

Studies on HIV Preventive Vaccines in China

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HIV/AIDS Epidemic in China

- **Up to the end of Sep. 2005, the cumulative reported HIV infections in China have reached 135,630, including 31,143 AIDS cases and 7,773 death cases**
- **HIV-infected cases have been identified in all 31 provinces of China**

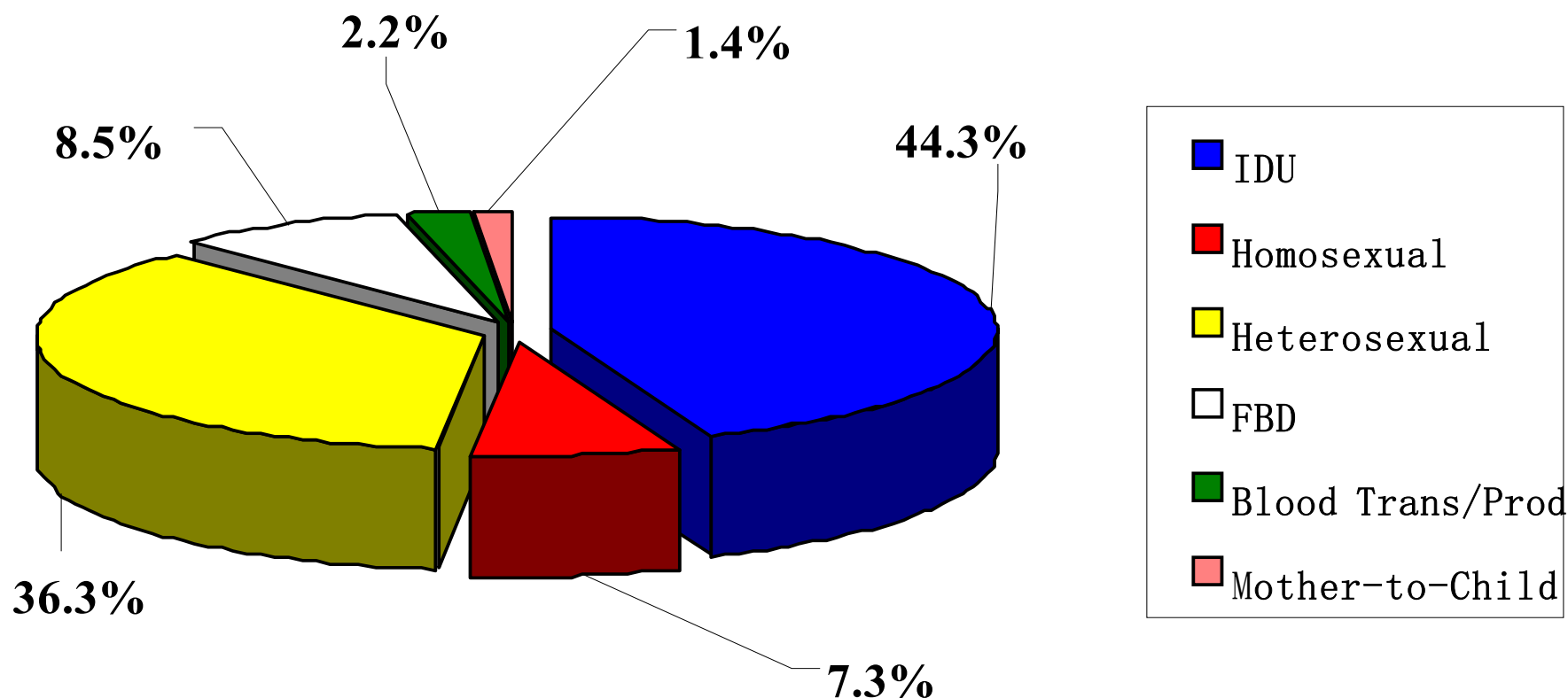
Geographic Distribution

- **The highest cumulative number of HIV infections came from Yunnan Province, consecutively followed by Henan, Guangxi, Xinjiang, Guangdong Provinces. Qinghai was the last province to identify HIV infection**
- **Most of the HIV-infected cases identified in the southwestern and northwestern parts of China came from the drug-using population**
- **Most of the HIV-infected cases identified in the southeastern China and the major cities of China came from the commercial sex population**

