Novel Immunotherapeutic Approaches for Head and Neck Cancer

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Conflicts of Interest

• Duane Sewell, MD
  – No financial conflict of interest

• Scott Strome, MD
  – Gliknik Corporation
Edward Jenner (1749-1823)
Diseases eliminated/controlled by vaccination

• Diphtheria
• Tetanus
• Yellow Fever
• Pertussis
• Poliomyelitis
• Measles
• Mumps

• Mumps
• Rubella
• Hepatitis B
• Influenza
• Haemophilus
• Smallpox
Prophylactic Cancer Vaccines

• Hepatocellular carcinoma (HCC)
  – Vaccination against Hepatitis B in Taiwan since 1984
  – Decreased seroprevalence HBsAg
  – 68% decline in mortality from hepatitis in infants
  – 75% decrease in the incidence of HCC in children 6-9 years old

Chien YC, et al, 2006
Prophylactic Cancer Vaccines

• Cervical Cancer
  – Koutsky, et al, 2002
    • 2392 women aged 16-23
    • 2 groups: vaccine (VLP L1 capsid) or placebo
    • Placebo group: 3.8% HPV+ (18 months later)
    • Vaccine group: 0.0% HPV+
Prophylactic Cancer Vaccines

• Cervical Cancer
  – Ault, et al, 2007
    • 468 women
    • > 4 year follow-up
    • 96% effective against HPV colonization
    • 100% effective against CIN1
Prophylactic Cancer Vaccines

- **Gardasil**
  - Merck
  - HPV 6, 11, 16, 18 VLP
  - Aluminum adjuvant
  - 0, 2, 6 months

- **Cervarix**
  - GlaxoSmithKline
  - HPV 16, 18 VLP
  - Aluminum, LPS adjuvant
  - 0, 1, 6 months

**NEJM, 2007**
- No therapeutic efficacy
- No difference in rate of progression from HPV infection to CIN (~11%)
T cells

- **CD8 T cells**: peptide + MHC class I
  - Cytotoxic (killer) T cells
    - Kill virus-infected cell
  - Apoptotic cell

- **CD4 T cells**: peptide + MHC class II
  - T_{H1} cells
    - Activate T_{H1}
    - Intra-cellular bacteria
  - T_{H2} cells
    - Activate T_{H2}
    - Antigen-specific B cell
    - Bacterial toxin
    - Anti-toxin antibody
    - B lymphoblast
    - Dead intracellular bacteria
Therapeutic Vaccines

• Melanoma
  – Peptide vaccination (MAGE, MART-1, etc)
  – 381 NCI treated between 1995-2004
  – 11 patients partial or complete response

• Multiple cancer review
  – Various vaccination strategies
  – 35 trials, 765 patients
  – 29 patients partial or complete response

Rosenberg, 2004
New Vaccine Strategies

• Obstacles
  – Lack of CD4 help
  – Immunogenicity of vaccine
  – Immune tolerance

• Possible solutions
  – Listeria vaccine
  – Trojan peptide vaccine
Listeria monocytogenes

- Gram positive intracellular bacteria
- Ubiquitous
- Attenuated strains being tested as vaccine vector for HIV
- Unique life cycle ideal for antigen presentation
Listeria Life Cycle
Lm-ActA-E7 Construction

```
| Xba I | PHYL | ss | ActA | E7 | prfA | Sal I |
```

pDP-2028

CAT

Listeria (prfA-)

Methods

E7-expressing Tumor

• TC-1
  – E7-expressing tumorigenic cell line
  – Established from primary lung epithelial cells of C57BL/6 mice immortalized with HPV 16 E6 and E7 and transformed with an activated ras oncogene

TC-1 cell line courtesy of T.C. Wu, PhD, Johns Hopkins University, Baltimore, MD
Experimental Design

- Establish HPV+ tumors
- Vaccinate Lm-ActA-E7 i.p.
- Second vaccination
- Measure tumors

Day 0: Establish HPV+ tumors
Day 7: Vaccinate Lm-ActA-E7 i.p.
Day 14: Second vaccination
Day 14-28: Measure tumors
Tumor regression data

Sewell, et al, 2004
Animal models for tolerance

• E7 transgenic animal model
  – Embryonic injection of E7
  – Will be a “self” protein
  – Immune system will not react strongly to E7 as a foreign protein
  – Will Listeria or peptide vaccinations work in this model?
Thyroid transgenics
Thyroid Transgenics

Experiment #1

Experiment #2

Tetramer data

WT

activated T Cells
WT tu ActA 50
Event Count: 3127

activated T Cells
WT tu ActA 300
Event Count: 3586

activated T Cells
WT tu ActA 3000
Event Count: 2963

Trans

activated T Cells
Trans tu ActA 50
Event Count: 5921

activated T Cells
Trans tu ActA 300
Event Count: 5221

activated T Cells
Trans tu ActA 3000
Event Count: 4776

1:50

1:300

1:3000

Phase I clinical trial in cervical cancer

- 5 unresectable cervical cancer patients
- All patients experienced mild to moderate fever, chills and nausea
- One patient exhibited a protracted fever unresponsive to NSAIDs for 48 hours and was given a course of antibiotics
- No patient exhibited a positive blood culture for *Listeria* when tested on days 3 and 5.
- Immune response data pending
Trojan Peptide Vaccines

• Peptide-based vaccines have demonstrated few objective clinical responses

• Obstacles to success include:
  – Lack of immunogenicity
  – Lack of Class II epitope utilization

• Our Trojan peptide vaccine strategy is designed to address these obstacles
Background – Tumor Antigens

• MAGE-A3
  – Cancer testis antigen
  – Expressed on surface of tumors
  – Detected in 44% of SCCHN by PCR
  – Class I and II epitopes identified

• HPV-16 E7
  – Viral oncogene
  – Required for maintenance of transformation
  – Expressed in 20-25% SCCHN
  – Class I and II epitopes identified
Background – Trojan Vaccines

**MAGE-A3 Vaccine**

KVAELVHFL / RVKR / FLWGPRALV / RVKR / VIFSKASSSLQL / RKKRRQRRR

Class I Epitope
Furin Linker
Class I Epitope
Furin Linker
Class II Epitope
HIV TAT Domain

**HPV-16 Vaccine**

TLGIVZPI / RVKR / PAGQAEPDRAHYNIVTFZZKZD / RKKRRQRRR

Class I Epitope
Furin Linker
Class II Epitope
HIV TAT Domain

Antigen Processing and Presentation...

