Regulatory Issues for Cell and Gene Therapy in China

Post Conference
ISCT 2007 Annual Meeting
Chunming Rao
NICPBP, Beijing, China
Overview

1. Current Development of Cell and Gene Therapy in China
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4. Content for Application of Somatic Cell Therapy in China
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6. Quality Control Requirements Research for Gene Therapy Products in China
1. Current Development of Cell Therapy in China

<table>
<thead>
<tr>
<th>Products</th>
<th>Indication</th>
<th>Progression stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine induced killer cell</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Antigen activated dendritic cell</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Autoallergic dendritic cell</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Erythroid progenitor derived from human umbilical blood</td>
<td>aplastic anemia</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Megakaryocytic progenitor derived from human umbilical blood</td>
<td>aplastic anemia</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Bone marrow stroma derived stem cell</td>
<td>Skeleton disease</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Cytokine sensitized dendritic cell</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
</tbody>
</table>
1. Current Development of Cell Therapy in China

<table>
<thead>
<tr>
<th>Products</th>
<th>Indication</th>
<th>Progression stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2/neu mRNA acitved dendritic cell</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>nonsmall-cell lung cancer autoallergic</td>
<td>nonsmall-cell lung cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>dendritic cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro actived PBMC</td>
<td>bone marrow depression</td>
<td>preclinical</td>
</tr>
<tr>
<td>Autoallergic RAK cell</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>Mesenchymal stem cell</td>
<td>hepatic fibrosis</td>
<td>preclinical</td>
</tr>
<tr>
<td>Mesenchymal stem cell derived from human</td>
<td>hematopoiesis disorder</td>
<td>preclinical</td>
</tr>
<tr>
<td>umbilical blood</td>
<td></td>
<td></td>
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</tbody>
</table>
# 1. Current Development of Gene Therapy in China

<table>
<thead>
<tr>
<th>Products</th>
<th>Indication</th>
<th>Progression stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adv – P53</td>
<td>cancer</td>
<td>come into the market</td>
</tr>
<tr>
<td>**ONCORINE ( Ad5- E1B-E3, H101)</td>
<td>cancer</td>
<td>come into the market</td>
</tr>
<tr>
<td>Adv - VEGF</td>
<td>arterial block</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Adv-TK</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Adv – heat shock protein</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Adv – interleukin 2</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
</tbody>
</table>
1. Current Development of Gene Therapy in China

<table>
<thead>
<tr>
<th>Products</th>
<th>Indication</th>
<th>Progression stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl – 2 antisense oxynucleotide</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>HIV DNA Vaccine</td>
<td>AIDS</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>HIV DNA and vaccinia vaccine prime – boost strategy</td>
<td>AIDS</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>VEGF DNA gene thread</td>
<td>fibrin clot</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>therapeutic double plasmid HBV DNA vaccine</td>
<td>hepatitis B</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>Adv – Endostatin</td>
<td>cancer</td>
<td>Clinical Trial</td>
</tr>
</tbody>
</table>
1. Current Development of Gene Therapy in China

<table>
<thead>
<tr>
<th>Products</th>
<th>Indication</th>
<th>Progression stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adv-GM-CSF</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>Adv- INF-γ</td>
<td>liver cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>recombinant replicative Adv</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>Adv –B7-1</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>V-erbB antisense oligonucleotide</td>
<td>cancer</td>
<td>preclinical</td>
</tr>
<tr>
<td>AAV-2/hFIX</td>
<td>hemophilia B</td>
<td>Clinical Trial</td>
</tr>
</tbody>
</table>
2. Guidelines for New Drug Application in China

“Drug Administration Law of the People’s Republic of China” passed on September 20, 1984; Newly Revised in Feb, 28, 2001;

“Guide for Evaluation and Approval of New Drug” April 22, 1999

“Guide for Evaluation and Approval of New Biological Products” April 22, 1999

“Drug Manufacturing Practice for Pharmaceutical Products (Revised in 1998)” June 18, 1999

“Good Clinical Practice” September 1, 1999

“Good Laboratory Practice for Non-Clinical Laboratory Studies” (trying) October 14, 1999