Demographic Distribution

- **Males comprise of the major part of the whole HIV-infected population in China. Of all the cumulative reported cases, males make up 65%**
- **Adults continue to be the major target population of HIV infection. Most infected cases are found in the age group of 20-29, which comprises of about 50% of all those infected, while the age group of 30-39 and that of 40-49 comprise of 30% and 10%, respectively**

Transmission Routes



Transmission Routes Composition of the Estimated Population Living with HIV/AIDS, 2005

The distribution about different subtypes of HIV-1 in China's 1st NHMES (1996-1998)

Province	Total	A		B'		B		C		D		F		CRF-BC		CRF01-AE		CRF02-AG		AE/B		A/J	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Anhui	48			48	100.00																		
Beijing	25	4	16.00	4	16.00	10	40.00	1	4.00					1	4.00	5	20.00						
Chongqing	38			1	2.63									33	86.84	3	7.89	1	2.63				
Fujian	49	1	2.04	3	6.12	5	10.20	1	2.04					4	8.16	35	71.43						
Gansu	28	1	3.57	7	25.00			1	3.57					19	67.86								
Guangdong	97			4	4.12	7	7.22					2	2.06	35	36.08	49	50.52						
Guangxi	24			10	41.67									6	25.00	8	33.33						
Guizhou	65			8	12.31									54	83.08	3	4.62						
Hainan	5			2	40.00											3	60.00						
Hebei	22			18	81.82			2	9.09					2	9.09								
Henan	109			109	100.00																		
Heilongj	12			7	58.33	1	8.33							4	33.33								
Hubei	61	1	1.64	60	98.36																		
Hunan	21			6	28.57									3	14.29	12	57.14						
Jilin	20	2	10.00	18	90.00																		
Jiangsu	56	7	12.50	21	37.50	2	3.57	2	3.57	2	3.57			19	33.93	3	5.36						
Jiangxi	36			5	13.89			1	2.78					4	11.11	26	72.22						
Liaoning	53	6	11.32	27	50.94	3	5.66	2	3.77					6	11.32	6	11.32	2	3.77			1	1.89
Neimong	4			4	100.00																		
Ningxia	8	1	12.50	1	12.50									6	75.00								
Qinghai	2													2	100.00								
Shandong	62	1	1.61	44	70.97	2	3.23	7	11.29					5	8.06	3	4.84						
Shanxi	29			28	96.55	1	3.45																
Shannxi	41	2	4.88	33	80.49			1	2.44					4	9.76	1	2.44						
Shanghai	42	2	4.76	18	42.86	4	9.52	4	9.52					3	7.14	9	21.43	1	2.38	1	2.38		
Shenzhen	49	1	2.04	13	26.53	5	10.20							3	6.12	27	55.10						
Sichuan	117			65	55.56	2	1.71	1	0.85	1				45	38.46	3	2.56						
Tianjin	11			3	27.27									7	63.64	1	9.09						
Tibet	3			1	33.33			2	66.67														
Xinjiang	318			2	0.63			6	1.89					310	97.48								
Yunna	89			47	52.81	2	2.25	5	5.62					16	17.98	19	21.35						
Zhejiang	32			5	15.63	1	3.13	2	6.25					13	40.63	10	31.25	1	3.13				
Total	1576	29	1.84	622	39.47	45	2.86	38	2.41	3	0.19	2	0.13	604	38.32	226	14.34	5	0.32	1	0.06	1	0.06

