Peptide Approaches to Melanoma Vaccines: Innovations and Challenges

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Do vaccines work?

What is their job?
To induce immune responses……..
……YES they work
To induce clinical responses
……YES they work (3-5%)
Do they work well for clinical outcome?
…… No
Does any other systemic therapy work well for melanoma?
…… No
What is the best cancer vaccine?

• One that hasn’t been developed yet.
Dendritic cells: targeting ex vivo and in vivo

melanoma peptides

+ cytokines

+ Adjuvant

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In the diagram:
- Dendritic cells
- Melanoma peptides
- Cytokines
- Adjuvant

The diagram illustrates the interaction between dendritic cells and various components such as melanoma peptides, cytokines, and adjuvants, highlighting their role in targeting both ex vivo and in vivo.
Why study peptide vaccines?

1. Pure………….Avoid tolerizing cellular antigens; exclude normal protein, avoid autoimmunity.
2. Processed…..Avoid effects of immunoproteasome.
3. Cheap……….Feasible to study without corporate support.
4. Easier………..Lower regulatory hurdles
5. Evaluable……Excellent cancer vaccine model, allowing direct evaluation of response to the specific immunogen
6. Modifiable……Create synthetic peptides better than native peptides
7. Immunogenic..Induce T cell responses in patients
8. Combinable….Multipeptide vaccines may mimic immune effects of whole cell vaccines.
Early concept for vaccine therapy

- Immunologic ignorance
- Tumor antigens as weak antigens

Vaccine ➔ Immune Response ➔ Clinical Response ➔ Improved Survival
Immunologic pathways in melanoma progression

- p16
  - RAS
    - BRAF
    - AKT
  - MAPK
  - mTOR

- MHC loss
  - Ag/APM loss
    - IL-10
    - Arginase
    - IDO

- Pre-existing tolerance
  - Ag-spec Tc
  - T_{reg}

- Escape variants
- Anergy

Failure of immune rejection
Or immune adaptation

Concept only
Approaches for Immune Therapy of Melanoma

Antibody
- carbohydrates and proteins

CD8 killer T-cells
- MHC Class I + peptide

CD4 helper T-cells
- MHC Class II + peptide

Adoptive vs Active Immunotherapy
Typical Regimen for Multipeptide Vaccines – University of Virginia

Resected stage IIB-IV melanoma. HLA-A1, A2, or A3. Tumor gp100+, tyrosinase+

Randomize

4 MELANOMA PEPTIDES + tetanus helper peptide in 110 ug GMCSF + Montanide ISA51 adjuvant

Vaccinate weekly x 3 ID and SQ in 2 sites

Harvest

Vaccinate weekly x 3 ID and SQ in 1 site

Harvest

12 MELANOMA PEPTIDES + tetanus helper peptide in 110 ug GMCSF + Montanide ISA51 adjuvant

Vaccinate weekly x 3 ID and SQ in 2 sites

PBL

SIN

PBL
### 12-peptide: MDPs and CTAs; 3 index peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Protein</th>
<th>MHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAEKSDICTDEY</td>
<td>tyrosinase</td>
<td>HLA A1</td>
</tr>
<tr>
<td>SSDYVPIPIGTY</td>
<td>tyrosinase</td>
<td>HLA A1</td>
</tr>
<tr>
<td>EADPTGHSY</td>
<td>MAGE-A1</td>
<td>HLA A1</td>
</tr>
<tr>
<td>EVDPIGHLY</td>
<td>MAGE-A3</td>
<td>HLA A2</td>
</tr>
<tr>
<td>YMDGTMSQV</td>
<td>tyrosinase</td>
<td>HLA A1</td>
</tr>
<tr>
<td>YLEPGPVTA</td>
<td>gp100</td>
<td>HLA A2</td>
</tr>
<tr>
<td>IMDQVPFSV</td>
<td>gp100</td>
<td>HLA A2</td>
</tr>
<tr>
<td>GLYDGMEHL</td>
<td>MAGE-A10</td>
<td>HLA A2</td>
</tr>
<tr>
<td>ALLAVGATK</td>
<td>gp100</td>
<td>HLA A3</td>
</tr>
<tr>
<td>LIYRRRLMK</td>
<td>gp100</td>
<td>HLA A3</td>
</tr>
<tr>
<td>SLFRAVITK</td>
<td>MAGE-A1</td>
<td>HLA A3</td>
</tr>
<tr>
<td>ASGPGGGGAPR*</td>
<td>NY-ESO-1</td>
<td>HLA A3</td>
</tr>
</tbody>
</table>