MAGE Trojan-vaccine

HLA Class I and II

HLA Class II

HLA Class I

S Strome

=CTL

=HTL
Phase I Clinical Trial

• Part A
  – Unresectable, end stage SCCHN
  – HLA – A2 positive
  – MAGE A3 or HPV 16 positive
  – 4 vaccinations (300 micrograms) at monthly intervals with adjuvants Montanide and GMCSF

• Endpoints
  – Safety
  – *In vitro* evidence of immune response
Phase I trial – Part A

- 32 patients screened
- 5 patients vaccinated
- 3 (of 4) patients generated measurable immunologic responses to the vaccine
- No patient experienced tumor regression
- One patient experienced a serious adverse event (SAE) most likely related to the vaccine
Phase I Trial – Part A

- Neurological symptoms resolved with steroids
Phase I Trial – Part A

Pre Treatment

CD4, 20x

TUNEL, 20x

Post Treatment

Metastatic lymph node
Trojan peptide-based vaccines stimulate Trojan-specific T cells

A. Patient 1

B. Patient 3

C. Patient 5
Trojan vaccine-specific T cells are found at the vaccine administration site

A. 

H & E  CD3  CD4  CD8  CD68

B. 

CD3  CD4  CD8

C. 

Number of spots

Antigen (10µg/ml)
## Phase I Trial – Part A

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>No. of immunizations</th>
<th>Trojan vaccine</th>
<th>Trojan CD8 response</th>
<th>Trojan CD4 response</th>
<th>Serious Adverse Event</th>
<th>Reason early removal from trial</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Mage-A3</td>
<td>-</td>
<td>+</td>
<td>Cerebral edema, hemiplegia</td>
<td>SAE</td>
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<tr>
<td>2</td>
<td>2</td>
<td>Mage-A3</td>
<td>-</td>
<td>_</td>
<td>Nausea</td>
<td>Disease progression</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Mage-A3</td>
<td>-</td>
<td>+</td>
<td>None</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>HPV-16</td>
<td>?</td>
<td>?</td>
<td>None</td>
<td>Pelvic fracture</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>HPV-16</td>
<td>-</td>
<td>+</td>
<td>None</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Phase I Trial – Part B

• Part B
  – Unresectable, end stage SCCHN
  – No exclusion based on HLA type
  – MAGE A3 or HPV 16 positive
  – 4 vaccinations at biweekly intervals with adjuvants Montanide and GMCSF
  – Dose escalation from 500 to 1500 micrograms

• Endpoints
  – Safety
  – In vitro evidence of immune response
Phase I trial – Part B

• Ongoing
• 6 patients screened
• 3 positive for HPV, 2 positive for MAGE
• Safety
  – 1 patient removed after one vaccination (hospice)
  – 1 patient developed a pharyngocutaneous fistula
• Immune response
  – Data is pending
Based on stringent inclusion criteria 5 patients received one or more Trojan vaccinations

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>AJCC stage</th>
<th>HLA A2, HLA A2 (tumor)</th>
<th>Trojan vaccine</th>
<th>No. of immunizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>F</td>
<td>IV</td>
<td>A2, A24, B77, B44, DR4, DR12</td>
<td>A0201 60%-80% Mage-A3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>M</td>
<td>IV</td>
<td>A2, A66, B7, B71, DR15, DR9</td>
<td>A0205 60%-80% Mage-A3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>M</td>
<td>IV</td>
<td>A2, A-, B7, B13, DR15, DR-</td>
<td>A0201 40%-60% Mage-A3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>M</td>
<td>IV</td>
<td>A3, A33, B-, B35, DR1, DR17</td>
<td>n/a n/a</td>
<td>HPV-16</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>IV</td>
<td>A2, A3, B7, B35, DR12, DR15</td>
<td>A0206 80%-100% HPV-16</td>
<td>4</td>
</tr>
</tbody>
</table>
Clinical observation: CD8$^+$ and CD4$^+$ T cells infiltrate the tumor environment.
Specific Aim 1 – Feasibility and Safety

• Eligibility Criteria
  – Stage III or IV SCCHN
  – MAGE A3 or HPV 16 positive
  – No evidence of disease after treatment via PET scan and/or biopsy at primary site

• Vaccination schedule
  – 300 μg appropriate Trojan antigen with adjuvants at bi weekly intervals
  – Adjuvant alone at bi weekly intervals
Future Directions - Phase II

Informed Consent

Tumor biopsy and MAGE/HPV testing

MAGE or HPV positive
ELIGIBLE

MAGE and HPV negative
INELIGIBLE

Standard SCCHN treatment

PET/CT or biopsy negative
ELIGIBLE

PET/CT and biopsy positive
INELIGIBLE

Treatment with appropriate Trojan vaccine with adjuvants

Treatment with adjuvants only

Immunological assays
Determination and comparison of disease-free and overall survival
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