mRNA vaccine; CCHFV

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Crimean – Congo Haemorrhagic fever- Is in the blue Print list of WHO (highlight the emergency of vaccine and Antiviral for this disease)

CCHFV is one of the formidable viral haemorrhagic fevers.

- Cases reported from 40 countries.
- Ticks has been found even in North Europe such as Sweden since 2 years ago

Distribution of CCHF correlates with principal vector of virus, ticks belonging to genus Hyalomma
Hyalomma marginatum, are “two-host” ticks. Hyalomma are “hunting” ticks, which can quest up to 400 m to find their hosts (including humans).
DNA plasmids coding for different CCHFV proteins.
- Nucleoprotein
- Precursor glycoprotein M (include non-structural proteins)
- Glycoprotein Gc
- Glycoprotein Gn
WP 3 – A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a Cynomolgus macaque model.

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid(s)</td>
<td>M + N</td>
<td>Control</td>
</tr>
</tbody>
</table>

- Non Human Primates (NHP)
- 6 per group, 2 groups
- 3 immunizations
- Total of 2 mg DNA/animal at each immunization (1 mg M and 1 mg N)
- End point – 6 days post challenge

Partner 1, FoHM; partner 7, KI, NIH
WP 3 – A DNA-based vaccine protects against Crimean-Congo haemorrhagic fever virus disease in a Cynomolgus macaque model.

- **Immunological results**
  - Vaccination induced CCHFV specific antibodies after 2 immunizations
  - CCHFV-specific T-cell responses against N and G

- **Clinical results**
  - Vaccination prevented changes in blood chemistry often associated with poor outcome of CCHF in humans
  - Vaccination significantly reduced CCHFV viral shedding and viral burden in several tissues tested.

Partner 1, FoHM; partner 7, KI, NIH
WP 3 – DNA based vaccine protects NHP against CCHF.

### Group 1
- **Plasmid(s):** M + N
- **1st immunization and blood sample**
- **2nd immunization and blood sample**
- **3rd immunization and blood sample**
- **4th blood sample**

### Group 2
- **Plasmid(s):** N
- **1st immunization and blood sample**
- **2nd immunization and blood sample**
- **3rd immunization and blood sample**
- **4th blood sample**

### Group 3
- **Plasmid(s):** M
- **1st immunization and blood sample**
- **2nd immunization and blood sample**
- **3rd immunization and blood sample**
- **4th blood sample**

### Group 4
- **Plasmid(s):** Control
- **1st immunization and blood sample**
- **2nd immunization and blood sample**
- **3rd immunization and blood sample**
- **4th blood sample**

- **Non Human Primates (NHP)**
- **6 per group**
- **2 OR 3 immunizations**
- **Total of 2 mg DNA/animal at each immunization**
- **End point – 6 days post challenge**

Partner 1, FoHM; partner 7, KI, NIH
WP 3 – DNA based vaccine protects NHP against CCHF

Before challenge

Whole Virion ELISA: IgG

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day</th>
<th>A 405nm</th>
<th>Dilution (Log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>-49</td>
<td>0.0</td>
<td>-6</td>
</tr>
<tr>
<td>NP</td>
<td>-28</td>
<td>0.0</td>
<td>-5</td>
</tr>
<tr>
<td>GPC</td>
<td>-7</td>
<td>0.0</td>
<td>-4</td>
</tr>
<tr>
<td>NP + GPC</td>
<td>2</td>
<td>0.0</td>
<td>-3</td>
</tr>
</tbody>
</table>

Day 0

Day 6

Day 3 Blood

Day 5 Blood

Day 6 Blood

Copy #/mL (Log10)

Partner 1, FoHM; partner 7, KI, NIH
WP 3 – DNA based vaccine protects NHP against CCHF

Day 6 Tissues  Difference compared to sham

Partner 1, FoHM; partner 7, KI, NIH
Hepatic functions (ALP, ALT, AST, and Tbilirubin) in vaccinated group were normal during the study. The control animals elevated ALT and AST after inoculation.

- Major conclusions is that double antigen conferred the most protection while single antigen was not as effective.
Nucleoside-modified mRNA vaccines protect IFNAR<sup>-/-</sup> mice against Crimean Congo hemorrhagic fever virus infection

- IFNAR<sup>-/-</sup> mice lacking type I interferon receptor and immunocompetent mice
- 4 different immunization groups: 1: GcGn; 2: N; 3: GcGn+N and 4: Control
- Intradermal injections of mRNA-LNP
- Immunocompetent mice euthanized 5 weeks after last immunization
- IFNAR<sup>-/-</sup> mice was challenged with 400 pfu CCHFV IbAr10200 (i.p)
Survival and viremia

- Immunization, independent of vaccine candidate, induced 100% protection against CCHFV infection

- Significant more viral RNA in serum, spleen and liver from control mice compared to immunized
mRNA-LNP induced antibody titers and NT antibodies

IFNAR−/− mice

- Ab aganist N
- Ab aganist Gc
- Ab aganist Gn
- Neutralizing Ab

Dilutions: 1:64, 1:256, 1:1024, 1:4096

% inhibition compared to control

OD 450 nm

Immunocompetent mice

- Ab aganist N
- Ab aganist Gc
- Ab aganist Gn

Dilutions: 1:10000, 1:20000, 1:40000, 1:80000, 1:160000, 1:320000

% inhibition compared to control

OD 450 nm

T1: □ N  △ GcGn+N  ○ GcGn  ▼ Control
T2: ■ N  ▲ GcGn+N  ● GcGn  ▼ Control
mRNA-LNP activated T cells in WT mice

mRNA-LNP immunization induces CCHFV-specific cellular response

- Spleens from immunized immunocompetent mice was homogenized
- Cells counted and mixed with pools of peptides based on the CCHFV N, Gc or Gc proteins
mRNA-LNP vaccine induced proteomic changes

- To identify and better understand more large-scale potential differences between the two candidates and, in addition, compare to unvaccinated mice after CCHFV infection
- Liver samples from GcGn, N and control immunized mice after CCHFV infection
- No difference in protein expression between GcGn and N vaccinated mice.
- Clear separation between control and GcGn or N vaccinated mice.
- Most of the effected proteins were associated to “metabolic pathways.” For example:
  - oxidative phosphorylation,
  - propanoate metabolism
  - valine leucine and isoleucine degradation
  - carbon metabolism
- The results indicate a metabolic recovery in the liver of vaccinated mice.
Summary and conclusion

- Two immunizations with mRNA vaccine encoding for only CCHFV GcGn or N, or the combination of the two, induces a 100% protection of IFNAR\(^{-/-}\) mice against CCHF.

- Both vaccine candidates induce:
  - high antibody levels (anti-N and anti-Gc).
  - cellular immunity. Result indicate that a large part of N can act as an antigen, while only a specific part of the glycoprotein induces a cellular response.

- No difference in the protein profile between the two vaccine candidates, but a distinct shift in metabolism compared to unvaccinated mice.

- Survival of mice immunized with only N mRNA-LNP strongly indicate that neutralizing antibodies is not necessary.
Next Step

- Doseing (Ongoing)
- Durability (ongoing)
- NHP Data
- Clinical Phase I
Thank you

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