LM-LLO-E7, a Novel Vaccine for the Treatment of Cervical Cancer

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Our Team

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Today’s Presentation

• Description of our technology

• Data in animal models

• Summary of our proposed clinical trial
1. Clinical cancer is a failure of the immune system to recognize tumor-associated antigens and eliminate neoplastic cells.

2. Cell-mediated immunity is the key to the elimination of solid tumors.

3. Listeria monocytogenes is an excellent vector to deliver a specific tumor antigen in a way that will stimulate a cell-mediated response.
Why Listeria monocytogenes?
Innate Immunity

Immune System

- Bacteria
- Virus

Adaptive Immunity

- Humoral (B Cells)
- Cell mediated (T Cells)
Bacteria induce an inflammatory cytokine cascade early after infection

MΦ → IL-12 → NK

MΦ → TNF-α → IL-12

MΦ → IL-1 → Neu.

IL-12 → Th1

Th1 → IL-2 → IFN-γ

Th0 → IL-12 → IL-12

Activated MΦ → IL-12
Life Cycle of Listeria monocytogenes

Antigen Processing Cell

Listeria, Salmonella, BCG etc

Listeria

CD4 T-cell

CD8 T-cell
LM-LLO-E7 Expression System

$p$  $hly$  $E7$  $p$  $prfA$

$prfA^-$
Listeria monocytogenes as a Delivery Vector

- Turns on innate immunity
- Generates powerful cell-mediated immunity
- Readily engineered to produce antigens
- No gram-negative endotoxins
- Sensitive to antibiotics
- Stable vaccine that may be stored frozen
- Can be grown on completely synthetic media
A Platform Technology

- LM-LLO-E7 is our first application
- This technology can be adapted to other antigens
- Potential value in other cancers and infectious disease
Mouse Data
Tumor Regression Study

5 × 10^5 Tumor cells

1 week

0.1 LD_{50} of vector (twice; 1 week apart)

Measure Tumors every 3 days

Sacrifice ~ day 90 or when tumors reach 2.0 cm
TC-1 tumor

- C57Bl/6 derived epithelial tumor
- Immortalized with HPV-16 E6 and E7
- Transformed with oncogenic ras
- Expresses E6 and E7 constitutively at low levels similar to human cervical tumors
- Grows rapidly in syngeneic mice compared other HPV-immortalized tumors
- Used extensively to test therapeutics for HPV transformed tumors.
LM-LLO-E7 induces regression of established TC-1 tumors

![Graph showing tumor size over time for different groups: Naive, Lm-LLO-E7, Lm-E7, Lm-Gag, Lm-LLO-NP. The graph displays data points at various days post tumor inoculation, with a noticeable trend towards regression in the Lm-LLO-E7 group.](image-url)
Clinical Protocol

• We have a vector that generates significant anti-tumor activity in animal models of cervical cancer.

• Next step is human clinical testing.
Human Papillomavirus and Cancer

- > 95% of cervical cancers are associated with HPV
- 2nd leading cause of cancer death for women world-wide
- About 5,000 deaths p.a. of cervical cancer in the USA
- HPV strains 16 and 18 most commonly linked to cancer
- HPV E6 and E7 gene products immortalize cells
- E6 and E7 are constitutively expressed in HPV-associated tumors
- 25% head & neck cancers express HPV antigens; may also be expressed in lung, prostate and other tumors.
Protocol-Outline

- Give an attenuated vector to patients with advanced cervical cancer under inpatient hospital evaluation.

- Monitor for adverse events, treat promptly as indicated.

- Incrementally increase dose as tolerated.

- Purpose is to determine the safety and immunogenicity of the vector in a patient population.
Patient Population

- Adult women with stage III or stage IV disease who have failed standard therapy
- Must be HPV-16 positive
- Must be 4 weeks or more out from last chemotherapy or radiation
- Exclusions: steroid use, active brain mets, valvular disease, bone marrow or renal insufficiency, anergy, pregnancy, severe debilitation, HIV
Clinical Protocol Summary

- 20 patients in 4 dose cohorts
- Dose escalation based on tolerance in lower cohort
- Day 1: admit to hospital, vaccination
- Day 5: 1 dose IV ampicillin and discharge on 10 days of oral ampicillin
- Return on Day 21 for second dose, repeat above
Clinical Protocol Endpoints

- Safety
- Immunogenicity
- Clinical response
Is it safe to give Listeria to Cancer Patients?

