Phase 1 Clinical Trial of DNA Vaccines for Hemorrhagic Fever With Renal Syndrome Delivered by Intramuscular Electroporation

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4th International Conference on Vaccines & Vaccination
Valencia, Spain
September, 2014
Hantaviruses and Their Hosts

Hantaviruses have been detected in >50 species of rodents, shrews, moles and bats

Hantaviruses

Pathogenic Hantaviruses
- Persistently infected rodents
- Transmitted in aerosols of rodents’ urine, feces, saliva

NIAID Category A Priority Pathogens

Hantaan virus
Dobrava virus
Seoul virus
Puumala virus

Old World Rodents

HFRS
Mortality ~1%-15%

Sin Nombre virus
Andes virus
And more…..

New World Rodents

HPS
Mortality ~40%
Phylogeny and Rodent Hosts

Coevolution Postulated for >100 MY*

DNA Vaccines
Desirable Characteristics

- **Easily Manufactured**
  - Can be quickly designed and produced in response to emerging or genetically engineered threats
  - DNA has established and approved manufacturing procedures

- **Safe**
  - Plasmids are replication defective
  - Not transmissible person to person or into the environment

- **No Pre-existing Vector Immunity**

- **Flexible Platform**
  - Easily combined to form multivalent vaccines
  - Can be delivered by a variety methods
    - Gene gun
    - Electroporation
Hantaviruses

enveloped, ~100 nm
ssRNA, (-),
3 segments

Bunyaviridae

Immunity: Neutralizing antibodies
Hantaan Virus M Segment DNA Vaccine

Immune precipitation of HTNV or DNA vaccine expression products with polyclonal mouse sera (HMAF) or monoclonal antibodies (G_N, G_C)
Hamster Protection Studies
HTNV DNA Vaccine

- Elicits neutralizing antibodies in hamsters
- Protects hamsters from infection with HTNV
- Protects most hamsters from SEOV or DOBV infection
- Does not protect hamsters from PUUV infection

Hantaviruses

HFRS

<table>
<thead>
<tr>
<th>Hantaan</th>
<th>Seoul</th>
<th>Dobrava</th>
</tr>
</thead>
</table>

PUUV

<table>
<thead>
<tr>
<th>Puumala (Finland)</th>
<th>Puumala (Russia)</th>
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</table>

HPS

<table>
<thead>
<tr>
<th>New York</th>
<th>Sin Nombre</th>
<th>Bayou</th>
<th>Black Creek Canal</th>
<th>Andes</th>
<th>Laguna Negra</th>
</tr>
</thead>
</table>

% $G_N + G_C$ amino acid identities

- Some neutralization: 77%
- Low or no neutralization: 53%

HTNV

- pWRG7077

PUUV

- pWRG7077
Mixed HTNV and PUUV DNA vaccines elicit neutralizing antibodies in hamsters only to PUUV.

Could not overcome this with higher ratio of HTNV:PUUV DNA

For Phase 1 (Gene Gun) study the HTNV and PUUV DNAs were administered separately.
Phase 1 Study

Gene Gun Delivery of HFRS DNA Vaccines

- Phase 1 Study: 3 vaccine groups of 9 subjects
  - HTNV DNA
  - PUUV DNA
  - Both DNAs delivered as separate administrations

- The vaccines were well tolerated and immunogenic

- Some volunteers produced high-titer neutralizing antibody responses (PRNT$_{50}$ >1000)

- Overall sero-conversion rate for study was <50%

- Improved delivery needed

Electroporation-based DNA Vaccination

DNA Administration

Electrical Pulse creates temporary membrane pores

Antigen Expression in Transfected Tissue
GLP Preclinical Safety Studies
Neutralizing Antibody Responses

N=20
2 mg DNA
IM-EP days 1, 15, 29, 57

- No vaccine-related mortalities or systemic clinical abnormalities
- No notable changes in mean body weights or food consumption
- No vaccine-related effects in mean body temperatures
- No observed changes during ophthalmic examinations

Phase 1 Clinical Study
IM-EP Delivered HTNV + PUUV DNA Vaccines

- Three study groups: HTNV, PUUV, HTNV+PUUV DNA Vaccines
  - Determine if electroporation delivery improves seroconversion rate
  - Assess potential interference between hantavirus DNA vaccines in humans

