LEAPS Vaccines: A Peptide Platform for Creating T Cell Immunogens for Viruses, Cancer and Autoimmunity

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Publications on LEAPS Vaccines

- DH. Zimmerman, PTaylor, ABendele, RCarambula, YDuzant, VLowe, SP O'Neill, E Talor and KS Rosenthal. 2010. CEL-2000: A Therapeutic Vaccine for Rheumatoid Arthritis Arrests Disease Development and Alters Serum Cytokine / Chemokine Patterns in the Bovine Collagen Type II Induced arthritis in the DBA Mouse Model, Intl Immunopharm. 10:412-421
- DH. Zimmerman, PTaylor, ABendele, RCarambula, YDuzant, VLowe, SP O'Neill, E Talor and KS Rosenthal. 2010. CEL-2000: A Therapeutic Vaccine for Rheumatoid Arthritis Arrests Disease Development and Alters Serum Cytokine / Chemokine Patterns in the Bovine Collagen Type II Induced arthritis in the DBA Mouse Model, Intl Immunopharm. 10:412-421
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Today’s Talk

• Overview: LEAPS
  – Ligand Epitope Antigen Presentation System
• J-LEAPS vaccines initiate Th1 immune responses by promoting the development of Dendritic Cells
• J-LEAPS antimicrobial vaccines
• J-HER vaccine for HER-2/neu Breast Cancer
  – Prevention
  – Treatment
• J-LEAPS vaccine therapies for autoimmune diseases
• derG-LEAPS vaccine initiates Th2 immune responses
  – derG-PG-70
<table>
<thead>
<tr>
<th>ICBL/TCBL</th>
<th>PEPTIDE SEQUENCE/ MOLECULAR SOURCE</th>
<th>RECEPTOR</th>
<th>RESPONSE</th>
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</thead>
<tbody>
<tr>
<td>derG</td>
<td>DGQEEKAGVSTGLI MHC-IIβ2 (135-149)</td>
<td>CD4</td>
<td>Th2</td>
</tr>
<tr>
<td>J</td>
<td>DLLKNGERIEKVE β-2 Microglobulin (aa38-50)</td>
<td>??</td>
<td>DC1:Th1</td>
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<tr>
<td>F</td>
<td>VQGEESNDK IL1β (163-171)</td>
<td>IL-1 receptor</td>
<td>MIXED</td>
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<tr>
<td><strong>INFECTIONIOUS DISEASE VACCINES</strong></td>
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<tr>
<td><strong>HIV</strong></td>
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<tr>
<td>H (HGP-30)</td>
<td>YSVHQRIDVKDTKEALEKIEEEQDNKSKKKA</td>
<td>HIV-1 B p17 (aa 85-115)</td>
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<td>m-HGP-30</td>
<td>ATLYSVHQRIDVKDTKEALEKIEEEQN</td>
<td>HIV-1 B p17 (aa 82-112)</td>
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<tr>
<td><strong>MYCOBACTERIA TUBERCULOSIS</strong></td>
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<tr>
<td>M</td>
<td>DQVHFQPLPPAVVKLDAL</td>
<td><em>M. tb.</em> 38 kDa (aa350-369)</td>
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<tr>
<td><strong>HERPES SIMPLEX VIRUS</strong></td>
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<tr>
<td>H1</td>
<td>LYRTFAGNPRA</td>
<td>HSV 1 ICP27 (aa 322-332)</td>
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<tr>
<td>gB1</td>
<td>SSIEFARL</td>
<td>HSV 1 gB (aa 498-505)</td>
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<tr>
<td>gD1</td>
<td>SLKMAEPNRFRGKDLP</td>
<td>HSV 1 Glycoprotein D (8-23)</td>
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<td><strong>INFLUENZA</strong></td>
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<tr>
<td></td>
<td>NP, M2 proteins</td>
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<tr>
<td><strong>THERAPIES</strong></td>
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<td><strong>MYOCARDITIS</strong></td>
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<tr>
<td>My-1</td>
<td>DSAFDVLSFTAAEKAGVYK</td>
<td>Murine Cardiac Myosin (334-352)</td>
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<tr>
<td><strong>RHEUMATOID ARTHRITIS</strong></td>
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<tr>
<td>CEL-2000</td>
<td>TGGKPGIAGFKGEQGPKGEP</td>
<td>Human Collagen type 2 (254-273)</td>
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<tr>
<td><strong>CANCER</strong></td>
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<tr>
<td>Breast: HER</td>
<td>TYVPANASL</td>
<td>Rat HER-2/neu</td>
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<tr>
<td>Melanoma: BRAF</td>
<td>B16 melanoma</td>
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*All vaccines tested in mouse models of disease*
## Published Mouse Studies with J-LEAPS Vaccines: Protection from lethal HSV infection

