The Good, the Bad and the Ugly:

Clinical trials which assess vaccine characteristics

ISBT Meeting, San Francisco, CA
November 4-8, 2004
Ideal cancer vaccine trial

1. An informative immune assay
2. Ability to derive data on immune response
3. Toxicity and clinical response/survival data

4. Correlate ability of the assay in #1 to be a surrogate for #3.
5. Problem: #3 not the idea scenario for #2!
What is needed in a vaccine trial?

- Sufficient number of highly avid T cells that are antigen specific
- Ability of the T cells in question to traffic to lymph nodes and sites
- T cells generated must be in a proper state of activation and able to overcome both passive (antigen and MHC down-regulation) and active (Tregs, IL-10, TGF-beta) defences
What is the evidence that immune monitoring has clinical relevance?

- We need to determine if any immune assay correlates with relapse-free or overall survival.
- Is there a surrogate endpoint for survival and/or clinical benefit?
- If simple enumeration is not useful, why not?
- Immune monitoring, if it correlates with clinical benefit can help us decide what qualities are important for a therapeutic T cell.
Do we have the right assay, in the right type of trial?

- Different immune assays need to be prioritized
- Is there a place for pure immune surrogate trials, in patients without evidence of disease?
- Should we concentrate on patients with measurable disease, or are NED patients OK?
- What clinical endpoints are proper for vaccine trials; survival vs. response vs. stability?
Are we measuring the correct thing, and in the right place?

- Measurement of circulating T cells in PBMC is important, but what about draining nodes and tumor infiltrating T cells?
- Should we be measuring circulating or tumor Treg cells as well as effector cells?
- How important are circulating cytokines, both proinflammatory and suppressive?
- Are NK, NKT or DC relevant as a measure of immunity?
- Should we be measuring cytokine gene polymorphisms and cytokine gene epigenetic modifications and changes after vaccination as a surrogate marker for the ability to immunize?
Case studies in immune monitoring of clinical vaccine trials

- CanVaxin: cell based vaccine with BCG
- Peptide vaccines: melanoma differentiation antigens
- Dendritic cells: pulsed with peptides, lysates and fusion products
- This is not a comprehensive assessment, more a set of instructive examples to assess whether immune assays correlate with clinical benefit
Canvaxin: cellular vaccine
Chung et al JCO 2003 21: 313

- Three melanoma cell lines administered with BCG for two injections, then alone for 6 months total
- Induces antibody responses against a 90 kD tumor associated glycoprotein TA-90
- 54 patients: (-)SNB, all had >4 mm melanoma
- 43 got vaccine, 11 were observed
- DFS and OS correlated with maximal TA-90 IgM response (p=0.006 and 0.06) in the vaccine group, but not the observation group
- Non-randomized, but encouraging result
Canvaxin: cellular vaccine

- 2602 patients had complete lymphadenectomy in the period 1984-1998; 935 received Canvaxin, and 1667 did not
- Comparison group had no therapy or IFN 1971-1998
- They were matched for 7 co-variates
- Median OS was 49% vs. 37% favoring vaccine
- The authors claim OS was the same in the observed group pre-1985 and post-1985, which disagrees with SWOG data
- Canvaxin correlated with OS p= 0.001; RR death = 0.64
- Justifies a randomized phase III trial, just concluded in over 1100 patients of Canvaxin/BCG vs. BCG alone
CanVaxin: Phase III trials

- Two randomized trials, one ongoing, one just finished in resected stages III and IV melanoma
- A lower than expected rate of events will slow down the final interpretation of the trial; BCG effect?
- Evidence that Canvaxin/BCG may be beneficial:
  - Vaccinated patients have increased DTH to the vaccine, which correlates with survival
  - Vaccinated patients have a reduction in TA-90 IgM levels, which correlates with survival
  - Anti-ganglioside antibodies are induced by CanVaxin
  - T cell responses can be detected to known antigens

