Viral Diseases and New Vaccines Development in China

Li Dexin
National Institute for Viral Diseases Control and Prevention
China CDC
Infectious Diseases

Infectious disease are responsible for one-fifth of all deaths worldwide, and killing at least 11 million people every year;

There are about 40 new pathogens discovered since 1973.
Main Events of Viral Disease in China

1957: Flu A H3N2  The new virus contains 3 gene segments (HA, NA, PB1) from duck, others from human A H1 virus.
1968: Flu A H2N2  The new virus contains 2 gene segments (HA, PB1) from avian, others from human A H2 virus;
1960’s: HBV  Up to date, the chronic hepatitis and carriers are more than 120 million.
1977: Flu A H1N1: The 8 gene segments of the new virus are all similar to A HINI, which was prevalent in 1950’s.
1980’s: HFRS  Hemorrhagic fever with renal syndrome caused by Hantaviruses, more than 90% cases of the world in China
1988: HAV  Involved more than 300,000 persons transmitted by contaminated clam in Shanghai.
1997: HPAIV  In Hong Kong, 18 cases with 6 deaths.
2003: SARS  8098 cases with 774 deaths.
2005: HPAIV  In China mainland, 16 cases with 11 deaths
## Reported Cases of Infectious Diseases 2004 and 2005

<table>
<thead>
<tr>
<th>Disease</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis</td>
<td>1 249 783</td>
<td>1 320 166</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1 073 014</td>
<td>1 357 665</td>
</tr>
<tr>
<td>The others</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>4124 167</td>
<td>4 676 591</td>
</tr>
</tbody>
</table>
Geographical distribution of Cumulative reported AIDS cases in China, 1985-2004
Cumulative reported HIV/AIDS cases in China
1985----2004.12

- AIDS
- HIV(+)

<table>
<thead>
<tr>
<th>Year</th>
<th>AIDS</th>
<th>HIV(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>86</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>87</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>88</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>89</td>
<td>0</td>
<td>171</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>299</td>
</tr>
<tr>
<td>91</td>
<td>2</td>
<td>216</td>
</tr>
<tr>
<td>92</td>
<td>3</td>
<td>261</td>
</tr>
<tr>
<td>93</td>
<td>5</td>
<td>274</td>
</tr>
<tr>
<td>94</td>
<td>23</td>
<td>531</td>
</tr>
<tr>
<td>95</td>
<td>29</td>
<td>156</td>
</tr>
<tr>
<td>96</td>
<td>52</td>
<td>264</td>
</tr>
<tr>
<td>97</td>
<td>38</td>
<td>334</td>
</tr>
<tr>
<td>98</td>
<td>126</td>
<td>330</td>
</tr>
<tr>
<td>99</td>
<td>136</td>
<td>467</td>
</tr>
<tr>
<td>0</td>
<td>230</td>
<td>520</td>
</tr>
<tr>
<td>1</td>
<td>233</td>
<td>821</td>
</tr>
<tr>
<td>2</td>
<td>714</td>
<td>973</td>
</tr>
<tr>
<td>3</td>
<td>102</td>
<td>216</td>
</tr>
<tr>
<td>4</td>
<td>126</td>
<td>476</td>
</tr>
</tbody>
</table>

Importing | Spreading | Rapid spreading
Acute Phase Female Patient
A 25 year-old woman with hemorrhagic fever with renal syndrome (HFRS) 3 days after the onset of high fever.
(Hung Tao, 1988)

Conjunctival hemorrhage
Conjunctival hemorrhage in a 34 year-old man suffering from HFRS at 2 days of illness (Hung Tao, 1988).

Acute Phase Male Patient
A 20 year-old man with (HFRS) 3 days after onset of high fever severe hemorrhage of internal organs
(Hung Tao, 1988).

A: Purpura on legs
A 24 year-old woman, 4 days after the onset of (HFRS) showing typical purpuric stasis after needle puncture during injection
(Hung Tao, 1988).

B: Petechia on arm
Same patient, showing typical petechia and purpura on the arm. (Hung Tao, 1988).