The Distribution of Subtypes in Different Risk Groups

HIV-1 Subtype	The Risk Groups		
	Donors	Drug Users	Sex & Others
B	5(3.7%)	6(3.2%)	11(16.4%)
B'	125(91.9%)	51(27.6%)	7(10.4%)
B'/C	2(1.5%)	18(63.8%)	13(19.4%)
AE	4(2.9%)	8(4.3%)	26(38.8%)
A			8(11.9%)
D			1(1.5%)
F		2(1.1%)	
G			1(1.5%)
Total	136(100%)	185(100%)	67(100%)

Types of HIV vaccines

- **Whole killed vaccine:** has not been progressed into clinical trials due to unfavorable benefit/risk ratio
- **Live attenuated vaccine:** formidable safety concerns have limited research in human
- **Peptide vaccine:** based on the identification and chemical synthesis of B cell and T cell epitopes
- **Protein vaccine:** including recombinant HIV protein and VLP that closely resemble the intact HIV virion without HIV genome
- **DNA vaccine:** using plasmid as vector
- **Virus vectored vaccine:** MVA, Tiantan vaccinia, fowlpox, Canarypox, AAV, Adeno

The Main Concerns for the Development of HIV Vaccine in China

- Several kinds of HIV vaccine should be developed in China, including Protein vaccine, DNA vaccine, virus-vectored vaccine, etc.
- Several subtypes such as subtypes B' and BC may exist in China. The vaccine should include the genomic regions of those genotypes to insure the vaccine may prevent infection of those genotypes
- A successful HIV vaccine should induce both cellular and humoral (cytotoxic T responses and neutralizing antibody) immune responses
- In order to induce neutralizing antibodies, the envelope proteins (gp 160, gp 120 and gp 41) should include in vaccine, but they are very variable
- In order to induce cellular immune response, the proteins such as core, pol as well as regulatory proteins such as nef, tat etc should be included

The Main Concerns for the Development of HIV Vaccine in China

- Both CTL (cytotoxic T responses) and neutralizing antibody responses should be evaluated in animals before clinical trials
- The concern for DNA vaccine is the source of the DNA incorporated into the vector including eukaryotic promoters and enhancers et al. In order to limit the possibility for chromosomal integration, homology of plasmid DNA sequences to known sequences in the human genome should be avoided
- The volunteers shall be evaluated for systemic toxicity by physical and laboratory examination. The anti-vector antibody (anti-nuclear antibody) as well as specific antibody in the volunteers after vaccination shall be evaluated in laboratory

Updates on HIV vaccine research in China

- More than 10 research groups focus on HIV preventive vaccine research
- Different groups focus on different kinds of vaccine
- Several DNA vectors have been used to develop DNA vaccine
- Several virus vectors such MVA, Ad5, replicating and non-replicating Tiantan Vaccinia have been used
- Genomes of subtype B', BC have been used in different groups
- Different research groups use different fragments of HIV, Some use structure genes and others may use structure and non-structure genes

Reference

- Drug administrative law of People's Republic of China
- Provisions of drug registration
- Requirements for biological products
- Guideline for preventive DNA vaccine
- Guideline for virus-vectored vaccine
- Guideline for clinical trial of HIV vaccine
- Guideline for preclinical study of preventive vaccine
- Guideline for defining level of side effects preventive vaccine clinical trial
- Guideline for clinical study of preventive vaccine
- Chinese GCP

General QC tests for DNA and MVA vectored vaccine

- Physical examination
- pH determination 7.2 ± 0.5
- Quantity, including concentration and volume
- Sterility test, to detect aerobic and anaerobic bacteria and fungi.
- General safety, performed in mice and guinea pigs
- Pyrogen detection with rabbits or LAL test, $\leq 0.01 \text{EU}/\mu\text{g}$

Specific QC test for DNA vaccine

➤ Identity test:

- Restriction enzyme digestion
- Detecting expressed protein

➤ Purity:

