Combination Immunotherapies

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Rationale and foundation for discussion

• Important disclaimer: we are not suggesting that “vaccines don’t work” and therefore combinations of vaccines plus other therapies may show synergy
• Rather, our view is that an anti-tumor immune response is a complex and multi-stage process that can become dysregulated at several levels in the context of a growing tumor
• Overcoming each of these defects may require a distinct intervention, and therefore combination therapies may be important in order to translate immune responses into tumor regression
• Another way to look at it: with cancer vaccines, the “drug” is not the vaccine itself—rather, the therapeutic entity is the properly generated tumor antigen-specific effector T cell population that has penetrated the tumor microenvironment and maintained effector function there
An effective anti-tumor immune response is a multistep process

1. Priming phase (vaccination)
   - Magnitude and breadth of T cell response
   - Qualitative aspects of T cell differentiation (effector functions)
   - Issue: limitations of available repertoire

2. Expansion and persistence
   - Survival of effector cells in periphery
   - Immunologic memory

3. Trafficking to tumor sites
   - Chemokines and homing receptors

4. Executing effector function
   - Overcoming negative regulatory pathways
     - Anergy, CTLA-4, PD-1, IDO, Tregs
   - Maintenance of effector function
     - Regeneration of cytotoxic granules

5. Tumor cell susceptibility to recognition and killing
   - Expression of antigens, processing machinery, MHC
   - Overcoming anti-apoptotic mechanisms
   - Interface with tumor cell-intrinsic biology
Complexities of anti-tumor immune responses: Taking into account the effector phase

Lymph node (Priming phase)
Vaccine

APC
nCD8
IL-2
eCD8

Blood

Tumor microenvironment (Effector phase)

Inhibitory mechanisms

APC
Chemokines
IFN-γ
eCD8

Granzymes perforin
I. Priming phase/vaccine: considerations for combinations

• Antigen choice(s)
  – Peptides, protein, DNA, RNA, bulk tumor cells
  – Type of antigen (e.g. necessary for malignant phenotype)
  – Class I MHC, class II MHC, non-classical (glycolipids)

• Adjuvant components
  – Emulsions in oil-based formulations
  – TLR agonists (LPS/MPL + CpG)
  – Cytokine additions—differentiation promoters
  – Microbial vectors
  – Dendritic cell-oriented

• Dose, schedule, route of administration
  – Issue of tissue-specific homing of T cells
Example 1: α-GalCer

Administration of protein and α-GalCer can synergistically expand CD8+ T cells

Example 2: IL-12
Superior induction of specific CTL responses in mice using peptide-loaded APCs + IL-12

Superior immune responses with IL-12 + peptides in Montanide in patients with melanoma

Potent T cell response against multiple antigens post-immunization of melanoma patients with peptide-pulsed PBMC + IL-12
II. T cell expansion and persistence: considerations for combinations

- Survival/homeostatic cytokines
  - IL-7
  - IL-15
  - IL-21
- Costimulatory receptors
  - B7 family members
  - 4-1BB
  - Other TNFR family members
Example 3: Anti-4-1BB

Co-administration of anti-4-1BB mAb with adoptively transferred T cells induces superior tumor rejection and T cell survival in mice

III. T cell trafficking: considerations for combinations

- Intratumoral chemokines
  - Mig, IP-10, MIP-1α
  - CCL21
  - (Blockade of TARC/MDC?)

- Intratumoral LIGHT
  - Promotes secondary generation of chemokines

- Homing receptors/adhesion molecules
  - Intratumoral ICAM-1 (component of TRICOM)
  - Immunizing via optimal route (tissue specific homing)

- Angiogenesis targeting
Only a subset of melanoma metastases appear to have the appropriate signature for T cell recruitment.

Example 4: LIGHT

Intratumoral LIGHT can induce T cell recruitment and tumor rejection in multiple tumor models

Fu et al, submitted
IV. Negative regulatory pathways: considerations for combinations

- Inhibitory receptors on T cells
  - CTLA-4
  - PD-1
  - KIRs
- Inhibitory cytokines
  - TGF-β
  - IL-10
- Inhibitory cell populations
  - CD4+CD25+FoxP3+ Tregs
  - Other Tregs
  - Myeloid suppressor cells
  - B cells
- Metabolic regulation
  - IDO
  - Arginase
  - Nutrient deprivation (glucose)
Example 5: CD25$^+$ Tregs
CD25 depletion can partially control B16 melanoma growth in vivo

