Immunotherapy/Immunotherapy combinations

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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 will automatically be required.
• 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 T cell-based immunotherapy, the “drug” is not necessarily the product administered (e.g. vaccine)—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.
At what levels can a spontaneous anti-tumor T cell response fail?

**Lymph node**

1. **innate immune awareness**
2. **Antigens/Ag processing innate immune awareness**
3. **APC maturation/costimulation**
4. **T cell repertoire/activation**
5. **T cell differentiation/expansion/persistence**

**Blood**

6. **Effector T cell trafficking**
7. **T cell effector function (negative regulation)**

**Tumor microenvironment**

2. **Antigens/Ag processing innate immune awareness**
3. **APC maturation/costimulation**
4. **T cell repertoire/activation**
5. **T cell differentiation/expansion/persistence**
6. **Effector T cell trafficking**
7. **T cell effector function (negative regulation)**
8. **Target cell apoptosis**
Hypothetical barriers point towards strategies for intervention

**Vaccines**
1. Innate immune awareness/Ag presentation/APC maturation
   - Are there “danger” signals to ensure productive antigen display?
2. T cell repertoire/initial activation
   - Repertoire may be restricted or of low avidity
   - Immune suppression may carry over to DLN compartment

**Adoptive Tx**
3. T cell differentiation/expansion/persistence
   - Proper T cell phenotype might not be induced (Th1/CTL/memory)
   - Magnitude or duration of T cell response may be inadequate
4. T cell trafficking into tumor sites
   - Lack of proper chemokine receptors on T cells, or chemokines at tumor site
   - Signals for penetrating extracellular matrix?
5. Executing effector function in tumor microenvironment
   - Dominant negative regulatory pathways
   - Poor maintenance of effector function (e.g. regeneration of cytotoxic granules)
6. Tumor cell susceptibility to recognition and killing
   - Loss of antigens, processing machinery, MHC
   - Anti-apoptotic mechanisms: tumor cells can be resistant
   - Interface with tumor cell-intrinsic biology: oncogenic pathways orchestrating resistance
Candidate approaches to overcome these barriers

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)

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?)
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**Example 1: α-GalCer**

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

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Example 2: 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
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)

a: 13Mar06: SAE - Hypophysitis (7 mo)
b: 03Feb06: Hypophysitis (5 mo)
c: 09Feb06: SAE – Hypophysitis (5 mo)

Gerritsen et al. ASCO 2006
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Example 3A: IL-12
Superior induction of specific CTL responses in mice using peptide-loaded APCs + IL-12

Fallarino et al. Int. J. Cancer 1999
Potent T cell response against multiple antigens post-immunization of melanoma patients with peptide-pulsed PBMC + IL-12

Direct ex vivo IFN-γ ELISPOT

3 patients with CR post-vaccination
Superior immune responses with IL-12 + peptides in Montanide in patients with melanoma

Lee, Weber et al. JCO 2001
Example 3B: 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

Example 3C: Homeostatic proliferation

Anergic 2C T cells reject tumors after homeostatic proliferation in RAG2^{-/-} hosts

Brown et al., J. Immunol., 2006
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T cell transcripts in melanoma metastases are associated with expression of specific chemokine genes

Cell Lines

CCL5/RANTES

TCR-α

Expression Units

Sample Number

Expression Units

Sample Number

Cell Lines
Example 4: Intratumoral LIGHT adenovirus in B16 melanoma promotes greater recruitment of CD8$^+$ T cells in primary tumor and leads to rejection of non-injected distant metastases

Yu et al, J. Immunol. 2007
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Example 5A: PD-1⁻/⁻ 2C TCR Tg T cells are superior at tumor rejection in vivo

Blank et al, Cancer Research, 2004
Example 5B: 1-methyltryptophan reverses immunosuppression by IDO and improves tumor control in vivo

Example 5C: CD25\(^+\) Tregs

CD25 depletion can partially control B16 melanoma growth in vivo

Jones, Gallimore et al. Cancer Immunity 2002
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Example 6: 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
Multiple combinations:
Another layer of complexity and excitement through combined manipulation of regulatory checkpoints

- 1-MT + lymphodepletion
- Anergy reversal + Treg-depletion
- Anti-4-1BB + anti-CTLA-4
- Anti-4-1BB + anti-PD-L1
- Anti-CTLA-4 + Treg depletion
- TLR agonist + Treg-depletion
1-MT + lymphodepleting chemotherapy: Partial control of B16 melanoma

Hou, Munn et al. Cancer Res. 2007
Treg depletion + anergy reversal CD25-depleted T cells transferred into lymphopenic hosts gives long-lived rejection of B16 melanoma and vitiligo