- A260/A280 Ratio
- Residual host protein
- Residual host DNA
- Residual RNA

➤ Homogeneity: The circular form is more than 90%

➤ Cellular response

➤ Humoral response

Specific QC test for MVA vaccine

- Identity test for Vector: PCR Method
- Identity test for target gene:
 - PCR method for target gene
 - Detecting expressed target protein
- Relative purity:
- Special toxicity test:
- Residual BSA
- Humoral response
- Cellular response

Our Responsibility

- **Review QC test which provided by manufacture**
- **Validate the QC test methods when needed**
- **Review the specification for the product**
- **Test each item for the product with QC test**
- **Issue the Quality Control report and comments**

Validation of QC tests

- Precision (repeatability and intermediate precision)
- Accuracy
- Specificity
- Detection limit and quantitation limit
- Linearity

Preclinical Toxicity

- General toxicity study
- Bio-distribution of HIV DNA vaccine and MVA vaccine
- Potential integration study
- Detect anti-DNA antibody which may contribute to autoimmune disorders
- Detect duration of DNA plasma in mice or guinea pigs, may regard the possibility of tolerance

Preclinical efficacy studies

- Expressing HIV target genes of DNA and MVA vaccine in cell culture
- Immunogenicity of DNA and MVA vaccine
- Rationale for dose and dosing regimen in the proposed clinical protocol

The First HIV vaccine under phase I clinical trial in China

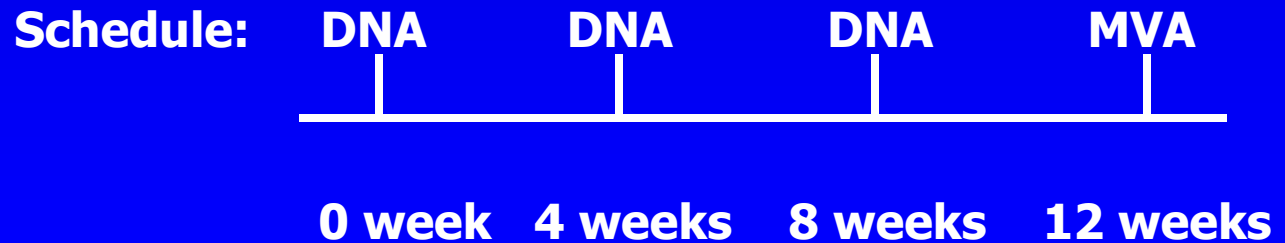
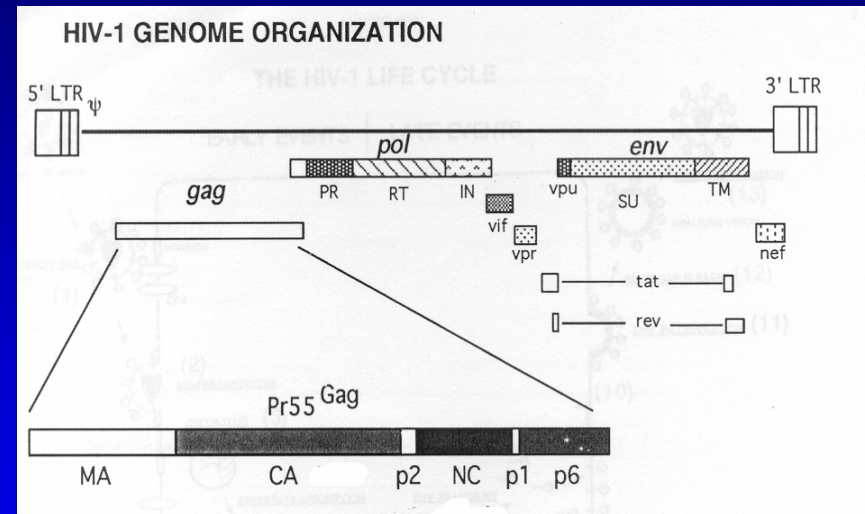
Subtype: B/C