2. Guidelines for New Drug Application in China

- “Guide for Drug Registration” (trying) October 30, 2002
- “Regulatory Guide for Drug Manufacturing” (trying) December 11, 2002
- “Regulatory Guide for Approval of Biological Products” December 13, 2002
- “Good Laboratory Practice for Non-Clinical Laboratory Studies” August 6, 2003
- “Good Clinical Practice” August 6, 2003
- Guidelines for Study of Somatic Cell Therapy and Quality Control, 2003, SFDA
- “Regulatory Guide for Drug Manufacturing” August 5, 2004
- “Guide for Drug Registration” February 28, 2005
SFDA Regulation: Approval for Clinical Trial in China

Submission of application

Provincial drug administration organizes (<5d) and Completes spot inspection & sampling (<30d)

SFDA Acceptance (5d)

Sample testing & standard verification by NICPBP (60d)

Technical evaluation by CDE, SFDA (120/100d*)

Supplement data by implementer (<4m)

Evaluation of supplement data by CDE (40/25*)

Evaluation by SFDA (40/20d*)

Rejection or return

Approval for clinical trial (195/155d*)

Submission of clinical protocol & medical centers to SFDA

Conduct trial

Notify implementer

Check R&D and documentations

NICPBP: National Institute for the Control of Pharmaceutical & Biological Products
CDE: Center for Drug Evaluation
*: accelerated approval time
SFDA Regulation: Approval for New Drug Manufacturing in China

1. Implementer
   - Submit data of clinical trial & other changed and supplemented documentations

2. Status check, organize and complete spot inspection & sampling by PDA (30d)

3. SFDA acceptance (5d)
   - Sample test by NICPBP (30d/60d)

4. Technical evaluation by CDE (120/100d*)
   - Supplement data by Implementer (<4m)

5. Evaluation by SFDA (40/20d*)
   - Evaluation of supplement data by CDE (40/25*)
   - Rejection or return

6. Approval of manufacturing (195/155d*)

PDA: Provincial Drug Administration

NICPBP: National Institute for Control of Pharmaceutical and Biological Products

*Note: The numbers in parentheses indicate the time required for each step.
3. Review of Regulations for Cell and Gene Therapy in China

- An Outline of Quality Controls for Clinical Study of Somatic cell therapy and gene therapy, 1993, Ministry of Health
- Guiding Principles for Human Gene Therapy Clinical Trials, 1999, SFDA,
- Guidelines for Study of Somatic Cell Therapy and Quality Control, 2003, SFDA
4. Content for Application of Somatic Cell Therapy in China

- The names, aims, and foundations of R&D
- R&D status in worldwide of the selected somatic cell preparations
- Collection, separation, and quality control of somatic cell
- In vitro operation of selected somatic cells
- Quality control of somatic cell preparations
- Pre-clinical study of somatic cell preparations
- Clinical protocol of somatic cell therapy
- Qualification affirmation to R&D and probation institution also assure the qualification of their directors.
- Ethnics considerations of somatic cell therapy
4.1 The names, aims and foundations of R&D, R & D status in worldwide of somatic cell preparations

- Application form
- Title of cellular therapy products and the nominative evidence
- Aims and background
- Current situation in home and broad including research, producing and clinical application (as well as patent information)
4.2 Collection, separation and quality control of somatic cell

Collection of cells: Safety, feasibility and stability of collection techniques should be clearly demonstrated, details includes SOP, place and environment, devices used, storage and delivery condition, measures for preventing microorganism infection, measures for preventing cross-infection from share-used facilities.

Isolation of cells: All materials, devices and methods involved in the processes of cell isolation should be exactly stated. If source of cell is available from commercial way, detail datasheet and qualified certification from commercial providers should be presented.

Quality control of cells: Quality control tests should be performed during the processes of cell collection and cell isolation, such as recovery, viability, purity and homogeneity.
4.3 In vitro operation of selected somatic cells

Culture medium: All components used should meet the requirements of adequate purity, no visible risks for recipients due to the possible residues of culture medium remained should be guaranteed. All substances used as medium component should meet the requirements of defined standards.

Serum: Use of serum is not recommended unless the necessity for cell culture or activation can be proofed. It is prohibit to use any allogenic serum or plasma.

Human blood components: Information about the origin, batch number, certification of quality inspection should be presented, products with SFDA authenticated is recommended.
4.3 In vitro operation of selected somatic cells

Conditioned medium: Detailed information about the origins, production and datasheet should be presented. Screen tests for infectious pathogens should be done according to national standard of blood. Risk for transmit of virus diseases should be prevented.