- 35 patients received gp100 209-217 (210M) with Montanide ISA 51
- Tetramer staining shows median of 0.36% post-vaccine (0.05 to 8.9%)
- Cells were CCR7(-) CD45RA (+) or (-) > suggesting effector or effector-memory type
- Virtually all cells expressed gamma interferon after ex vivo expansion
Peptide vs. DC vaccine for stage IV melanoma: Slingluff et al *J Clin Oncol* 2004

- 26 patients, stage IV melanoma, 13 each randomly allocated to receive peptides with Montanide/GM-CSF or pulsed onto DC
- Higher overall immune response with restimulated ELISPOT in peptide arm $p<.02$
- Vitiligo seen in 2 peptide but no DC patient
- 4 SD + PR in the peptide arm, versus 2 SD + PR in the DC arm
- Immune response appeared to correlate with PR/SD
Evaluation of CTL Responses to Vaccination with GMCSF-in-Adjuvant or DC+peptide in Patients with Substantial Tumor Burden (Stage IV)

% patients with CTL response

- Dendritic Cells
  - SIN (Node): 1/8
  - PBL: 1/9

- GMCSF/adjuvant
  - SIN (Node): 8/10
  - PBL: 5/12

UVA-MEL31

Vaccine
Peptide vaccines for melanoma: Clinical data

- gp100/tyrosinase/IFA+IL-12 trial for resected stage III/IV patients: 26 with stage III, 22 with stage IV disease; median relapse-free survival 20 months, median survival greater than 57 months, 85% had augmented immunity to gp100 by tetramer staining, with increase from 0.03 to 0.08% IL-12 vs. no IL-12 Lee et al J Clin Oncol 2001

- In an ongoing trial, three peptides with IFA were used to vaccinate stage III/IV resected patients with low dose IL-12/alum, low dose IL-12+GM-CSF or high dose IL-12/alum.
Reactivity to melanoma antigen gp100: are higher doses of IL-12 with alum a superior adjuvant?
Reactivity to melanoma antigen MART-1: are higher doses of IL-12 with alum a superior adjuvant?
Conclusions: Peptide vaccines with Montanide, alum and IL-12

- ELISPOT responses greater for both gp100 and MART-1/Melan-A heteroclitic and wild type in high dose IL-12 than either low dose group, \( p \) values ranging from 0.04 to 0.005
- WT immune responses equal to heteroclitic
- More deaths (3 versus 1) and more relapses (10 vs. 4) in low dose groups than high dose group; correlation seen with immune response and time to relapse
Fowlpox gp100 vaccine: no correlation of immunity with response

- Three consecutive trials were done with 7, 14 and 16 pts who received a fowlpox-native gp100, fowlpox modified gp100, and folwpox –gp100 minigene (ER targeted)
- Rosenberg et al *Clin Can Res* 2003
- Responses to gp100 seen in 0/7, 10/14 and 12/16 patients respectively
- Restimulation assays done for cytokine release
- No correlation of assays with response and benefit
- The group immunized with the fowlpox gp100 minigene later received IL-2 with a 50% response rate

- Five biweekly SC vaccinations with peptide pulsed mature DC; only 16 received all DC
- Good responses seen to MAGE-3 243-258 by fresh ex vivo ELISPOT, and to KLH
- No clear correlation of immune response with clinical response; 1 CR with very low immunity seen, also 7 stable disease patients with no clear pattern of immunity
hTERT peptide-pulsed DC induce functional T cell responses

• Four of seven patients immunized with hTERT peptide/KLH pulsed DC demonstrated an immune response.

• The only objective response in a breast cancer patient was associated with a potent CD8 T cell response. Vonderheide et al. *Clin Can Res* 2004.

