INACTIVATED, CELL-BASED YELLOW FEVER VACCINE-SAFETY, IMMUNOGENICITY AND EFFICACY in ANIMAL MODELS
Yellow fever

- Mosquito-borne flavivirus
- Severe viral hemorrhagic fever, 50% case fatality rate
- Significant public health problem in South America and Africa
- Travelers are at risk
- Vaccination certificate required under IHR for travel to many countries
Travelers to risk areas require YF vaccination
New maps of areas with risk of yellow fever, WHO, 2010

- Endemic/high risk, vaccination recommended
- Transitional/moderate-high risk, vaccination recommended
- Low risk, vaccination not recommended (except prolonged rural exposure)
Live, attenuated 17D Vaccine

• Precautions
  – Age >60 years

• Contraindications
  – Age <6 months
  – Immune deficiency, immune suppression
  – Thymic disease/thymectomy
  – Egg allergy
  – Pregnancy

• Possible future contraindications
  – Autoimmune disorders
  – Breast-feeding
Live, attenuated YF 17D – Serious Adverse Events

• Yellow fever vaccine associated neurotropic adverse events (YEL-AND)
  – Previously infants, now seen in adults
  – Reporting rate as high as 0.8 per 100,000
  – CFR 1-2%

• Yellow fever vaccine associated viscerotrophic adverse events (YEL-AVD)
  – Similar to wild-type YF disease
  – Reporting rate 0.4 per 100,000 (Peru 2007, 7.9/100,000)
  – CFR 64%

• Anaphylaxis (egg, gelatin allergy)
  – Incidence 1.8 per 100,000
Indication for a Safer Vaccine

• Prevention of yellow fever in travelers, military, (endemic zone populations)

• Safer vaccine than current live 17D
  – No serious AEs related to replicating virus (neurotropic, viscerotropic adverse events)
  – No egg or other animal derived allergens
  – No age restriction (indicated for infants, elderly)
  – Not contraindicated for immune suppressed, thymic disease, pregnant, lactating patients
Formulation

• Whole virion vaccine, BPL-inactivated
• Liquid, adsorbed (0.2% aluminum hydroxide)
• Proprietary excipient formulation
  – GRAS substances
• Single dose vial (0.65mL)
• Shipment/storage 2-8° C
Original Selection of the Clone

• Starting material: YF-VAX®, Lot # NDC49281-915-05, Sanofi Pasteur, Swiftwater PA.

• The virus was adapted for increased replication in Vero cells by 10 serial virus passages at terminal dilution.

• At Virus Passage 10, a single plaque was picked and passed in fluid culture to produce a mini-seed stock at Virus Passage 11.

• This virus showed a 3-7 fold increased replication capacity in Vero cells compared to the YF 17D at Virus Passage 1.

• The Virus Passage 11 virus stock was used for RNA extraction and the RNA used to produce cGMP grade virus seeds

• Full genome sequencing showed that P11 contained a single mutation in the E protein associated with increased replication.
Increased Yield, Vero cell Adapted Seed P1 (unadapted) vs. P11 (adapted)
Yields, Virus Harvest (Lot 010)

Proprietary process

Drain, refeed

Yield limit without refeed
Virus Harvest

Clarification
90 um sieve tube
Depth filtration

Benzonase Digestion

Concentration and Buffer Exchange
Ultrafiltration and Diafiltration, sterile filtration

Live Virus Bulk

Virus Inactivation
0.1% BPL, 16h, RT

Purification
Cellufine Sulfate Chromatography
Dilution to target potency

Bulk Drug Substance

Formulation with Alum and proprietary stabilizers

Bulk Drug Product
Potency: Relationship between YF Titer by ELISA and Protein Concentration

XRX-001 similar to other alum adsorbed flavivirus vaccines (JE, TBE)

Lot 006
8.3 log_{10} VE/0.5mL
4.4 \mu g/0.5mL

R^2 = 0.998

IXIARO® JE Dose

XRX-001

FSME-Immun® TBE Dose

YF antigen concentration
VE/0.5mL
Immunization and Challenge Hamster Model

Group DAY (N=10)

0

1
XRX-001 9E+07 VE

2
XRX-001 8E+06 VE

3
XRX-001 6E+05 VE

4
XRX-001 9E+07 VE

5,6
YF-VAX Full or 1/10

7
Saline

21

XRX-001 9E+07 VE

XRX-001 8E+06 VE

XRX-001 6E+05 VE

XRX-001 9E+07 VE

6 log_{10} IP

49

Viremia

+6

Survival

70

ALT

weight

Saline

Saline

Xcellerex
Hamster Results

Single dose of XRX-001 (high dose group) elicited antibody response similar to YF-VAX® after single dose, higher after two doses, and was fully protective against challenge.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Schedule days</th>
<th>Day 49 PRNT$_{50}$</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seroconversion</td>
<td>GMT</td>
</tr>
<tr>
<td>XRX-001 High dose</td>
<td>0,21</td>
<td>100%</td>
<td>20,480</td>
</tr>
<tr>
<td>XRX-001 Mid dose</td>
<td>0,21</td>
<td>80%</td>
<td>453</td>
</tr>
<tr>
<td>XRX-001 Low dose</td>
<td>0,21</td>
<td>30%</td>
<td>37</td>
</tr>
<tr>
<td>XRX-001 High dose</td>
<td>21</td>
<td>100%</td>
<td>2,195</td>
</tr>
<tr>
<td>YF-VAX</td>
<td>21</td>
<td>100%</td>
<td>1,940</td>
</tr>
<tr>
<td>YF-VAX 1/10 dose</td>
<td>21</td>
<td>100%</td>
<td>905</td>
</tr>
<tr>
<td>Saline+alum</td>
<td>0,21</td>
<td>0%</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>
Survival – Hamsters