Antibiotics: Application of lactam in culture medium should be avoided. Skin test should be performed if penicillin is used. Final products must be labeled with the name of antibiotics that was used.

Other components: Mitogens, antibodies, cytokines, chemicals and other culture medium should be stated in details if they are applied for cell culture or activation. Products with national approved for clinical usage are highly recommended.
4.3 In vitro operation of selected somatic cells

Production: All procedures involved in cell production should have detailed SOP and an adequate program for on-time revising. After cell harvest, small amount of cell sample and culture medium should be kept for further inspection.

If any methodology improvement on cell culture is made, re-evaluation of the new protocol should be done in cell availability, biological effect, homogeneity and purity.
Each batch of somatic cell must be controlled

- Control for somatic cell preparations:
  - Harvest rate and survival rate;
  - Purity, uniformity or specific markers;
  - Biological effect;
  - Tests for adventitious agents including bacteria, fungi, mycoplasmas, viruses, endotoxin, etc;
  - Tests for other residual materials related to process including: BSA, antibodies, serum, antibiotic, solid particles, etc.

- Original documents for the process of production and quality control tests

- Check reports of NICPBP
4.5 Preclinical Study of somatic cell therapy

- Safety evaluation
  - To cells depending on growth factors, their growth behavior must be monitored.
  - To transplant of allogenic cells, safety data must be provided from immunological aspects.
  - To heterogenous cells, their survival time in vivo and safety data must be presented.
- Toxicity tests
- Tumorigenicity tests
- Components added to final preparations should be considered as a part of the product, animals toxicity tests must be carried out.
4.5 Preclinical Study of somatic cell therapy

- Efficacy evaluation
  - Phenotype of somatic cells: Cell morphologic characters, surface markers should be described.
  - Tests in vitro: Test for biological effects, such as cytotoxic effect, immuno induction and proliferating ability of hematopoietic cell
  - Tests in vivo: Use of animal model if possible, tests for the biological effects and treatment effectiveness
4.6 Clinical protocol of somatic cell therapy

Clinical protocol must include the types of involved diseases, age range and sex of patients, the stage of disease (clinical phases), the number of cases in probation, the pre-formulated standards of enrollment and elimination.

Types, dosage, time and course of administration. If introduce the preparations by operation, its details must be provided.

Objective standards for evaluating efficacy, including testing items in clinical test and lab test.

Objective standards for evaluating toxicity and side effects and their degree, the criterion of therapy termination, testing items in clinical test and lab test to detecting toxicity and side effects.
4.7 Qualification for R&D, probation institution and their directors

- Provide professional background and experience for somatic cell therapy of all participant including doctors in charge, nurses and operators in lab.

- Provide GMP compliance and medical conditions certification of institutions which participate in the somatic cell therapy.
Two Basic Principles

- The safety and efficacy of the product should be guaranteed. A comprehensive assessment of the benefit and risk of the product should be conducted.

- New and innovative ideas should be promoted when sponsoring gene therapy product development. Considering the uniqueness of gene therapy relative to traditional chemically synthesized and genetically engineered protein medicines, there will be certain flexibilities for the regulation of novel gene therapy products.
《Points to Consider for Human Gene Therapy and Product Quality Control》

(2003) by SFDA is providing national guidance for research on and commercialization of gene therapy products in China.

The document was published in Biopharm International in May, 2004.
5. 1 Review Items of R&D of Relevant Gene Therapy Field for Application Clinical Trial of New Gene Therapy Drug in China

- Delivery vector
- Gene delivery system and method
- In vitro study efficacy data
- Preclinical animal study including toxicity safety and efficacy data
- Clinical trial investigation plan including safety and efficacy study
- Overview of production process
- Overview of quality control
- Discussion of the novelty of the product
- Discussion of the product commercialization strategy
5.2 Content for Application of Human Gene Therapy in China

- Construction of the DNA expression cassette and the gene delivery system
- Generation and characterization of cell banks and engineered bacteria banks
- Manufacture of gene therapy products
- Quality control in product manufacture and release test
- Evaluation of efficacy for gene therapy product
- Safety evaluation
- Clinical trial for gene therapy products
- Ethics study
5. 3 Clinical trial for gene therapy products