* A31/A3
**Arm A**
(4 peptide mix):

Reactivity to Index peptide only

**Arm B**
(12 peptide mix):

Reactivity to Index peptide + 2 others
Immunogenicity of the 12 peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>HLA-A1</th>
<th>HLA-A2</th>
<th>HLA-A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr (240)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyr (146)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGE-A1 (161)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGE-A3 (168)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyr (369D)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gp100 (280)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gp100 (209-2M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGE-A10 (254)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gp100 (17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gp100 (614)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGE-A1 (96)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NY-ESO-1 (53)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Persistent vaccine-induced responses to GP100-209-2M [IMD] & MAGE-A10 [GLY], transient response to tyrosinase [YMD]

ELISPOT VMM490 (Mel 39-GroupB) Stim1x

#IFNg secreting cells/10^5

- YLE
- YMD
- IMD
- GLY
- C1RA2
- C1RA2+GAG

Pre          1         2         SIN         3         3A  4          5        6 +5mo

Vaccine number

0  200  400  600  800  1000  1200  1400  1600

#IFNg secreting cells/10^5
Sustained systemic immune response to DAEKSDICTDEY in VMM 150 (HLA-A1)
UVA-Mel 43 Fresh ELIspot. With CD8+ No Stimulation

#IFNg secreting cells/10^5 CD8

Patient 1

Patient 2

Patient 3

Pre 3 6 Pre 2 7 Pre 3 6
3wk 9mos 2wk 9mos 3wk 3mos

ALLA gp100
LIY gp100
SLF MAGE-A1
ASG NY-ESO-1
C1RA3+GAG
C1RA3
## T cell responses to HLA-A3 / ALLAVGATK (gp100 17-25)

<table>
<thead>
<tr>
<th></th>
<th>PBL</th>
<th>SIN</th>
<th>PBL</th>
<th>PBL</th>
<th>PBL</th>
<th>PBL</th>
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<tbody>
<tr>
<td></td>
<td>Prevaccine</td>
<td>3 vaccines</td>
<td>3 vaccines</td>
<td>4 vaccines</td>
<td>5 vaccines</td>
<td>6 vaccines</td>
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<tr>
<td></td>
<td>Week 0</td>
<td>Week 3</td>
<td>Week 3</td>
<td>Week 4</td>
<td>Week 5</td>
<td>Week 6</td>
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<tr>
<td><strong>Tetramer</strong></td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
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<tr>
<td><strong>CD8</strong></td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
<td><img src="image9" alt="Graph" /></td>
<td><img src="image10" alt="Graph" /></td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
</tr>
<tr>
<td>% tet+/CD8+</td>
<td>0.10%</td>
<td>0.85%</td>
<td>1.77%</td>
<td>0.98%</td>
<td>1.52%</td>
<td>1.39%</td>
</tr>
<tr>
<td>Staining of tetramer+ cells for markers of effector or memory phenotype:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD28</strong></td>
<td><img src="image13" alt="Graph" /></td>
<td><img src="image14" alt="Graph" /></td>
<td><img src="image15" alt="Graph" /></td>
<td><img src="image16" alt="Graph" /></td>
<td><img src="image17" alt="Graph" /></td>
<td><img src="image18" alt="Graph" /></td>
</tr>
<tr>
<td>% CD45RO+/CD28+</td>
<td>47.2</td>
<td>29.3</td>
<td>20.4</td>
<td>10.8</td>
<td>4.5</td>
<td>16.6</td>
</tr>
<tr>
<td>% CD45RO-/CD28-</td>
<td>8.9</td>
<td>15.4</td>
<td>29.6</td>
<td>11.4</td>
<td>32.2</td>
<td>18.3</td>
</tr>
<tr>
<td>% CD28</td>
<td>54.6</td>
<td>32.6</td>
<td>23.4</td>
<td>11.6</td>
<td>5.6</td>
<td>17.2</td>
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<tr>
<td>% CD45RO</td>
<td>70.8</td>
<td>68.5</td>
<td>55.7</td>
<td>69.3</td>
<td>53.8</td>
<td>64.8</td>
</tr>
<tr>
<td>% CD62L</td>
<td>3.3</td>
<td>17.3</td>
<td>20.7</td>
<td>19.1</td>
<td>11.9</td>
<td>23.4</td>
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<tr>
<td>% CD27</td>
<td>78.0</td>
<td>58.0</td>
<td>70.4</td>
<td>59.7</td>
<td>38.2</td>
<td>51.5</td>
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Tumor-induced response to YMD (Tyrosinase) 
Transient vaccine-induced response to IMD, GLY

