Local, regional and systemic effects of TLR agonists and incomplete Freund’s adjuvants for peptide vaccines

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Disclosures

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The rebirth of cancer vaccines

• Immune checkpoint therapy proves the relevance of immune surveillance

• Failures of checkpoint blockade in some patients highlight the need to augment antitumor efficacy with combination immunotherapies, which can include vaccines.

• Technologies for high throughput sequencing of patient tumors have enabled more rapid identification of mutated neoantigens that show promise as ideal antigens for vaccines.
Vaccination with mutated neoantigens
Is there consensus on how to vaccinate?

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>PI (Site)</th>
<th>Mutated neoantigens</th>
<th>Adjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00001703</td>
<td>Khleif, NIH</td>
<td>mutated VHL peptides</td>
<td>IFA</td>
</tr>
<tr>
<td>NCT00019006</td>
<td>Khleif, NCI</td>
<td>mutated RAS peptides</td>
<td>DETOX-B</td>
</tr>
<tr>
<td>NCT00003959</td>
<td>Stephen Nimer, MSKCC</td>
<td>mutated RAS peptides</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>NCT00019331</td>
<td>Barry Gause, Magnuson Ctr</td>
<td>RAS peptides</td>
<td>GM-CSF, DETOX-PC, systemic IL2</td>
</tr>
<tr>
<td>NCT01885702</td>
<td>Jolanda IM de Vries; Radboud Univ, Netherlands</td>
<td>Mutated neoantigens in MSI-H CRC and germline MMR-gene mutation</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>NCT01970358</td>
<td>Patrick Ott, DFCI</td>
<td>personalized mutated NeoAg</td>
<td>polyICLC</td>
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</tbody>
</table>

- Wide range of selected adjuvants reflects lack of consensus on the best adjuvant(s) to use, locally or systemically, with these seemingly ideal neoantigens.
- There is a major need to define optimal local and systemic adjuvants, for use with all vaccines.
**Neo-antigen:**

A newly acquired and expressed antigen

- Mutated neo-antigens: Epitopes in normal proteins arising by somatic mutations in cancer cells, not in normal cells.
  - Unique (driver vs passenger)
  - Shared driver (EGFR, KRAS, BRAF)

- Phosphopeptides: expressed by cancer cells as a reflection of their transformed nature (Victor Engelhard and Donald Hunt).

- Cancer-testis antigens (eg: NY-ESO-1)
  - Expressed only in mature spermatids (lack MHC)

- Cancer-oocyte antigen (eg: SAS1B - John Herr)
  - Expressed only in mature oocytes (lack MHC)

- Viral antigens (eg: HPV, HCV, Merkel polyomavirus)
Factors impacting efficacy of cancer vaccines

**Antigen selection:** Unique vs shared
- Source proteins
- Selection
- Length

**VSME**
- Vaccine site micro-environment

**SIN**
- Sentinel immunized node

**PBMC**
- Peripheral blood

**Tumor**
- Metastasis micro-environment

**T CELL ACTIVATION** (Vaccine adjuvants):
- Local, regional, systemic effects

**T CELL HOMING TO TUMOR**
- Barriers to T cell infiltration
- T cell dysfunction
Antigens for use in cancer vaccines

Crude antigen

*Constructs:* protein, DNA, RNA, or viral constructs

*Presented* on DC/APC or injected directly, or on nanoparticles

*Route:* SQ, IM, ID, transdermal, IV

Tumor cells or lysates

Whole protein

Long peptides

Minimal epitope (short) peptides

Defined antigen
Excision of the sentinel immunized node (SIN) draining the vaccine site, 1 week after vaccine #3.

Identification of the sentinel immunized node in the groin draining a cutaneous vaccine site in the left thigh by Tc99-sulfur colloid injected intradermally around the vaccine site. Gamma camera scan demonstrates radioactivity at the injection site, the lymphatic channel draining toward the sentinel immunized node, and the hot spot representing the location of the SIN in the left groin.