![Graph showing survival rates of hamsters over days post-virus injection. Different lines represent different treatments and doses.]

- XRX001-High
- XRX001-Mid
- XRX001-low
- XRX001-High, Single dose
- YFVax, 1:10
- YFVax, undilute
- Saline + Alum
Viremia Post-challenge – Hamsters
ALT post-challenge – Hamsters
Nonhuman Primates (cynomolgus macaques) Clinical lot (-006) tested

<table>
<thead>
<tr>
<th>Group #</th>
<th># Monkeys</th>
<th>Vaccine</th>
<th>Dose</th>
<th>Route and volume</th>
<th>Vaccinations</th>
<th>Blood collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>XRX001 Lot M-900-03-09-006H Inactivated YF vaccine, alum adsorbed</td>
<td>$2.3 \times 10^8$ VE/0.5mL</td>
<td>IM, 0.5mL</td>
<td>Days 0, 21</td>
<td>Day 0, 21, 42</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>XRX001 Lot M-900-03-09-006H Inactivated YF vaccine, alum adsorbed</td>
<td>$2.3 \times 10^8$ VE/0.5mL</td>
<td>IM, 0.5mL</td>
<td>Days 0, 10, 21</td>
<td>Day 0, 21, 42</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>YF-VAX®</td>
<td>$\sim 5 \times 10^4$ PFU/0.5mL</td>
<td>SC, 0.5mL</td>
<td>Day 0</td>
<td>Day 0, 21, 42</td>
</tr>
</tbody>
</table>
Nonhuman Primates (cynomolgus macaques)

Single dose of XRX-001 elicited a rapid antibody response similar to a single dose of YF-VAX®

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animal #</th>
<th>PRNT\textsubscript{50} Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>1</td>
<td>XRX-001 2 doses</td>
<td>60964</td>
<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td>Day 0, 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29162</td>
<td>&lt;40</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td>29448</td>
<td>&lt;40</td>
<td>5120</td>
</tr>
<tr>
<td>2</td>
<td>XRX-001 3-doses</td>
<td>61325</td>
<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td>Day 0, 10, 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10443</td>
<td>&lt;40</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>26367</td>
<td>&lt;40</td>
<td>640</td>
</tr>
<tr>
<td>3</td>
<td>YF-VAX® Day 0</td>
<td>26372</td>
<td>&lt;40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26390</td>
<td>&lt;40</td>
<td>640</td>
</tr>
</tbody>
</table>
## Passive Antibody Protection From Virus Challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Pool Dilution</th>
<th>GMT nAb titer 4hrs before challenge</th>
<th>GMT nAb titer 4 days after challenge</th>
<th>GMT nAb 21 titer days after challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1:10 XRX-001</td>
<td>75</td>
<td>32</td>
<td>520/33%</td>
</tr>
<tr>
<td>2.</td>
<td>1:100 XRX-001</td>
<td>20</td>
<td>20</td>
<td>640/44%</td>
</tr>
<tr>
<td>3.</td>
<td>1:1,000 XRX-001</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>403/75%</td>
</tr>
<tr>
<td>4.</td>
<td>1:10,000 XRX-001</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>1,110/83%</td>
</tr>
<tr>
<td>5.</td>
<td>Saline + Alum</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>1,140/86%</td>
</tr>
<tr>
<td>6.</td>
<td>1:10 XRX-001</td>
<td>NT</td>
<td>20</td>
<td>&lt;1:160</td>
</tr>
</tbody>
</table>

(no virus challenge)

Confidential
Results, passive immunization and challenge, hamsters: ALT and passive titer pre-challenge

Indiv. sera tested
GMT 74.6
Range 40-160
Conclusions

- XRX-001 YF vaccine is highly immunogenic, eliciting neutralizing antibodies (nAb) in 100% of animals immunized.
- Two inoculations of ~4.4 mcg of vaccine are required for effective immunization.
- Hamsters immunized with XRX-001 developed nAb that protected them from disease after virulent YF virus challenge.
- Passive transfer of XRX-001 hyperimmune and YF-VAX® serum to hamsters protected them against YF disease following virulent YF virus challenge.
- Minimum serum nAb titers of ≥ 40 before challenge protected hamsters from YF disease when infected with virulent YF virus.
- Some animals treated with low doses of nAb were protected from YF disease w/o detectable YF virus nAb in the serum.
- Antibodies to XRX-001 and YF-VAX® were equally effective in protecting against YF virus disease in the hamster model.