Hemorragic Fever with Renal Syndrome (HFRS) Caused By Hantavirus
The Incidence and Mortality of HFRS in China (1950 - 2004)
SARS outbreak in 2003

- 5327 probable cases, 349 deaths reported in 24 provinces in China mainland
- 2102 probable cases, 336 deaths reported in Hong Kong, Macao, and Taiwan, China

8437 cases with 813 deaths in the world
AI Human cases in China, 2005~2006

(as of March 29, 2006)

- Dead cases (11)
- Survival cases (5)
- Clinical cases (2)
- Avian Flu Vaccine
- SARS Vaccine
- Hantavirus Vaccine
“Mock-up” H5N1 vaccine

- Establishment of the production processes for H5N1 vaccine
- Fulfillment of necessary clinical trials
- Establishment of fast track for licensing procedure
H5N1 vaccine R&D

- Inspection of strain;
- Establishment of seed lots;
- Studies of virus infectivity, antigenicity, toxicity, immunogenicity, stability;
- Genome sequence

- Inspection of manufacturing eggs

- Inactivation process

- Purification technique;
- Purifying products analysis

- Studies of formulation processes;
- Manufacturing scale technique

- Inspections of biochemistry, antigenicity & 20 other items;
- Processes of quality control;
- Processes of manufacturing inspection

- Evaluations of safety

- Vaccine stability

- Vaccine dose and type

- Vaccine potency

- Dossier preparation
Vaccine strain

NIBRG-14 (A/Viet Nam/1194/2004)

Schematic construction of NIBRG-14 strain
H5N1 vaccine R&D

Virus purification

Technique: multiple filtration and chromatographies

ultra-filtration (300KD), Fractoprep DEAE chromatography, Sepharose 4FF chromatography.
H5N1 vaccine R&D

**Virus purification**

Single harvest

bulk
Preparation of final bulk

The bulk was diluted with 0.01M PBS according to the HA contents, mixed with same volume of aluminum, with less than 0.10 mg/ml merthiolate as antiseptic in final concentration.
H5N1 vaccine R&D

**Potency assays**

*Methodology:*

- one dose and two doses,
- *ICR mice intraperitoneally and SD rats intramuscularly*
- evaluation of serum HI antibody.

- All tested animals were in good health during observation periods, no adverse reaction had been noted.
- Serum HI antibodies in all negative controls were lower than 1:10
- HI titer equal to and/or higher than 1:80 were taken as sero-positive
H5N1 vaccine R&D

Immunogenicity assays

Comparison of GMT curves between one and two immunizations of Al-absorbed inactivated vaccines (whole virion)
H5N1 vaccine R&D

Safety assays

According to related national and international requirements

- Finished 4 batches preparations (1500 ampoules/batch) of pre-clinical testing vaccines.
- Finished single dose toxin assays, abnormal toxin assays and irritability (allergy) assays in rodents.
- All tested vaccines were confirmed to be good safety.
H5N1 vaccine R&D

*Based on the assays of various types, doses and schedules of immunization onto more than 3000 mice and rats, the strategies of the type, doses and immunization schedule of testing vaccine for clinical trial have been done.*

**Testing vaccines for clinical trial**

- Immunization dosage: 1.25, 2.5, 5.0, 10.0 \( \mu \text{g/ml} \)
- Type: Inactivated H5N1 Influenza Vaccine (egg embryo derived, whole virion, Al-absorbed)
- Immunization schedule: 0, 28 day
Development of SARS Vaccine

- Cell Culture (Vero)
- Inactivated Vaccine
- Regulations and Standards
- Protection test in animal model
- Phase I clinical trial (36 Persons)
SARS Inactivated Vaccine

Culturing and Inactivation

Culture and Inactivate
- Inactivation effect
- Protein Content
- Antigen Content

Harvest

Harvest
- Protein Content
- Antigen Content

Purification

Purify
- Protein Content
- Antigen Content

Sterilization

Sterilized purified Lot
- Protein Content
- Residual Vero DNA
- Antigen Content
- Inactivation effect
- Residual Bovine serum

Formulation

Final bulk
- Sterility
- Al content

Filling and Packing

Final Batch
- Identity test
- Appearance
- pH
- Abnormal Toxicity
- Al content
- Endotoxin
- Sterility
- Effectiveness
- Volume
Preparation of SARS Antiserum In-House

- **Standard:** WHO TRS626
- **Batch amount:** Master ampoule lot 163 vials (freeze-dried)
- **Lot number:** 20031009
- **Potency:** 53u
- **u:** potency of neutralizing antibody
- **1u** is the antibody amount that neutralizes 100 CCID$_{50}$/100 μl virus
Evaluation on the Vaccine

Evaluation on safety
- Follow Chinese Criteria on Adverse Reactions to Vaccination
- Refer to U.S. FDA’s criteria on adverse reactions and lab. Indexes to phase-I clinical trial for vaccine products

Assessment on immunogenicity
- Sera conversion rate of the neutralization antibody
- GMT of neutralization antibody
Inactive SARS-CoV induces neutralizing antibodies in mice

Table 3-2. Detections of neutralization activities in the sera from mice with different immunizing dosages, times collected 4 and 6 weeks post-immunization