<table>
<thead>
<tr>
<th>Bullet Points</th>
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<tbody>
<tr>
<td><strong>Anti-HSV vaccines</strong>: 4 different peptides (n = 8-16aa) containing <strong>CD8 T cell epitopes</strong></td>
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<td><strong>ICP27 (JH1, JH2), glycoprotein B (JgB), glycoprotein D (JgD)</strong></td>
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<td>Requires an oil-water emulsion depot: slow release</td>
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<td>Promotes protective Th1 responses in mice</td>
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<td>Protection from lethal HSV infection <strong>without detectable antibody</strong></td>
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<td>CD8 cells and IFN-gamma important to initiate immunity</td>
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<td>T cell response sufficient for protection</td>
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<tr>
<td><strong>Cytokine kinetics (JgD) (obtained without virus challenge)</strong></td>
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<td>Initial phase: IL-12p70, but <strong>not</strong> TNFα or IL-1 (present days 3,10,24)</td>
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<td>Transient Th17: IL-17 (days 3,10)</td>
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<td>Long term Th1: IFN-gamma, IL-2 (days 10,24)</td>
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Published Ex vivo Mouse and human Studies

Activation of Mouse and Human DC precursors

- Sufficient to promote differentiation of precursors into IL12 producing unique type of Dendritic Cells (DC) in <48h.
  - Mouse bone marrow (without or with GM-CSF, IL4)
    - HSV: JgD
    - HIV: JH
    - Influenza A: J-M2e, J-NP
    - Produce IL-12 but not TNFa or IL1
  - Human monocytes (without or with GM-CSF, IL4)
    - JgD and JH
    - Produce IL-12 but not TNFa or IL1

- JgD-DC, JH-DC are sufficient to induce Th1 cytokines in naïve T cells or promote an antigenic boost from immune T cells.
  - Human cells: Activated T cell MLR like response
  - Mouse cells: Booster Response: JgD-DC, not JH-DC enhances IFNgamma production by splenic T cells obtained from JgD immunized mouse
Published Mouse Studies with J-LEAPS Vaccines:

Adoptive transfer of J-LEAPS-DCs elicit protection

- Antigen specific immunity and protection to lethal HSV challenge
  - JgD-DC (HSV), not JH-DC (HIV)
  - JgD-DCs administered within 48 h of preparation
  - Cells washed free of unbound vaccine and other molecules

- J-M2e-DC and J-NP-DC treatment after infection protects mice from lethal influenza infection
  - (Boonnak, J Clin Invest. 2013;123(7):2850–2861.)
  - http://www.jci.org/articles/view/67550
How do J-LEAPS Vaccines Work?

- **Required covalent attachment of ICBL and epitope**
  - Extends and enhances receptor binding
  - Promotes internalization of epitope
  - Cross-linking activation

- **Interacts with myeloid lineage cells**
  - Receptor present on mouse and human cells

- **Promotes the differentiation and activation of precursor cells**
  - Consistent change in activation of transcription factors occurs within 1.5 h
    - Activation of 6 transcription factors for mouse bone marrow, human monocytes, human THP1 cells.
    - Expression of new mRNA consistent with phagocytic cell (SuperArray)

- **J-LEAPS DCs activate CD8 T cells**
  - Peptides contain CD8 not CD4 epitopes
Preventive and Therapeutic Anti-Cancer Vaccines
Switching to an Anti-Tumor (M1/DC1/Th1) Response Through Vaccination

- J-LEAPS promote the development of DC1 cells
  - IL-12p70 production generates Th1 immune responses
- Th1 immune responses are better anti-tumor (CD4/CD8)
  - Interferon gamma
  - More sensitive to tumor antigen expression
  - Cytolytic T cells
  - Antibody??????
LEAPS HER-2/neu Based Breast Cancer Vaccine: J-HER

• J-HER
  – DLLKNGERIEKVE-GGG-TYVPANASL
    • minimal CD8 T cell epitope from HER2/neu

• Mouse model for Breast Cancer
  – TUBO cells: obtained from spontaneous tumors from transgenic Balb/c mice expressing rat HER-2/neu from MMTV promoter.
    • Subq implantation of $5 \times 10^5$ total cells, divided into 2 sites
J-HER TUBO-Tumor Challenge Model

**PREVENTION MODEL**

J-HER in Montanide ISA-51

-3 weeks

Repeat

-1 week

TUBO cells

**THERAPY MODEL**

TUBO cells

1 week

J-HER in Montanide ISA-51

3 weeks

Repeat
Cluster of tumors in mesenteric lymph node of mouse. Note the vascularization of the lymph nodes.