Kline et al. Submitted.
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
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, tumor vasculature)
  - Molecular targets (FoxP3, LAG-3, GITR, IDO, arginase, PD-1, LIGHT, T cell signaling)

- How to prioritize combinations?
  - Many permutations => 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 tumor antigen-specific 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
Conclusions

• Spontaneous anti-tumor T cell responses may fail at one of several levels
• Specific mechanisms of failure have identified new targets and strategies for intervention
• There is a strong scientific basis for combination therapies with the aim of overcoming specific barriers and immunologic checkpoints to increase the therapeutic efficacy of T cell-based immunotherapy of cancer
• Some agents are becoming available for clinical translation, but others need broad-based community support to be made available for clinical studies based on a sound rationale and preclinical data
Agents prioritized by scientific community for clinical development

Table 1. Final Rankings of Agents with High Potential for Use in Treating Cancer

<table>
<thead>
<tr>
<th>Rank</th>
<th>Agent</th>
<th>Agent Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL-15</td>
<td>T-Cell Growth Factor</td>
</tr>
<tr>
<td>2</td>
<td>Anti-Programmed Death-1 (PD1) and/or Anti-B7-H1 (PD1 Ligand)</td>
<td>**T-Cell Checkpoint Blockade Inhibitor</td>
</tr>
<tr>
<td>3</td>
<td>IL-12</td>
<td>Vaccine Adjuvant</td>
</tr>
<tr>
<td>4</td>
<td>Anti-CD40 and/or CD40L</td>
<td>Antigen Presenting Cell Stimulator</td>
</tr>
<tr>
<td>5</td>
<td>IL-7</td>
<td>T-Cell Growth Factor</td>
</tr>
<tr>
<td>6</td>
<td>CpG</td>
<td>Vaccine Adjuvant</td>
</tr>
<tr>
<td>7</td>
<td>1-Methyl Tryptophan</td>
<td>Enzyme Inhibitor</td>
</tr>
<tr>
<td>8</td>
<td>Anti-CD137 (anti-41BB)</td>
<td>T-Cell Stimulator</td>
</tr>
<tr>
<td>9</td>
<td>Anti-TGF-beta</td>
<td>Signaling Inhibitor</td>
</tr>
<tr>
<td>10</td>
<td>Anti-IL-10 Receptor or Anti-IL-10</td>
<td>Suppression Inhibitor</td>
</tr>
<tr>
<td>11</td>
<td>Flt3L</td>
<td>Dendritic Cell Growth Factor/Vaccine Adjuvant</td>
</tr>
<tr>
<td>12</td>
<td>Anti-Glucocorticoid-Induced TNF Receptor (GITR)</td>
<td>T-cell Stimulator</td>
</tr>
<tr>
<td>13</td>
<td>CCL21 Adenovirus</td>
<td>T-Cell Attracting Chemokine</td>
</tr>
<tr>
<td>14</td>
<td>Monophosphoryl Lipid A (MPL)</td>
<td>Vaccine Adjuvant</td>
</tr>
<tr>
<td>15</td>
<td>Poly IC and/or Poly ICLC</td>
<td>Vaccine Adjuvant</td>
</tr>
<tr>
<td>16</td>
<td>Anti-OX40</td>
<td>T-Cell Stimulator</td>
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<tr>
<td>17</td>
<td>Anti-B7-H4</td>
<td>T-Cell Checkpoint Blockade Inhibitor</td>
</tr>
<tr>
<td>18</td>
<td>Resiquimod and/or 852A</td>
<td>Vaccine Adjuvant</td>
</tr>
<tr>
<td>19</td>
<td>LIGHT and/or LIGHT vector</td>
<td>T-Cell Stimulator</td>
</tr>
<tr>
<td>20</td>
<td>Anti-Lymphocyte Activation Gene-3 (LAG-3)</td>
<td>T-Cell Checkpoint Blockade Inhibitor</td>
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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/anti-CTLA4 trial Contributors

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Prostate Cancer Foundation

MEDAREX
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

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

2 CR, 1 MR, 4 mixed responses
Complexities of anti-tumor immune responses: Taking into account the effector phase

Vaccine

Lymph node (Priming phase)
- APC
- nCD8
- IL-2
- eCD8

Blood

Tumor microenvironment (Effector phase)
- APC
- Chemokines
- IFN-γ
- eCD8
- Granzymes perforin

Inhibitory mechanisms
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
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)
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
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
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 4: LIGHT

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

Fu et al, submitted
Only a subset of melanoma metastases appear to have the appropriate signature for T cell recruitment.