• The same hTERT I540 peptide with Montanide did not induce immune responses with CD8 T cells that recognized native cell lines; 0 responses were observed. Parkhurst et al. *Clin Can Res* 2004.
Peptide-pulsed CD34+ derived DC

• 18 patients were treated with multiple melanoma peptide-pulsed DC generated from CD34+ progenitor cells
• 16/18 responded by ELISPOT to *ex vivo* or restimulated cells
• 6/7 pts with response to 2 peptides or less progressed, versus only 1 of 9 with an immune response with p=0.02; the authors felt that response correlated with benefit
• Follow-up suggests that survival does correlate with immune response to more than 2 antigens
• Palucka et al *Cancer Res* 2001
CEA peptide-pulsed flt3L derived DC: immune response correlation

- Patients were treated with heteroclitic CEA peptide-pulsed DC after flt3L treatment
- 2 clinical responses of 12 seen
- Correlation of clinical response with CD8 tetramer-specific immune response to CEA
- Fong et al. *P.N.A.S.* 2001
Immune Assays for tumor specific T cells: strengths and weaknesses

• Choice of surrogate assay is important to guide future development
• ELISPOT methodology is limited in its reliability, flexibility and reproducibility, but is today’s choice
• Flow assays can be standardized and easily controlled, but are not functional assays
• New tetramer assay generates functional CD8 T cell data; it is based on staining with CD107a, a lysosomal membrane protein, to denote lytic T cells
• Tetramer array in development yields quantitative data on T cell phenotype and function
High avidity T cell clones are CD107a positive  Lee et al Nat Med 2003

- CD8 T cell clones were raised from gp100 peptide-vaccinated melanoma patients
- Most were low avidity and did not recognize tumor cells or APC pulsed with low peptide concentration; some were high avidity but all bound gp100 tetramer
- The high avidity clones were lytic, recognized tumor cells and expressed CD107a
Tetramer+ CD8 high avidity T cell clones are CD107a positive and recognize tumor cells

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<tr>
<th>Average % cytotoxicity</th>
<th>Functional avidity (M)</th>
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<tr>
<td></td>
<td>CD107+</td>
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<td>35.3</td>
<td>-3.3</td>
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CD107a/tetramer flow assay: high avidity T cells recognize tumor cells
Functional status of TAA specific immune response: endogenous* vs. vaccine induced T cells:

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<tr>
<th>Patient ID</th>
<th>TAA</th>
<th>T2-peptide</th>
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<th>Malm e-3M</th>
<th>A-375</th>
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<td>422</td>
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<td>27.2</td>
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<td>54.9</td>
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<th>Patient ID</th>
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<th>% tetramer</th>
<th>mel526</th>
<th>Malm e 3M</th>
<th>A-375</th>
<th>% functional response</th>
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<td>21.5</td>
<td>6.1</td>
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MHC-Cytokine Arrays -
Cytokine Sandwich Assays

Secondary cytokine Detection antibody
Conjugated to a fluorophore

Co-spotted Cytokine Capture antibody

Cytokine secreted by T cell after recognition of Peptide/MHC

Chen, DS 2004
## T Cell Functional Profile

**Capture Probes:** αCD8, gp100 209/A2, MART1 25/A2, CMVpp65/A2, αCD3/αCD28

**Cytokine Detector Probes:**

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<thead>
<tr>
<th>IL4</th>
<th>IFNγ</th>
<th>IL12</th>
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<tr>
<td>IL5</td>
<td>TNFα</td>
<td>IL15</td>
</tr>
<tr>
<td>IL10</td>
<td>GranzymeB</td>
<td>VEGF</td>
</tr>
<tr>
<td>IL13</td>
<td>GM-CSF</td>
<td>VEGF-D</td>
</tr>
<tr>
<td>TGFβ</td>
<td>IL1b</td>
<td></td>
</tr>
<tr>
<td>IL2</td>
<td>IL6</td>
<td></td>
</tr>
<tr>
<td>IL7</td>
<td>No Co-Spot</td>
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</table>