Node sectioned for 4 preservation conditions within 1-5 minutes at the bedside

Immune response in SIN, not in random node

Increasing the number of peptides targeted with a multipeptide vaccine – Mel39

- **DAEKS DICT DEY**
  - tyrosinase
  - gp100
  - MAGE-A1
  - MAGE-A3

- **YMDGT MSQV**
  - tyrosinase
  - gp100
  - MAGE-A10

- **YLP GP VTA**
  - tyrosinase
  - gp100
  - MAGE-A10

- **ALLAVGTK**
  - gp100
  - MAGE-A1

**Index peptides**

**A1**

**A2**

**A3**

**Randomize**

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

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

**Randomize**

- Vaccinate weekly x 3
- ID and SQ in 2 sites

**Harvest**

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

**Randomize**

- Vaccinate weekly x 3
- ID and SQ in 2 sites

**Randomize**

- Vaccinate weekly x 3
- ID and SQ in 1 site

**SIN**

**PBL**

**Immune response (ratio over background)**

**VMM484**

12 peptide vaccine: Reactivity to:
- tyrosinase (369-377)
- gp100 (209-2M)
- MAGE-A10 (254-262)

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- gp100 (209-2M)
- MAGE-A10 (254-262)

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12 peptide vaccine: Reactivity to:
- gp100 (209-2M)
- MAGE-A10 (254-262)
Randomized trial testing effect of GM-CSF as vaccine adjuvant combined with IFA (Mel43)

Direct ELIspot responses

- **Ratio:** 0.47 x 0.81 x
- **P:** <0.001 0.005
- **N:** 60 58 61 57

<table>
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<tr>
<th>Slingluff CCR 2009</th>
<th>No GM</th>
<th>+ GM</th>
<th>No GM</th>
<th>+ GM 1 site</th>
<th>1 site</th>
<th>2 sites</th>
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<tr>
<td>2.55%</td>
<td>N = 53</td>
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</table>

**Tetramer data for HLA-A2 peptides**

- **Ratio:** 1.3 x 0.92 x
- **P:** 0.093 0.28
- **N:** 60 58 59 59

- Addition of GM-CSF significantly decreases circulating T cell responses to the vaccine.
  - **CD8^+** T cell responses: ELIspot & tetramer
  - **CD4^+** T cell responses: ELIspot
- Concurrent paper by Faries ... Morton showed negative clinical effect of GM-CSF added to a whole cell vaccine/BCG.
- GMCSF induces MDSC/MSC in melanoma patients when given with HSP vaccine: Filipazzi... Rivoltini, JCO 2007

Slingluff CCR 2009
Mel 41: Vaccination with Peptides to induce CD4+ helper T cells: 6 melanoma helper peptides (6MHP) in IFA (+/- GM-CSF)

Class II-MHC Restricted Melanoma Peptides (6MHP)

<table>
<thead>
<tr>
<th>Protein (residues)</th>
<th>Allele</th>
<th>Peptide Sequence (14-23 aa)</th>
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<tr>
<td>Tyrosinase 88-100</td>
<td>DR4</td>
<td>(A)QNILSNAPLGFPQ (Topalian)</td>
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<tr>
<td>Tyrosinase 298-408</td>
<td>DR15</td>
<td>FLLHAFVDSIFEGWLQRHHP (Kobayashi)</td>
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<tr>
<td>MelanA 81-103</td>
<td>DR4</td>
<td>RNYRALMDKSLHVGTQCALTRR (Zarour)</td>
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<tr>
<td>MAGE-3 231-244</td>
<td>DR11</td>
<td>TSYVKVLHMMKVIGS (Manici)</td>
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<tr>
<td>MAGE-1-3, 6421-144</td>
<td>DR13</td>
<td>LLKYRAREPVTKAEE (Chaux)</td>
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<tr>
<td>gp100 44-80</td>
<td>DR1, DR4</td>
<td>WNRQLYPEWTEAQRLED (Haldar/LI)</td>
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Helper T cell immune responses (n = 39)
- 57% in PBMC
- 81% in node + PBMC

Clinical responses (n = 17 measurable):
- PR (2/17, 12%) Duration 1, 3.9+ years
- SD (2/17, 12%) Duration 1.8, 7 years
- Durable PR or SD (24%)  

J Clin Oncol 2008

Epitope spreading to CD8+ cell epitopes in 5/11 patients (45%) tested  

Hu, Y. CII 2014
Associations of immune response to 6MHP with survival

ECOG 1602 Landmark analysis (2 months) of overall survival associated with CD4+ T cell response to 6MHP, but not to tetanus peptide (CCR 2013)

Mel41 trial: Improved overall survival associated with (i) early Ab responses (week 5-8, p=0.009), (ii) CD4+ T-cell responses (p=0.03), and (iii) Ab + CD4+ T cell responses, compared to T-cell response alone (p=0.0264)

Reed CM in preparation
Biopsies to test for T cell recruitment and function/dysfunction in the vaccine site microenvironment (VSME)

1 x 3 cm or 2 x 6 cm full thickness excision

Digest portion for viable cells, Some for FFPE, QF, RNA later

Sectioning of vaccine site biopsies
3 punch biopsies (4 mm)

formalin-fixed for paraffin embedding and histology, IHC.