- The following information should be included when clinical trial plans are submitted.
  - Sponsor GMP compliance certificate
  - Clinical study site and biography of the principal investigator
  - The route of administration, dose, time and period of treatment (If surgery is required for product administration, detailed description of the surgery procedure is required.)
  - General clinic status index and laboratory test.
  - The signed consent forms of patients and family members
  - The molecular biology analysis of target and non-target tissues
  - Recording and reporting of side effects
  - Follow-up strategy and plan
  - A medical intervention plan should be provided to deal with unexpected immune reactions
6. Quality Control Requirements Research for Gene Therapy Products in China

- Characters of Quality System for Gene Therapy Products
- Foundation of quality control standard for gene therapy products
- Total Process Quality Control of Gene Therapy Products
6.1 Characters of Quality System for Gene Therapy Products

- Quality system for gene therapy products, especially for viral vector products are different from that for recombinant polypeptide.

- Every thing should be considered, such as character of vector, expression cassettes; etc.
6.2 Foundation of quality control standard for gene therapy products in China

International Guidelines

- Guidelines established by WHO, FDA, ICH
- U.S. Pharmacopoeia
- European Pharmacopoeia, etc.
6.2 Foundation of quality control standard for gene therapy products in China

Domestic Rules and Guidelines

- Pharmacopoeia of the PRC (Vol. III), 2005 edition
- Requirements for Biologics of the PRC, 2000 edition
- Good Manufacturing Practice in Drug Production
- Guidelines for quality control of recombinant DNA products
- Guidelines for Gene Therapy Research and Quality Control
- Other rules and guidelines promulgated by SFDA
6.3 Process Quality Control of Gene Therapy Products in China

- Quality Systems of gene therapy products
- Construction of the DNA expression cassette and the gene delivery system
- Generation and characterization of cell banks and seed lots
- Description of manufacturing process of gene therapy products and process control
- Control of gene therapy products
- Other content related with quality of gene therapy products
6.3.1 Construction of the DNA expression cassette and the gene delivery system

- Origin and sequence of target gene
- Vector: origin, character, regulatory element, restriction map, etc.
- DNA expression cassette: detailed construction procedure, DNA sequence of expression cassette, etc.
- Construction of the gene delivery system (viral and non-viral gene delivery systems): Adv, retrovirus, AAV, naked DNA, etc.
6.3.2 Generation and characterization of cell banks and seed lots

- Establish three-level cell banks and seed lots: primary cell bank/seed lots, master cell bank/seed lots, working cell bank/seed lots
- Requirement of cell banks and seed lots: definite origin, clear passage history, comprehensive identification
- Identification of cell banks and seed lots: susceptibility to virus infection, expression level of target gene, character of host cell, purity and stability, sequence of gene expression cassette, containment of wild virus, containment of adventitious agents
6.3.3 Description of manufacturing process of gene therapy products and process control

- Detailed batch record: cell culture and harvesting, purification
- Control of critical step and intermediates
- Control of raw material: material of human or animal origin
- Demonstrate Control of the Manufacturing Process
  - Specifications
  - GMPs
  - Documentation
  - Quality Assurance/Quality Control
6.3.5 Control of gene therapy products in China

- Bulk and final products have their own specifications respectively. The product quality can be monitored in different production stage.

- Establishment and validation of quality control methods:
  A. Physiochemical characters
  B. Bioactivity
  C. Impurities
  D. Safety

- Reasonable and scientific quality standards have been established for Adv based products, AAV based products.
A . Assay of Physicochemical Characters

Adv based products

- Genome molecular weight
- Restriction endonuclease map
- Characteristic gene region and expression cassette
- Count of virus particles
- Virus titer: CPE, TCID50
- UV spectroscopy
- Identification
- Virus particles purity: anion exchange HPLC
A . Assay of Physicochemical Characters

AAV based products

- Characteristic gene and expression cassette: PCR
- Molecular weight of virus capsid protein
- Purity : cation exchange HPLC
- Count of virus particles : dot blotting
Specifications of recombinant Adv-p53 Injection

- Before the establishment of standards of recombinant Adv-p53, there is no gene therapy product licensed for market. There is insufficient information on quality control standards and analytical methods;

- It is important to investigate the quality control of gene therapy products systematically, and establish scientific, normative quality standards and test methods. It will ensure the drugs’ safety, efficacy, and fast move to clinical trial.
### Specifications of recombinant Adv-p53 Injection