Regions: gag-pol, env

Vectors: DNA plasmid, MVA

Schedule: DNA prime and MVA boost

Immunization routes:
Delta muscle of arm for DNA



Objectives for phase I clinical study

➤ Primary objective

To evaluate the safety and tolerability in human for HIV DNA vaccine, MVA vaccine and DNA-MVA prime-boost vaccine respectively

➤ Secondary objective

To evaluate the immunogenicity

To Monitor the social impact of participating in an HIV vaccine clinical trial

Staff and Clinic for Phase I

➤ Investigator

- National Institute for the Control of Pharmaceutical and Biological Products
- Guangxi Center for Disease Control

➤ Sponsor

Changchun Baike Pharmaceutical Inc.

➤ Clinical Base

Guangxi Center for Disease Control, Guangxi

Qualification of clinical trial bases for HIV vaccine

- Approved by SFDA
- Meet the requirement of China GCP
- Laboratory which is qualified for the related tests
- Establish the system of safety surveillance
- Ethics Committee

The personal integrity and welfare of the trial subjects must be fully protected during the process of the clinical trial. Ethics Committee and informed consent form are major measures to ensure the protection of the rights and benefits of human subjects.

Groups for phase I of HIV vaccine

Groups	Dose (pfu)	Vaccine
A	Low	MVA
B	Middle	MVA
C	High	MVA
D	Low	DNA
E	Middle	DNA
F	High	DNA
G	Middle	DNA-MVA
H	High	DNA-MVA

Design of Phase I Clinical Trial

- The random, double-blind and placebo control methods was used
- The blind will be broken only after discussion when an acute safety occurred
- Unblinding of each cohort will occur when all of the studies have been performed.

Criteria for Participant

- Be between 18 – 50 years of age
- Healthy individuals from lower risk behavior for HIV infection
- Be negative for HIV antigen and antibody
- Be negative for HBsAg and anti-HCV antibody
- Be healthy by physical examination
- Be normal by Chemical examination
- Agree to sign the informed consent
- Availability for follow-up for duration of the study (14 months)
- For female participants: negative pregnancy test at the screening and agree to use barrier contraception

Exclusion Criteria

- Pregnant or lactating women
- Prior receipt of HIV vaccine
- Receipt of immunosuppressive medicines within 6 months
- Receipt of blood products within 120 days, live attenuated vaccines within 30 days, other vaccine within 14 days, experimental agents within 30 days
- History of immunodeficiency, chronic illness, malignancy, autoimmune disease
- Medical or psychiatric conditions which preclude subject compliance

Criteria for stopping study

- If serious adverse events which are probably or definitely related to the study vaccine occurred
- The subjects should be withdrawn as following accident occurred during trial period:
 - Infected with HIV
 - Become pregnant
 - Not immunized accordingly

Safety observation

- **Participants should be observed for a minimum of 60 minutes in clinic after immunization. Local and system reactions should be recorded**
- **The participants should record the temperature and any symptoms themselves during following period**
- **Any disease or events should be reported to investigators by participants**

Serious Adverse Events

- To treat the participants with serious adverse events**
- Report to State, local drug administration, to sponsor and IRB within 24 hrs**
- Fill in forms related to serious adverse events and submit to above the institute rapidly**

Immunological testing

- Detect the antibodies against HIV target proteins and calculate positive rate among vaccinees
- Detect the INF- γ with ELISPOT and calculate the positive rate among vaccinees

Data analysis for phase I

- Analyze rate of system and local reactions after vaccination within 3 days
- Analyze rate of local and system reactions during follow-up period
- Analyze the serious adverse events
- Analyze the immunological results

Summary

- HIV preventive vaccine is needed in China to control the spread of HIV infection
- Several research groups focus on development of HIV preventive vaccine with different strategies
- The administrative system has been established for approval and evaluation of HIV vaccine
- One HIV preventive vaccine has been under evaluation in phase I clinical trial in China

Thanks