Multipeptide vaccines for melanoma – Strategies for increasing the breadth of the T cell repertoire

Craig L. Slingluff, Jr., M.D.
Department of Surgery
Human Immune Therapy Center
University of Virginia, Charlottesville, VA

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Challenges for Peptide Vaccines

- Heterogeneity of antigen expression
  - Downregulation of melanocytic differentiation protein (MDP) expression in metastases
  - Expression of cancer-testis antigens (CTA) in subsets of melanomas, increased in metastases
    → Include peptides from MDPs and CTAs
- Heterogeneity of HLA types in patient populations
  → Target HLA-A1, A2, and A3 (80% of population)
12-peptide: MDPs and CTAs; 3 index peptides

- DAEKSDICTDEY tyrosinase 240-251S
- SSDYVIPIGTY tyrosinase 146-156
- EADPTGHSY MAGE-A1 161-169
- EVDPIGHLY MAGE-A3 168-176
- YMDGTMSQV tyrosinase 369-376D
- YLEPGPVTA gp100 280-288
- IMDQVPFSV gp100 209-217M
- GLYDGMEHLM MAGE-A10 254-262
- ALLAVGATK gp100 17-25
- LIYRRRLMK gp100 614-622
- SLFRAVITK MAGE-A1 96-104
- ASGPPGGGAPR* NY-ESO-1 53-62
**UVA-Mel 39: Evaluation of Immunogenicity of a Multi-Epitope Vaccine Incorporating Differentiation Proteins and Cancer-Testis Antigens**

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

  **Randomize**
  - **Yes**
    - Eligible for HD-IFN?  
      - **No**
        - Ineligible for trial
      - **Yes**
        - Vaccinate weekly x 3 ID and SQ in 1 site
  - **No**
    - 4 MELANOMA PEPTIDES + tetanus helper peptide in 110 ug GMCSF + Montanide ISA51 adjuvant
      - Vaccinate weekly x 3 ID and SQ in 2 sites
    - 12 MELANOMA PEPTIDES + tetanus helper peptide in 110 ug GMCSF + Montanide ISA51 adjuvant
      - Vaccinate weekly x 3 ID and SQ in 2 sites
    - Vaccinate weekly x 3 ID and SQ in 1 site

  **Harvest**
  - **PBL**
  - **SIN**
Mel39: Hypotheses

1) A 12-peptide melanoma vaccine will be safe.
2) Each of the 12 peptides in this mixture will be immunogenic.
3) Vaccination with 12 peptides will increase the total CTL reactivity against tumor antigens, when compared to 4 peptides.
4) Addition of 3 peptides competing for binding to the same MHC molecule will not significantly inhibit immunogenicity of an index peptide.
# Study Population

<table>
<thead>
<tr>
<th></th>
<th>Group A 4 peptide</th>
<th>Group B 12 peptide</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>26</td>
<td>25</td>
<td>51</td>
</tr>
<tr>
<td>Age (median)</td>
<td>49</td>
<td>56</td>
<td>53</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>54%</td>
<td>68%</td>
<td>61%</td>
</tr>
<tr>
<td>Stage IIB/C</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Stage III</td>
<td>19</td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td>Stage IV</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>HLA-A1</td>
<td>10</td>
<td>6</td>
<td>16 (31%)</td>
</tr>
<tr>
<td>HLA-A2</td>
<td>15</td>
<td>13</td>
<td>28 (55%)</td>
</tr>
<tr>
<td>HLA-A3</td>
<td>7</td>
<td>14</td>
<td>21 (41%)</td>
</tr>
</tbody>
</table>
Ex vivo Reactivity to gp100\(_{17-25}\) (HLA-A3, ALLAVGATK) in 12-peptide vaccine

Pre-vaccine                 After 4 vaccines

0.1%                        1.39%

44.1                        44.7

55.8                        53.4
Ex vivo Evaluation of Patient Lymphocytes, ELIspot assay

ELIspot Ex Vivo
VMM427 & VMM437 CD8+ sep

#IFNg secreting cells/10^5

DAEK C1RA1 Alone GAG

Patient VMM427 Patient VMM437
Pre SIN after 4 Pre SIN after 4
ELIspot Assays on IVS Lymphocytes: Immune Responses against melanoma peptides

• All Patients (Group A & B) - PBL & SIN lymphocytes sensitized once *in vitro* with 12 Melanoma Peptide Mixture

• ELIspot assay day 14, against all 12 Melanoma peptides relevant to their HLA-type, individually.

• 48 of 51 patients evaluable for T cell response (94%)

• Positive response: > 2 x background, > 30 spots/100,000 cells, > 2x pre-vaccine response.