<table>
<thead>
<tr>
<th>Injected Dosage</th>
<th>0.15625 ( \mu ) g/ml</th>
<th>0.625 ( \mu ) g/ml</th>
<th>2.5 ( \mu ) g/ml</th>
<th>10 ( \mu ) g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>1:4</td>
<td>1:4</td>
<td>1:64</td>
<td>1:406</td>
</tr>
<tr>
<td>Group A 6 weeks</td>
<td>1:8</td>
<td>1:8</td>
<td>1:100</td>
<td>1:723</td>
</tr>
<tr>
<td>4 weeks</td>
<td>1:25</td>
<td>1:181</td>
<td>1:362</td>
<td>1:1200</td>
</tr>
<tr>
<td>Group B 6 weeks</td>
<td>1:37</td>
<td>1:144</td>
<td>1:406</td>
<td>1:1289</td>
</tr>
</tbody>
</table>

Figure 3-1. The titers of neutralizing antibody in the mice immunized with inactivated SARS-CoV vaccine
Safety and Immunogenicity of a SARS Inactivated Vaccine on Rats

Table 5-1. Rat Groups and the number of rat each group contained

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>25 Negative Control</td>
</tr>
<tr>
<td>Adjuvant Control</td>
<td>25 Adjuvant Control</td>
</tr>
<tr>
<td>16 SU group</td>
<td>25 16 SU group</td>
</tr>
<tr>
<td>32 SU group</td>
<td>25 32 SU group</td>
</tr>
<tr>
<td>Adjuvant Control satellite group</td>
<td>5 Adjuvant Control satellite group</td>
</tr>
<tr>
<td>16 SU group satellite group</td>
<td>5 16 SU group satellite group</td>
</tr>
<tr>
<td>32 SU group satellite group</td>
<td>5 32 SU group satellite group</td>
</tr>
<tr>
<td>Total</td>
<td>115 Total</td>
</tr>
</tbody>
</table>

Table 5-5. Neutralising Test Results

<table>
<thead>
<tr>
<th>Gender</th>
<th>Group</th>
<th>Neutralising Titre (1:) at different time during the test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 28</td>
</tr>
<tr>
<td>Male</td>
<td>Negative Control</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Adjuvant Control</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>16SU</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>32SU</td>
<td>48</td>
</tr>
</tbody>
</table>

- **Allergenicity Test**
- **Abnormal Toxicity Test**
- **Multiple Administration Toxicity Test**
Neutralizing antibody in serum changes between vaccine immunized 0,28 days (test group 4) and controlled group after virus attack.
Lung histopathology changes of animals after virus attack with different neutralizing antibody titre

<table>
<thead>
<tr>
<th>Neutralizing antibody titre (u)</th>
<th>Number of animals</th>
<th>Degree of lung tissue pathological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>—</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>(\leq 64)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>64-256</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>(\geq 256)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>21</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>
Protective study of inactivated SARS vaccine in monkeys

- **Good safety SARS inactive vaccine:** All the immunized animals had no clinical changes, and normal in blood biochemistry, lymphocyte, cytokines. No abnormal pathological response. Virus isolation was negative.

- **Good immunogenic SARS inactive vaccine:** Neutralization antibody was produced in all immunized animals, lymphocyte proliferate response enhanced

- **Good protective SARS inactive vaccine:** All the immunized animals remained alive and showed the normal pathological phenomenon after virus attack.
Phase I Clinical Trial of the SARS Vaccine

36 volunteer composed of 50% male and 50% female at age range from 21 – 40 were selected to receive the SARS vaccine.

- Healthy adults, aged 21-40
- Informed Consent signed beforehand
- The subjects can follow the requirement and instructions of clinical trial set by the investigator consistently
- Not vaccinated by any other vaccine product within one month before the trial.
- Oxter body temperature \( \leq 37^\circ C \)
- Laboratorial indexes normal, pregnancy test negative
Safety and Immunogenicity from a Phase I Trial of Inactivated SARS-CoV Vaccine

The inactivated SARS-CoV vaccine was well tolerated in humans. No severe adverse reactions (grade 3) were described.

Table 4. The Seroconversion at Different Time after Vaccination

<table>
<thead>
<tr>
<th></th>
<th>16 SU Group (n=12)</th>
<th>32 SU Group (n=12)</th>
<th>Placebo (n=12)</th>
<th>P Value (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Seroconversion</td>
<td>Rate (%)</td>
<td>No. of Seroconversion</td>
<td>Rate (%)</td>
</tr>
<tr>
<td>Day 0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Day 7</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Day 14</td>
<td>2</td>
<td>16.67</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Day 28</td>
<td>0</td>
<td>0.00</td>
<td>1</td>
<td>8.33</td>
</tr>
<tr>
<td>Day 35</td>
<td>6</td>
<td>50.00</td>
<td>7</td>
<td>58.33</td>
</tr>
<tr>
<td>Day 42</td>
<td>12</td>
<td>100.00</td>
<td>12</td>
<td>100.00</td>
</tr>
<tr>
<td>Day 56</td>
<td>12</td>
<td>100.00</td>
<td>11</td>
<td>91.67</td>
</tr>
</tbody>
</table>
Safety and Immunogenicity from a Phase I Trial of Inactivated SARS-CoV Vaccine
The Vaccines against Hantaviruses Licensed in China

Gold Hamster Kidney Cells Derived Monovalent Inactivated Seoul Virus Vaccine.
Gold Hamster Kidney Cell Derived Bivalent Inactivated Seoul and Hantaan Viruses Vaccine.