ELISpot VMM381 (Mel 39-GroupB) Stim 1x

<table>
<thead>
<tr>
<th>Vaccine number</th>
<th>YLE</th>
<th>YMD</th>
<th>GLY</th>
<th>C1RA2</th>
<th>C1RA2+GAG</th>
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<tbody>
<tr>
<td>Pre</td>
<td>2.5</td>
<td>3.1</td>
<td>2.9</td>
<td>0.15</td>
<td>4.9</td>
</tr>
<tr>
<td>1</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>SIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>3A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.5% tetrame</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 + 5 mo</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Vaccine number

#IFNg secreting cells/10^5
Stable Phenotype of Tumor-Induced CD8+ T cells reactive to Tyr \textsubscript{369-377D}

- **CD27**: negative (80%)
- **CD28**: negative
- **CD45RA**: positive (90%)
- **CCR7**: pos/neg (50/50)
Evaluation over time and in different compartments

- Three patterns of T cell response
  - Persistent vaccine-induced
    - SIN CD28+ CD45RO+
  - PBL progressive loss of CD28 and CD45RO with vaccination
  - PBL 6 weeks after last vaccine, regain CD28, and fewer total tetramer+ cells
    - May mimic acute antigen exposure, conversion to memory
  - Transient vaccine-induced
  - Tumor-induced
    - Response may be limited to PBL
    - Vaccine responses may be limited to SIN
    - CD45RA+, CD27 low, CD28-neg, CCR7 50%+
    - Central memory? Terminally differentiated effectors?
    - Regulatory function?
### Class II-MHC Restricted Melanoma Peptides (6)

<table>
<thead>
<tr>
<th>Protein (residues)</th>
<th>Allele</th>
<th>Peptide Sequence</th>
<th>Source</th>
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<tbody>
<tr>
<td>Tyrosinase 56-70</td>
<td>DR4</td>
<td>QNILLSNAPLGPQFP</td>
<td>Topalian</td>
</tr>
<tr>
<td>Tyrosinase 388-406</td>
<td>DR15</td>
<td>FLLHHAFVDSIFEQWLQRHRP</td>
<td>Kobayashi</td>
</tr>
<tr>
<td>MelanA 51-73</td>
<td>DR4</td>
<td>RNGYRALMDKSLHVGTQCALTRR</td>
<td>Zarour</td>
</tr>
<tr>
<td>MAGE-3 281-295</td>
<td>DR11</td>
<td>TSYVKVLHHMVKISG</td>
<td>Manici</td>
</tr>
<tr>
<td>MAGE-1-3, 6121-134</td>
<td>DR13</td>
<td>LLKYRAREPVTKAEB</td>
<td>Chaux</td>
</tr>
<tr>
<td>gp100 44-59</td>
<td>DR1, DR4</td>
<td>WNRQLYPEWTEAQRDL</td>
<td>Halder/Li</td>
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</table>
T cell proliferative response to tetanus peptide