• Among observed T-cell responses,
  - 86% > 5x, 72% > 10x, 53% > 20x background.
  - Pre-vaccine responses seen in only 3 cases.
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>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr (240)</td>
<td>87</td>
</tr>
<tr>
<td>Tyr (146)</td>
<td>50</td>
</tr>
<tr>
<td>MAGE-A1 (161)</td>
<td>25</td>
</tr>
<tr>
<td>MAGE-A3 (168)</td>
<td>50</td>
</tr>
<tr>
<td>Tyr (369D)</td>
<td>75</td>
</tr>
<tr>
<td>gp100 (280)</td>
<td>100</td>
</tr>
<tr>
<td>gp100 (209-2M)</td>
<td>75</td>
</tr>
<tr>
<td>MAGE-A10 (254)</td>
<td>100</td>
</tr>
<tr>
<td>gp100 (17)</td>
<td>75</td>
</tr>
<tr>
<td>gp100 (614)</td>
<td>50</td>
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<td>75</td>
</tr>
<tr>
<td>NY-ESO-1 (53)</td>
<td>25</td>
</tr>
</tbody>
</table>

HLA-A1: gp100 (209-2M), MAGE-A10 (254)
HLA-A2: Tyr (240), Tyr (146), MAGE-A3 (168), Tyr (369D), gp100 (280), gp100 (209-2M), MAGE-A10 (254), gp100 (17), gp100 (614)
HLA-A3: gp100 (209-2M), MAGE-A10 (254), gp100 (17), gp100 (614)
Max PBL and SIN responses (mean) by group restricted by HLA-A2

**PBL**

- # IFNγ secreting cells/100K
- Peptides: Tyr (YMD), Gp100 (YLE), Gp100 (IMD), MAGE-A10 (GLY)

**SIN**

- Group A (4)
- Group B (12)
- Peptides: Tyr (YMD), Gp100 (YLE), Gp100 (IMD), MAGE-A10 (GLY)
Immune Response to Index Peptides
Unchanged between groups

- T cell response to at least one index peptide in the PBL, SIN or either
- By treatment group (n = 24 per group; 48 overall)
- P values (Chi-sq):
  - PBL 0.75
  - SIN 0.79
  - Either 0.71
Immune Response to any Peptide increased in Group B (12 peptide)

- T cell response to at least one peptide in the PBL, SIN or either
- By treatment group (n = 24 per group)
- P values (Chi-sq):
  - PBL 0.30
  - SIN 0.11
  - Overall < 0.02 *
- Response to ~0.9 (A) vs ~2.8 (B) peptides
Cumulative responses to All Peptides (mean)
Ratio # IFN\(\gamma\)-secreting cells/ background

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<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio = PBL</td>
<td>0.88 x</td>
<td>0.81 x</td>
</tr>
<tr>
<td>P</td>
<td>0.58</td>
<td>0.80</td>
</tr>
<tr>
<td>Ratio = SIN</td>
<td>2.59 x</td>
<td>2.20 x</td>
</tr>
<tr>
<td>P</td>
<td>0.093</td>
<td>0.042</td>
</tr>
</tbody>
</table>
Findings

• Vaccination with this 12 peptide mixture is immunogenic in 100% of melanoma patients tested.

• Individually, 10 of the 12 peptides are immunogenic.

• Non-mutated peptide antigens can be reliably immunogenic.

• At least 4 peptides not previously tested in humans are immunogenic, expanding peptides available for clinical trials in HLA-A2+ and HLA-A3+ patients (A2: MAGE-A10254-262 (Huang); A3: gp100614-622 (Kawakami), MAGE-A196-104 (Chaux), NY-ESO-153-62 (Wang)).

• More peptides restricted by HLA-A1 are needed for clinical investigation.
Findings

• The 12-peptide vaccine mixture induces multivalent T-cell immune responses simultaneously, resulting in a 2-2.5x increase in the cumulative T cell response compared to a 4-peptide vaccine.

• Peptide competition for MHC does not appear to limit immunogenicity when 4 peptides of varied affinity for the MHC are administered in a single emulsion.

• Vaccines with multiple peptides can incorporate those peptides in single preparations of peptide mixtures.

• Ongoing and future studies will evaluate mixtures of peptides for helper T cells given in combination with this mixture of 12 Class I MHC restricted peptides.
Contributors

Human Immune Therapy Ctr
Kim Bullock
Elizabeth Woodson
Scott Boerner
Robyn Fink
Donna Barnd
Gina Petroni
Eric Bissonnette

Melanoma Team
William W. Grosh
James Patterson
Patrice Neese
Carmel Nail

Laboratory Analyses
Walter Olson
Galina Yamshchikov
Cheryl Murphy
Donna Deacon
Courtney Garbee
Jessica Thistlethwaite
Sarah Hibbitts
Naomi Anderson
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