Mongolian Gerbil Kidney Cells Derived Monovalent Inactivated Hantaan Virus Vaccine.
Mongolian Gerbil Kidney Cells Derived Bivalent Inactivated Hantaan and Seoul Viruses Vaccine.

Suckling mouse brain derived inactivated Hantaan virus vaccine

Vero cell Derived Bivalent Inactivated Hantaan and Seoul Viruses Vaccine.
# Efficiency of the Seoul virus vaccine

<table>
<thead>
<tr>
<th>Year after vaccination</th>
<th>Incidence of HFRS (1/100,000)</th>
<th>Protection Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinee</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0 (0/28,020)</td>
<td>130.53 (35/26,814)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0/27,895)</td>
<td>119.85 (32/16,700)</td>
</tr>
<tr>
<td>3</td>
<td>0 (0/27,870)</td>
<td>74.71 (20/26,770)</td>
</tr>
</tbody>
</table>

* The predominance is Seoul like virus
# Preventive Rate of Vaccines against Hantavirus

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Year</th>
<th>Incidence (1/100 000)</th>
<th>Prevention Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Vaccination</strong></td>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>GHKC</td>
<td>5</td>
<td>0</td>
<td>106.00</td>
</tr>
<tr>
<td>MGKC</td>
<td>5</td>
<td>0</td>
<td>38.45</td>
</tr>
<tr>
<td>SMB</td>
<td>4</td>
<td>0</td>
<td>49.48</td>
</tr>
</tbody>
</table>
Development of Recombinant Hantavirus Vaccines in China

Vaccinia virus vectored hantavirus vaccine

Hantavirus VLP vaccines produced in recombinant baculovirus/insect cells system

Hantavirus DNA vaccines

Hantavirus VLP produced in CHO cells
Hantaan Virus Particles and VLP
Immunofluorescence Assay of Hantavirus VLP expression in CHO Cells
Neutrilizing antibody induced by hantavirus DNA Vaccine
CTL response to Hantavirus DNA vaccine

DNA vaccine所诱发的CTL应答

CTL交叉反应 99-99

CTL交叉反应 84-84
Vaccine Development

- Vaccine are the keystone of the fight against pathogenic microbes. Various emerging infectious diseases continue to challenge vaccine efforts directed at improvement of public health.

- Safe, effective, high protective and economic acceptable vaccines against new and old plagues are considerably required in China.
谢谢

THANK YOU!
The Specification of The Vaccine

- The vaccine is prepared by a well-documented Sino3 coronavirus-SARS strain.

- The Sino3 strain is cultivated on Vero cell, harvested, inactivated by beta-propiolactone, purified and adsorbed by aluminum hydroxide.

- The vaccine has been released by China National Institute for the Control of Pharmaceutical & Biological Products (NICPBP), complying with the Applicable Requirements for SARS-CoV Vaccine Production and Quality Control.
The cases number of top 5 infectious diseases in China (last week, 2004)

<table>
<thead>
<tr>
<th>Diseases</th>
<th>No.of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Tuberculosis</td>
<td>24660</td>
</tr>
<tr>
<td>2.Hepatitis B</td>
<td>18011</td>
</tr>
<tr>
<td>3.Dysentery</td>
<td>4487</td>
</tr>
<tr>
<td>4.Gonorrhea</td>
<td>4137</td>
</tr>
<tr>
<td>5.Syphilis</td>
<td>1974</td>
</tr>
</tbody>
</table>
The number of death of top 5 infectious diseases in China (last week, 2004)

<table>
<thead>
<tr>
<th>Diseases</th>
<th>No.of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. AIDS</td>
<td>41</td>
</tr>
<tr>
<td>2. Rabies</td>
<td>30</td>
</tr>
<tr>
<td>3. Hepatitis B</td>
<td>12</td>
</tr>
<tr>
<td>4. Tuberculosis</td>
<td>10</td>
</tr>
<tr>
<td>5. HFRS</td>
<td>5</td>
</tr>
</tbody>
</table>
Infectious Diseases

and vaccines

- Infectious disease outbreaks can impact national security and global economy
- Emerging and re-emerging infectious diseases threat to public health in China.
- Vaccines development in China