- Exclusion of many high risk people
- Initial dose is equal to the lowest dose used in the rhesus study
- Attenuation: engineered bacteria has $LD_{50}$ in mice about $10^3$ times wild-type
- Patients will be monitored in hospital
- The bacteria is sensitive to ampicillin
Last Slide
1. Plasmid Transfer
Methods

- Mix LM-LLO-E7 with E. coli in vitro
- Grow in a high concentration chloramphenicol media
- Every two days, sample the mixture and culture on media specific for E. coli with chloramphenicol resistance
# Results

<table>
<thead>
<tr>
<th>Day</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no growth</td>
</tr>
<tr>
<td>3</td>
<td>no growth</td>
</tr>
<tr>
<td>5</td>
<td>no growth</td>
</tr>
<tr>
<td>7</td>
<td>no growth</td>
</tr>
<tr>
<td>9</td>
<td>no growth</td>
</tr>
<tr>
<td>11</td>
<td>E. coli</td>
</tr>
</tbody>
</table>
Conclusions

- Unrealistically high concentrations of bacteria (iv route)
- Full chloramphenicol pressure
- Still took over 9 days to push plasmid into E. coli (antibiotics at day 5)
2. Route of Administration
IP compared to SC administration

Tumor Size (mm) vs. Days Post Tumor Inoculation

- naive
- Lm-LLOE7 2x ip
- Lm-LLOE7 1x ip
- Lm-LLOE7 2x s.c.
- Lm-LLOE7 1x s.c.
IP compared to oral administration

![Graph showing tumor size over time for Naive, Lm-LLOE7 IP 1x10, and Lm-LLOE7 Oral 1x10 groups.](image)

Days Post Tumor Inoculation

Tumor Size (mm)
Comparison of I.V. versus I.P. immunization of Listeria based E7 vaccines
3. Dose Schedule
One vs Two doses of LM-LLO-E7

Tumor Size (mm)

Days Post Tumor Inoculation

- naive
- Lm-LLOE7 2x ip
- Lm-LLOE7 1x ip
- Lm-LLOE7 2x s.c.
- Lm-LLOE7 1x s.c.
4. Planned Studies
Biodistribution in Mice

- Dose route: iv and sc
- Check a cohort at days 2, 10, 20, 90
- Check blood, liver, spleen, brain, nodes, kidney, intestine, ovaries, injection site, bone marrow
- Use PCR to look for plasmid DNA
Acute Dose Toxicity

- Determine MTD in wild-type Listeria and LM-LLO-E7
- Compare iv and sc
Longer Term Toxicity in Mice

- 3 dose levels each for iv and sc
- Dose on days 1, 8, 15, and 22
- Monitor response and sacrifice on day 23 with full necropsy
Monkey dosing study

- Cynomolgus monkeys will receive LM-LLO-E7 intravenously
- Dose up to $10^{12}$ cfu iv
- Follow clinically and with blood tests
5. Dose Levels
Dose

- Starting dose $10^9$ cfu i.v. with LM-LO-E7
- $10^{10}$ LM-SIV i.v. tolerated in rhesus macaques with vector 2 logs more virulent
- Human study: $10^9$ tolerated p.o. with vector 1.5 logs more virulent
- Further studies planned
6. Misc
<table>
<thead>
<tr>
<th>Karnofsky Scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Normal, no complaints, no evidence of disease</td>
</tr>
<tr>
<td>90</td>
<td>Able to carry on normal activity: minor symptoms of disease</td>
</tr>
<tr>
<td>80</td>
<td>Normal activity with effort: some symptoms of disease</td>
</tr>
<tr>
<td>70</td>
<td>Cares for self: unable to carry on normal activity or active work</td>
</tr>
<tr>
<td>60</td>
<td>Requires occasional assistance but is able to care for needs</td>
</tr>
<tr>
<td>50</td>
<td>Requires considerable assistance and frequent medical care</td>
</tr>
<tr>
<td>40</td>
<td>Disabled: requires special care and assistance</td>
</tr>
<tr>
<td>30</td>
<td>Severely disabled: hospitalization is indicated, death not imminent</td>
</tr>
<tr>
<td>20</td>
<td>Very sick, hospitalization necessary: active treatment necessary</td>
</tr>
<tr>
<td>10</td>
<td>Moribund, fatal processes progressing rapidly</td>
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</tbody>
</table>