Example 6: CTLA-4

Anti-CTLA-4 mAb + GM-CSF-transduced B16 vaccine induces tumor rejection and leads to vitiligo

van Elsas, Allison et al. JEM 1999
Clinical development of anti-CTLA-4 mAb: Example of MDX-010 (Ipilimumab)

- Fully human IgG1 monoclonal antibody to human CTLA-4 created by Medarex
- Blocks binding of CTLA-4 to CD80 and CD86
- Augments immune responses in primate models
- Co-developed by Medarex and Bristol-Myers Squibb in multiple cancer indications
  - Phase III study in metastatic melanoma ongoing
  - Phase II studies in renal cell carcinoma, prostate cancer, ovarian cancer, and others
GVAX Immunotherapy (CG1940/CG8711) + Ipilimumab (MDX-010: anti-CTLA-4) for HRPC

VUmc Cancer Center Amsterdam
GVAX + anti-CTLA-4 in prostate cancer: PSA curves – Dose Level 3 (3 mg/kg)

Gerritsen et al. ASCO 2006

a: 13Mar06: SAE - Hypophysitis (7 mo)
b: 03Feb06: Hypophysitis (5 mo)
c: 09Feb06: SAE – Hypophysitis (5 mo)
Multiple combinations:
Another layer of complexity and excitement through combined manipulation of regulatory checkpoints

• Anergy reversal + Treg-depletion
• Anti-4-1BB + anti-CTLA-4
• Anti-4-1BB + anti-PD-L1
• Anti-CTLA-4 + Treg depletion
Example 8: Treg depletion + anergy reversal
CD25-depleted T cells transferred into lymphopenic hosts
gives long-lived rejection of B16 melanoma and vitiligo

Kline, Gajewski et al. Submitted.
Example 9: Anti-4-1BB + anti-PD-L1 Combination induces rejection of PD-L1-expressing tumors in vivo

Hirano, Chen et al. Cancer Res. 2005
V. Tumor cell susceptibility: considerations for combinations

- **Expression of “signal 1”**
  - Antigens
  - Antigen processing machinery
  - MHC, β2M

- **Overcoming anti-apoptotic mechanisms**
  - Survivin
  - Bcl2-family members
  - Serine protease inhibitors

- **Interface with tumor cell-intrinsic oncogenes**
  - Ras/MAP kinase pathway & DC activation
  - Stat3 pathway and chemokines
  - Notch pathway and survival, immune gene expression
Example 10: PI-9/Spi6
Serine protease inhibitor PI-9 is frequently expressed in human cancers

Introduction of the murine equivalent Spi6 into tumor cells decreases susceptibility to T cell-mediated lysis in vitro.
**Additional issues**

- **Tumor heterogeneity**
  - Different cancer types may have distinct dominant immunologic barriers
  - Different patients with the same cancer may have distinct dominant immunologic barriers

- **Opportunities for drug discovery and development**
  - Cellular targets (e.g. Tregs, MSCs)
  - Molecular targets (FoxP3, LAG-3, GITR, IDO, arginase, PD-1, LIGHT)

- **How to prioritize combinations?**
  - Too many choices => Ideally, should be based on sound mechanistic analysis of immunologic barriers in populations of patients with given cancer types
  - Preclinical models should show synergy

- **Patient selection**
  - Can we identify patients who have measurable expandable CTL precursors before enrolling on vaccine trials?
  - Similarly, can we identify patients with tumor microenvironment that can support effector phase of anti-tumor immune response before enrolling on immunotherapy trials?

Represents only 7 genes:
- 4 upregulated
- 3 downregulated

6 mos SD or better
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• The hurdles seem great—why keep exploring this area?
  – Elegant specificity of immune response
  – Memory—what other cancer therapeutic persists like an immune response?
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Affymetrix gene array analysis of pre-treatment biopsies from patients on melanoma vaccine sorted by clinical outcome

Represents only 7 genes:
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Has implications for patient selection on vaccine trials, and understanding biology
Differential chemokine expression in melanoma metastases with high versus low T cell transcripts
Co-expression of IDO, PD-L1, and FoxP3 transcripts in individual tumors
Summary of tumor microenvironment barriers: Need to promote T cell trafficking and overcome local immunosuppression.
Resolution of cutaneous metastases following immunization with melanoma peptide-pulsed PBMC + rhIL-12

After 3 vaccines

After 9 vaccines

Peterson, Gajewski et al. JCO 2003.
Greater increase in Melan-A-specific CD8\(^+\) T cells in clinical responders

\[ \Delta \text{IFN-\gamma producing cells/100,000 CD8}^+ \text{cells} \]

- Responders: 120
- Non-responders: 20

\[ p = 0.046 \]

2 CR, 1 MR, 4 mixed responses