Control (Unimmunized)

Representative presentation of TUBO-tumors in Balb/c mice
Immunized, prevention model

Representative presentation of TUBO-challenged, J-HER immunized mice.

Mesenteric lymph node is small, white, free of structures.
Trial 1: J-HER Prevention Model
Serum taken on day 7 post tumor challenge (day 28 of J-HER immunization (prevent model))

• Tumor bearing animals elicit antibody to HER-2/neu
• J-HER immunized animals make less antibody
  • Less tumor load, less antigen,
  • J-LEAPS vaccines switch the nature of the immune response to Th1, away from antibody production (Th2 responses).
Summary for Prevention Model

• J-HER elicits immune responses that **prevent** initiation of tumors.
  – J-ICBL converts CD8 T cell HER epitope into immunogen
  – J-HER activates and elicits protective response

• Less antibody production in J-HER immunized, tumor bearing animals.
  – Lower tumor load = less antigen
  – J-LEAPS vaccines promote Th1 responses
Trial 3  TREATMENT MODEL

Average tumor load (mm$^3$)

*Day 49, first death

Disease Score
- Tumor load: 1-3
- Necrosis: 1
- Ascites: 1
- Unkempt: 1
- Death: 4
- Maximum: 10

Survival

# survivors

Day
Mouse 1-3: Control
Tumor size: 4775m$^3$
(Died on day 75)

Mouse 2-7: Treated
Tumor size: 6780m$^3$
(alive on day 81)

Trial 3
Photos on Day 56
Mouse 2-1: Treated
Tumor size: 6390m^3

Mouse 1-4: Control
Tumor size: 1610m^3

NOTE: Immunized mouse has primary tumors, but no metastasis
Summary of Treatment Studies

- J-HER **treatment** of tumor bearing mice limits **metastasis**, morbidity and mortality.
  - Solid tumors may continue to grow despite booster immunizations.
Mice vaccinated with iDCs pulsed with J-LEAPS-braf challenged with $5 \times 10^4$ B16 melanoma cells

Survival of J peptide + B16V600E vs. No Rx: Survival proportions

Survival of J peptide vs. DC1 Ova + B16V600E: Survival proportions

Survival of Jpep V600E vs WT: Survival proportions

log-rank: $p=0.0016$

J peptide vs. DC1 Ova V600E

J peptide V600E vs. J peptide WT

J Peptide-BRAF

p=0.0134
Disease progression of untreated and CEL-2000 treated collagen-induced arthritis (CIA) mice

Serum Cytokines in CIA

<table>
<thead>
<tr>
<th></th>
<th>No Rx Day0/Day10</th>
<th>Enbrel Day 10</th>
<th>CEL-2000 Day 10</th>
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</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>2.1/3.08</td>
<td>0.74</td>
<td>0.77</td>
</tr>
<tr>
<td>IL12p70</td>
<td>1.11/1.10</td>
<td>1.58</td>
<td>2.21</td>
</tr>
<tr>
<td>IL17</td>
<td>2.81/3.60</td>
<td>1.78</td>
<td>1.83</td>
</tr>
<tr>
<td>IFN γ</td>
<td>1.81/1.84</td>
<td>1.28</td>
<td>1.84</td>
</tr>
<tr>
<td>IL10</td>
<td>0.87/0.92</td>
<td>1.62</td>
<td>1.96</td>
</tr>
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J-MY-1 LEAPS Prevention of Experimental Autoimmune Myocarditis
Summary of J-LEAPS Vaccines

• J-Leaps vaccines activate T cells to initiate Th1 immune responses
  – Peptide vaccine platform that promotes Th1 cell mediated responses to an incorporated antigenic epitope
  – Vaccines act on precursor cells to generate a unique type of DC
    • Produces IL12 but not TNFalpha or IL1
  – Activate T cell responses to CD8 T cell epitopes without antibody production
  – Demonstrated development of protective (anti-viral) or therapeutic (anti-inflammatory disease) immune responses
derG-LEAPS vaccines activate T cells to initiate Th2 immune responses.

• Immunization with derG-PG-70 prevents and treats proteoglycan induced rheumatoid arthritis. (Presented by Dan Zimmerman, later at this conference)
Future Studies

• Development of Human J-LEAPS Vaccines

• Development of J-LEAPS-DCs for adoptive transfer therapy

• Development of LEAPS immunomodulatory therapy
Thank you for your time and attention.