8β
CCL2
CCL4
CCL5
CXCL9
CXCL10
CCL19
CCL21
Superior recruitment of human CD8$^+$ effector T cells in NOD/scid mice bearing “chemokine-high” M537 melanomas

Harlin et al, Cancer Research, 2009
Why are melanomas that do attract CD8\(^+\) T cell not rejected spontaneously?

- Presence of immune inhibitory mechanisms:
  - IDO
  - PD-L1/PD-1
  - Tregs
  - T cell anergy

- Lack of migration
Co-expression of IDO, PD-L1, and FoxP3 transcripts in individual tumors

Note: these are more abundant in metastases that contain CD8+ T cells

Immunol. Rev. 2006
Strategies to uncouple immune inhibitory mechanisms for clinical translation

1. Promote increased migration into tumor sites
   - Chemokines, innate immune factors (e.g. type I IFNs), TLR ligands
   - LIGHT (Yu et al. J. Immunol. 2007)

2. Uncouple negative regulation
     - 1-methyltryptophan (RAID program); other inhibitors
   - Blockade of PD-L1/PD-1 interactions (Blank et al, Can Res 2004)
     - Anti-PD-1 or PD-L1 mAb (Medarex)
   - Depletion of CD4+CD25+FoxP3+ Tregs (Kline et al, CCR 2008)
     - Ontak (denileukin diftitox: IL-2/DT fusion; Daclizumab)
     - Ex vivo bead depletion of CD25+ cells from T cell product
     - Homeostatic cytokine-driven proliferation (lymphopenic recipient)
     - Inhibition of anergy factors (e.g. DGK-α)
   - **Combinations of negative regulatory pathway blockade**
     - Synergy between Treg depletion and anergy reversal with homeostatic proliferation (Kline et al, CCR 2008)
Intratumoral LIGHT adenovirus in B16 melanoma: Promotes chemokine production, CD8+ T cell recruitment, primary tumor control, and rejection of non-injected distant metastases

Yu et al, J. Immunol. 2007
1-methyltryptophan reverses immunosuppression by IDO and improves tumor control in vivo

PD-1^−/− TCR Tg T cells are superior at tumor rejection in vivo

Blank et al, Cancer Research, 2004
DGK as a drugable inhibitor of T cell activation in the anergic state

Zha et al., Nature Immunol. 2006
A pharmacologic inhibitor of DGK recovers IL-2 production by anergic T cells

Implies that it may be possible to develop small molecule immunopotentiating drugs to improve T cell function in the context of cancer and chronic infections
Anergic 2C TCR Tg T cells reject tumors after homeostatic proliferation in RAG2^{−/−} hosts

Brown et al., J. Immunol., 2006
Uncoupling multiple immune suppressive mechanisms: Combined Treg depletion and anergy reversal supports rejection of B16 melanoma and leads to vitiligo

Comprehensive view of levels at which a spontaneous anti-tumor T cell response can fail

Lymph node

3. APC maturation/costimulation

4. T cell repertoire/activation

5. T cell differentiation/expansion/persistence

Tumor microenvironment

2. Antigens/Ag processing innate immune awareness

8. Target cell apoptosis

6. Effector T cell trafficking

7. T cell effector function (negative regulation)
Candidate approaches to overcome these immunologic checkpoints

1. Innate immune awareness/Ag presentation/APC maturation
   - Innate immune cells and cytokines, TLR agonists, CD40 ligands, vaccination—novel Ag sources

2. T cell repertoire/initial activation
   - B7 and other costimulatory ligands
   - Interference with lymph node-based or systemic negative regulators (CTLA4, IDO, arginase, anergy, Tregs, IL-10)

3. T cell differentiation/expansion/persistence
   - Differentiation cytokines (IL-12, IL-18)
   - Expansion, survival factors (IL-2, IL-7, IL-15, anti-41BB; homeostatic proliferation)

4. T cell trafficking into tumor sites
   - Intratumoral chemokines, LIGHT
   - Pro-inflammatory treatments (XRT, TLR agonists, innate cytokines)

5. Executing effector function in tumor microenvironment
   - Blockade of tumor microenvironment-based negative regulators (IDO, PD-1/PD-L1, Tregs, anergy, TGF-β, IL-10, iNOS)
   - Promote effector cell proliferation (regenerate cytotoxic granules)

6. Tumor cell susceptibility to recognition and killing
   - Blockade of key anti-apoptotic molecules (Bcl2 and Spi inhibitors)
   - Inhibit oncogenic pathways that create resistant phenotype and/or resistant microenvironment (Stat3; MEK? Notch? Wnt?)
Anti-CTLA-4 mAb + GM-CSF-transduced B16 vaccine induces tumor rejection and leads to vitiligo

van Elsas, Allison et al. JEM 1999
Anti-4-1BB + anti-PD-L1
Combination induces rejection of PD-L1-expressing tumors in vivo

Hirano, Chen et al. Cancer Res. 2005
Vaccine + CpG + Treg depletion: Control of mammary tumors in Neu Tg mice

Nava-Parada, Celis et al. Cancer Res. 2007
Conclusions and implications

• The spontaneous natural host immune response against melanoma is heterogenous; mechanism unclear:
  – Somatic differences between tumors?
  – Germline polymorphisms in immunoregulatory genes?
• Implies that dominant barrier to T cell-mediated tumor rejection may be distinct in different subsets of patients, e.g.:
  – Failed T cell priming
  – Defective T cell recruitment to tumor sites
  – Immune suppressive factors blocking T cell effector phase
• Additional immunotherapeutic interventions beyond (or in place of) vaccination may be needed to maximize tumor rejection by anti-tumor T cells
• Importantly, clinical grade reagents and methodologies have been developed, enabling clinical testing of these concepts
• Many of these principles also likely apply to chronic infections
• Final point: in the meantime, can we better select patients for vaccine trials based on immunologic features of tumor microenvironment?
Gene expression profiling in context of Erlangen dendritic cell-based vaccine in melanoma

Survival based on clinical response

Survival groups

No correlation with immune response in blood

T cell markers and chemokines

Schuler collaboration, ASCO 2009
Impact of gene expression signature on clinical outcome in GSK MAGE3 vaccine trial

Median

GS-: 2.3 months [95% CI: 2.3 - 4.4]
GS+: 10.3 months [95% CI: 6.7 - 12.4]

HR: 0.31 [95% CI: 0.13 - 0.76]

Louahed, ASCO 2008
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