Proliferative Response to Tetanus Peptide

**Mel41 – Vax**
with 6 Melanoma Helper Peptides

**Mel31 – Vax**
with Tetanus Helper Peptide

<table>
<thead>
<tr>
<th>Trial / patient ID</th>
<th>Stimulation Index</th>
</tr>
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<tbody>
<tr>
<td>Mel41 582</td>
<td>0</td>
</tr>
<tr>
<td>Mel41 537</td>
<td>0</td>
</tr>
<tr>
<td>Mel41 504</td>
<td>0</td>
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<tr>
<td>Mel41 425</td>
<td>0</td>
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<tr>
<td>Mel41 550</td>
<td>0</td>
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<tr>
<td>Mel41 164</td>
<td>0</td>
</tr>
<tr>
<td>Mel31 182</td>
<td>0</td>
</tr>
<tr>
<td>Mel31 334</td>
<td>0</td>
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<tr>
<td>Mel31 326</td>
<td>0</td>
</tr>
<tr>
<td>Mel31 371</td>
<td>0</td>
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</tbody>
</table>
T cell proliferative response to 6 melanoma helper peptides

Mel41
Vax with 6 Melanoma Helper Peptides

Mel31 – Vax with Tetanus Helper Peptide
Cytokine release to melanoma helper peptides

VMM537 Cytokine Responses to Helper Peptides

Mel41 (SIN)

DR*0404 (AQN, RNG, WNR)
DR*1501 (FLL)

Cytokine conc (pg/ml)

Peptide

AQN, RNG, WNR
FLL
LLK
RNG
TSY
WNR

IFNg
TNFa
IL10
IL5
IL4
IL2
Cytokine release to melanoma helper peptides

VMM582 Cytokine Responses to Helper Peptides
Mel41 (SIN)

Cytokine conc (pg/ml)

Peptide

IFNg
TNFa
IL10
IL5
IL4
IL2

0  100  200  300  400  500  600  700  800  900  1000

AQN  FLL  LLK  RNG  TSY  WNR
Vaccines with poor T cell response

Vaccines with good T cell response

Stage III Mel 32 and 37

Stage III Mel 36 and 39

Survival of Stage IV Melanoma patients – Peptide vaccine trials
Clinical trials evaluating effects of helper peptides (6 MHP) on CTL response (to 12MP) and clinical outcome

• **UVA-Mel44**
  – (UVA, MD Anderson, Fox Chase)
  – Resected stages IIB-IV (n = 168)
  – 12 MP + tetanus peptide vs 12 MP + 6 MHP
  – Pretreatment with cytoxan (or not)

• **ECOG 1602**
  – Advanced stage IV melanoma (n = 176)
  – 4 arms: (i) 12 MP, (ii) 12 MP + tetanus peptide, (iii) 12 MP + 6 MHP, (iv) 6 MHP
Summary

• The lesion in the immune response to cancer is much more complex than simply a weak immune response to defined antigens.

• Given the complexity of the host:tumor relationship and the layers of regulatory control and immune escape mechanisms mediated by melanoma cells, it is remarkable that single interventions with vaccines have led to objective clinical responses in any patients.

• Thus, single digit response rates, though disappointing results for melanoma therapy, are an encouraging proof of principle for T-cell directed cancer vaccines.

• Furthermore, clinical data with peptide vaccines suggest the possibility of clinical benefit.

• These results should serve as a call to take a closer look at immune regulatory processes and principles, and to develop more comprehensive and multi-agent approaches to modulate the host: tumor relationship.