Phase 1 Study IM-Electroporation
Neutralizing Antibody Responses

HTNV Vaccine: 2 mg DNA/1ml PBS, 3X at 4 wk intervals
Seroconversions: 7/11 = 64%

![Graph showing HTNV PRNT50 over time with vaccinations at days 28, 56, and 84. The graph indicates the responses of different groups: 2 doses (4, 7, 9, 11, 19, 29, 30) and 3 doses.](image-url)
Phase 1 Study IM-Electroporation
Neutralizing Antibody Responses

PUUV Vaccine: 2 mg DNA/1ml PBS, 3X at 4 wk intervals
Seroconversions: 6/8 = 75%

Vaccinations
Phase 1 Study IM-Electroporation
Neutralizing Antibody Responses

HTNV+PUUV Vaccines: 1 mg each DNA/1ml PBS, 3X at 4 wk intervals

PUUV Seroconversions: 7/9 = 78%
HTNV Seroconversions: 3/9 = 33%

Vaccinations
Summary:

HFRS DNA Vaccines, IM-EP Delivery

- HTNV and PUUV DNA vaccines delivered by intramuscular electroporation were safe and immunogenic in a Phase 1 clinical study.

- Interference of mixed vaccines continued to be problematic.

- Mixed, gene-optimized HTNV and PUUV DNA vaccines developed and shown to be immunogenic in hamsters when given alone or as a mixture by IM-EP.
In progress: Phase 2a Dose ranging
Hantavirus DNA Vaccines Delivered by IM-EP

- Using modified HTNV DNA-no interference in animal studies
- Two schedules and two doses assessed

<table>
<thead>
<tr>
<th>Group #</th>
<th># of Subjects</th>
<th>Vaccine</th>
<th>Dose (mg)</th>
<th>Volume (ml)</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>HTNV/PUUV</td>
<td>2.0</td>
<td>1.0</td>
<td>Days 0, 28, 56 (180)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>HTNV/PUUV</td>
<td>2.0</td>
<td>1.0</td>
<td>Days 0, 56 (180)</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>HTNV/PUUV</td>
<td>1.0</td>
<td>1.0</td>
<td>Days 0, 28, 56 (180)</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>HTNV/PUUV</td>
<td>1.0</td>
<td>1.0</td>
<td>Days 0, 56 (180)</td>
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<tr>
<td>total</td>
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Funded by the Military Infectious Diseases Research Program
### Next: Phase 1 Clinical Study

Comparison of IM and ID EP with Mixed Optimized DNA Vaccines for HTNV and PUUV

All Groups to be vaccinated on days 0, 28, 56

<table>
<thead>
<tr>
<th>Group #total</th>
<th># of Subjects</th>
<th>Vaccine Candidate</th>
<th>Dose/route</th>
<th>Volume</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>HTNV</td>
<td>0.6 mg / ID EP</td>
<td>200 µl</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>HTNV</td>
<td>2.0 mg / IM EP</td>
<td>1000 µl</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>PUUV</td>
<td>0.6 mg / ID EP</td>
<td>200 µl</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>HTNV+PUUV</td>
<td>4.0 mg / IM EP</td>
<td>1000 µl</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>HTNV+PUUV</td>
<td>1.2 mg / ID EP</td>
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<tr>
<td>6a</td>
<td>5</td>
<td>none</td>
<td>- / IM EP</td>
<td>1000 µl</td>
</tr>
<tr>
<td>6b</td>
<td>5</td>
<td>none</td>
<td>- / ID EP</td>
<td>200 µl</td>
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<tr>
<td>total</td>
<td>60</td>
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NIAID Contract: HHSN272201200019C
Acknowledgements

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense. The research described herein was sponsored by the Military Infectious Disease Research Program.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

All clinical study procedures took place at the Walter Reed Clinical Trials Center. Recruitment was conducted according to current Good Clinical Practice (GCP) guidelines. The Clinical Protocol and Informed Consent forms were approved by the Walter Reed Army Institute of Research (WRAIR) Scientific Review Committee Sponsor’s Representative Team (Division of Regulated Activities and Compliance, USAMMDA), the WRAIR Institutional Review Board (IRB), Department of the Army’s Office of Research Protections, Human Research Protection Office (ORP, HRPO), Sponsor’s Representative (acting for the OTSG of the Army), USAMRMC Commanding General, Commander, WRAIR. The study was sponsored by the Office of the Surgeon General, Department of the Army under IND 13688 using an open-label, single-center design.