Quick-frozen for IHC and/or protein

RNA later For RNA
Lymphoid aggregates after repeated vaccination with multipeptide vaccine in IFA, without GM-CSF (Mel44), 2 weeks after the 3\textsuperscript{rd} vaccine.

Induction of lymphoid aggregates resembling tertiary lymphoid structures (TLS), with PNAd+ endothelium and mature DC (CD83) in T cell areas, and clustering of B cells. T cell proliferation (CD4+Ki67+ and CD8+Ki67+) counterbalanced by T regs (CD4+ FoxP3+), with chemokines CXCL12,13, CCL21.

Harris RC. J Immunother 2012
gp100-reactive T cells home preferentially to vaccine site with gp100/incomplete Freund’s adjuvant (IFA), rather than to tumor

Short peptide plus IFA induces T cell retention at the VSME and depletion from circulation.

Long peptides in IFA induce durable immune responses and tumor control.

Hailemaichael….Overwijk, Nat Med 2013 (MDACC) S.Fig 15)
Mel48: A multipeptide vaccine in melanoma patients with evaluation of the injection site microenvironment

Group 1
Vaccines: Wks 0, 1, 2, 4, 5, 6
Biopsy replicate site: Subgroup Week
A 0
B 1
C 3
D 6
E 12

Group 2
Vaccines: Wks 0, 1, 2, 4, 5, 6
Biopsy replicate site: Subgroup Week
A 0
B 1
C 3
D 6
E 12

Skin biopsies 2 x 6 cm excision
Flow, IHC, QF, RNA later

Cellular infiltrates in VSME (with IFA)

#### CD3

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<th>3</th>
<th>6</th>
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#### CD83

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#### T-bet (Th1)

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#### GATA-3 (Th2)

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</table>

**GATA3/T-bet ratio:**

- 2.8
- 6.6
- 0.9
- 1.8
- 2.9
Late formation of granulomas; accumulation of eosinophils, FoxP3+ T regs
Tetramer+ CD8 T cells accumulate in the VSME, but are largely dysfunctional, and express high levels of retention integrins.
**Mel 55: Clinical Trial to evaluate effects of recMAGE-A3 + AS15 Immunotherapeutic at the vaccine site microenvironment**

AS15: TLR4 agonist (MPLA), TLR9 agonist (CpG), saponin QS-21, liposomal

<table>
<thead>
<tr>
<th>Study arm</th>
<th>Adjuvant</th>
<th>Injection site</th>
<th>Abbreviation</th>
<th>N (eligible)</th>
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<tr>
<td>A</td>
<td>AS15</td>
<td>intramuscular</td>
<td>M15</td>
<td>12</td>
</tr>
<tr>
<td>B</td>
<td>AS15</td>
<td>intradermal/subcutaneous</td>
<td>C15</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

- Resected stage IIB-IV melanoma, MAGE-A3+ tumors
Perivascular lymphoid infiltrates induced by AS15 ASCI, with peripheral node addressin (PNAd) expression

VSME biopsies 1 week after the first vaccine

PNA\(_d\) antibody stains HEV-like vessels
MAGE-A3/AS15 immunotherapeutic induces a 4x more favorable Th1/Th2 balance in the VSME early, than vaccine with IFA.

**Mel48 (IFA) Week 1**
- GATA3/T-bet = 5.8x
- FoxP3 10%
- T-bet 13%
- GATA3 77%

**Mel55 (AS-15) Week 1**
- GATA3/T-bet = 1.4x
- FoxP3 16%
- RORγt 13%
- T-bet 30%
- GATA3 41%

- Th1/Th2 ratio 4x more favorable.
- Higher FoxP3 early.
- Better CD8/FoxP3 ratio wk 7.
- Higher early T cell accumulation.
- Much lower immune cell retention.
Long peptides for multipeptide vaccine (LPV7)