- **IL4**: Regulatory cytokine
- **IL5**: Regulatory cytokine
- **IL10**: Immunosuppressive
- **IL13**: Regulatory cytokine
- **TGFβ**: Growth factor (f)
- **IL2**: Stimulatory cytokine
- **IL7**: Cytokine growth f
- **IFNγ**: Stimulatory pleiotropic f
- **TNFα**: Stimulatory pleiotropic f
- **GranzymeB**: Mediator of CTL killing, apoptotic f
- **GM-CSF**: Hematologic growth f
- **IL1b**: Inflammatory cytokine
- **IL6**: Stimulatory cytokine
- **IL12**: Stimulatory cytokine
- **IL15**: Stimulatory cytokine
- **VEGF**: Angiogenic f
- **VEGF-D**: Angiogenic/lymphogenic

*Chen, DS 2004*
αCD8 Co-Spots

- IL4
- IL5
- IL10
- IL13
- IL2
- IL7

- IFNγ
- TNFα
- Granzyme B
- GM-CSF
- IL1b

- IL12
- IL15
- VEGF
- VEGF-D
- No Co-Spot

MART1/A2 Co-Spots

- IL4
- IL5
- IL10
- IL13
- IL2
- IL7

- IFNγ
- TNFα
- Granzyme B
- GM-CSF
- IL1b

- IL12
- IL15
- VEGF
- VEGF-D
- No Co-Spot

Chen, DS 2004
# Functional T Cell Responses to Peptide Vaccines

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<tr>
<th>Pt</th>
<th>ID</th>
<th>Vax</th>
<th>Adj</th>
<th>Stage</th>
<th>Outcome</th>
<th>IFNγ</th>
<th>TNFα</th>
<th>GranzB</th>
<th>IL-2</th>
<th>TGFβ</th>
<th>IL1b</th>
<th>IL6</th>
<th>GMCSF</th>
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<tbody>
<tr>
<td>1</td>
<td>68w</td>
<td>3 pep</td>
<td>High IL12</td>
<td>IV</td>
<td>Alive, 13m</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>2</td>
<td>76m</td>
<td>3 pep</td>
<td>High IL12</td>
<td>IV</td>
<td>Alive, 13m</td>
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<tr>
<td>3</td>
<td>72m</td>
<td>3 pep</td>
<td>High IL12</td>
<td>III</td>
<td>Recur m8/deceased</td>
<td></td>
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<tr>
<td>4</td>
<td>65w</td>
<td>3 pep</td>
<td>Low IL12</td>
<td>III</td>
<td>Relapsed m11, resected</td>
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</tr>
<tr>
<td>5</td>
<td>52w</td>
<td>3 pep</td>
<td>Low IL12</td>
<td>III</td>
<td>Alive, 16m</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>74m</td>
<td>3 pep</td>
<td>Low IL12</td>
<td>III</td>
<td>Alive, 16m</td>
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<th>IFNγ</th>
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<tr>
<td>8</td>
<td>42m</td>
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<td>GMCSF</td>
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<td>9</td>
<td>51m</td>
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<td>III</td>
<td>Alive, 5yrs</td>
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**Key**

- # of Blocks
- Denotes gp100
- Specific Activity

Chen, DS 2004
Overview

- Analysis of T cell specificity and function
- Single cell resolution
- High throughput
- Few peptide-specific T cells are responsive
- Different vaccination strategies result in different functional profiles
- Interferon-γ and TNF-α discordance correlates with poor outcomes
- IL-1β and IL-6 secretion is associated with good outcomes
- Representation of complex cellular interplay

Chen, DS 2004
Conclusions and Lessons Learned

• Immune monitoring is more rigorously and carefully done and ex vivo tetramer and ELISPOT assays are more widespread than when we last met in 2001.
• More evidence on the correlation between immune response and clinical benefit seen, but most trials have failed to show any correlation.
• State of the art functional *ex vivo* assays are necessary, and new assays and arrays are likely to be useful.
• Immune response assays provide feedback on optimal vaccine development and mechanistic understanding.
• High avidity, long lasting T cells capable of recognizing antigen on tumor cells are needed.
• We need to think outside the box on the development of new surrogate assays of immunity in cancer.