<table>
<thead>
<tr>
<th>HLA restriction &amp; protein source for short peptides</th>
<th>Amino acid sequences for short and long peptides</th>
<th>Residues for long peptides</th>
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<tbody>
<tr>
<td><strong>A1</strong> Tyrosinase 240-251s</td>
<td>FTIPYWDWR DAEKSDICTDEY MGGQHPTN</td>
<td>231-259 (29)</td>
</tr>
<tr>
<td><strong>A2</strong> Tyrosinase 369-377</td>
<td>SMHNALHI YMDGTMSQV QGSANDPIFLLHH</td>
<td>361-390 (30)</td>
</tr>
<tr>
<td>gp100 209-217-2M</td>
<td>VPLAHSSSAFT IMDQVPFSV SVSQLRALDG</td>
<td>198-227 (30)</td>
</tr>
<tr>
<td>MAGE-A10 254-262</td>
<td>VIWEALNMM GLYDGMEHL IYGEPRKLLTQD</td>
<td>245-274 (30)</td>
</tr>
<tr>
<td><strong>A3</strong> gp100 17-25</td>
<td>LLHLAVIG ALLAVGATK VPRNQDWLGVSRQL</td>
<td>9-39 (31)</td>
</tr>
<tr>
<td>MAGE-A1 96-104</td>
<td>SREEEGPSTSCILE SLFRAVITK KVADLVG</td>
<td>82-111 (30)</td>
</tr>
<tr>
<td><strong>B35/51</strong> NY-ESO-1 94-102</td>
<td>GARGPESRLLEFYLA MPFATPMEA ELARRS</td>
<td>79-108 (30)</td>
</tr>
</tbody>
</table>

Peptides selected for inclusion of known immunogenic epitope, polar residues (solubility), avoidance of cysteine residues, N-terminal glutamine or glutamic acid.
Enhanced immunogenicity of long vs short peptide (tyrosinase/HLA-A2), in TLR adjuvant

Albino HLA-A2 Tg mice were immunized with 100ug of the short or long tyrosinase peptide, 50ug FGK45 (anti-CD40) and 100ug CpG. Mice were revaccinated 6d later. CD8 T cells were enriched from the spleens and lymph nodes 5d after the 2nd vaccine. T cell response was evaluated by IFNg production and CD107a expression ex vivo.
Patients with resected high-risk melanoma will be randomized among 6 study arms. Each will be vaccinated wks 1, 2, 3, 6, 9, and 12 in an extremity (50% id/ 50% sc) with LPV7 plus tetanus helper peptide. PolyICLC will be injected at the vaccine site (1 mg). Resiquimod will be administered topically at the vaccine site, 112.5 mcg. Blood will be drawn wks 1, 2, 4, 7, 13, 26, 52, 104. Vaccine sites will be biopsied prevaccine (wk 1), and weeks 2 and 4.

**Goals:**
- Safety
- Durable CD8 T cell responses
Summary

• Cancer vaccines, including peptide vaccines, are weakly active clinically in melanoma and other cancers.
• Multipeptide vaccines can be administered safely with high rates of immune response.
• The role of GM-CSF as an adjuvant needs further scrutiny.
• Human data corroborate murine findings of T cell recruitment, retention to IFA vaccines, as possible explanation of transient immune responses.
• Improved outcomes may be anticipated with combination therapies to test optimized antigens, newer adjuvants including TLR agonists and CD40 agonists.
Future directions

• Optimized adjuvants
  – TLR agonists, role of IFA, cytokines

• Optimized antigen combinations
  – Neoantigens (Mutated antigens, Phosphopeptides, Cancer:testis, cancer:oocyte antigens, viral antigens)
  – Long peptides
  – Define role of helper peptides

• Define biologic effects of adjuvants on VSME and SIN to enable small trials to guide vaccine formulations.

• Modulate the tumor microenvironment
  – Combinations with intratumoral therapies
  – Combinations with systemic therapies
Research Team and Collaborators

Immune cell infiltrates in Vaccine Sites
Elise Salerno          Sofia Shea
Donna Deacon          Jochen Schaefer
Lynn Dengel          Gulsun Erdag
Ileana Mauldin       James Patterson
Tim Bullock          Vic Engelhard
Joel Pinczewski

Clinical Trials
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Alison Gaucher       Sasha White
William W. Grosh     Geoff Weiss
Patrice Neese        Carmel Nail
Kathleen Haden

Immunologic Monitoring
Walter Olson          Kelly Smith
Cheryl Murphy         Nadeja Galeassi
Chantel McSkimming

Public Health Sciences
Gina Petroni       Mark Smolkin
Nolan Wages

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Merrick Ross, Sapna Patel, MDACC
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Franco Marincola, NIH
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