<table>
<thead>
<tr>
<th>items</th>
<th>method</th>
<th>specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>pale white clear liquid, without visible particles</td>
<td></td>
</tr>
<tr>
<td>Filling quantity (ml/ vial)</td>
<td>Annex I A in CP</td>
<td>1.5-1.8</td>
</tr>
<tr>
<td>pH</td>
<td>Annex V A in CP</td>
<td>8.0-8.6</td>
</tr>
<tr>
<td>identification (restriction endonucleases map)</td>
<td>with restriction endonulease</td>
<td>consistency with reference substance</td>
</tr>
<tr>
<td>MW of p53 expression gene (Kb)</td>
<td>PCR</td>
<td>consistency with reference substance</td>
</tr>
<tr>
<td>A260nm/A280nm</td>
<td>UV</td>
<td>1.20 – 1.30</td>
</tr>
</tbody>
</table>
### Specifications of recombinant Adv-p53 Injection

<table>
<thead>
<tr>
<th>Specification</th>
<th>Method</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC purity</td>
<td>Anion HPLC</td>
<td>≥95.0</td>
</tr>
<tr>
<td>Number of virus particle (vp/vial)</td>
<td>UV</td>
<td>(1.0-1.2)×10^{12}</td>
</tr>
<tr>
<td>Virus titer (IU/vial)</td>
<td>TCID50</td>
<td>≥3.3×10^{10}</td>
</tr>
<tr>
<td>Specific activity (IU/vp)</td>
<td>Virus titer over number of virus particle</td>
<td>≥3.3%</td>
</tr>
<tr>
<td>Expression of p53 gene</td>
<td>Supernatant of infected Cell /ELISA</td>
<td>positive</td>
</tr>
<tr>
<td>Bioassay (MOI of IC50)</td>
<td>Cell infected /</td>
<td>100 - 500</td>
</tr>
<tr>
<td>AAV</td>
<td>PCR</td>
<td>negative</td>
</tr>
<tr>
<td>RCA</td>
<td>CPE</td>
<td>negative</td>
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### Specifications of recombinant Adv-p53 Injection

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Specification</th>
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<tr>
<td>mycoplasma test</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>endotoxin (EU/dose)</td>
<td>LAL</td>
<td>≤50</td>
</tr>
<tr>
<td>residual host cell DNA (ng/dose)</td>
<td>Solid-phase dot blot</td>
<td>≤10</td>
</tr>
<tr>
<td>residual host cell protein (ng/dose)</td>
<td>ELISA</td>
<td>≤100</td>
</tr>
<tr>
<td>residual bovine serum (ng/ml)</td>
<td>hemagglutination</td>
<td>≤50</td>
</tr>
<tr>
<td>residual benzonase (mg/ml)</td>
<td>ELISA</td>
<td>≤1.0</td>
</tr>
<tr>
<td>Sterility test</td>
<td>XII A in CP</td>
<td>complies with the test for sterility</td>
</tr>
<tr>
<td>Test for abnormal toxicity</td>
<td>mouse method of Annex XII F in CP</td>
<td>complies with the test for abnormal toxicity</td>
</tr>
</tbody>
</table>
QC Results of Recombinant Adv-p53 Injection

Restrict-enzyme digestive map of recombinant Adv-p53

Lane 1 - 3: batch No. 20030901, 20031001, 20031002;
Lane 4: Positive control;
PCR for detection of AAV in Recombinant Adv-p53 Injection
Lane 1 - 2: DNA Marker;
Lane 3: Positive control;
Lane 4: Negative control
Lane 5-7: ADV/p53 (batch No. 20030901, 20031001, 20031002);
QC Results of Recombinant Adv-p53 Injection

Standard curve for detection of number of Adv-p53 virus particles(*10^{10} VP/ml)
PCR for p53 gene of Adv/p53
Lane 1: DNA Marker DL 600;
Lane 2: Negative control;
Lane 3-5: ADV/p53 (batch No. 20030901, 20031001, 20031002); 
Lane 6: Positive control.
QC Results of Recombinant Adv-p53 Injection

Purity (HPLC Resource Q) ≥ 95.0%

batch No. 20030901, 20031001, 20031002)
Adv, anion HPLC purity

>95.0%

AAV, cation HPLC purity

>95.0%
AAV: single strand DNA virus, so restriction endonucleases map couldn’t be used to identify recombinant virus structure; PCR is used to identify different gene regions, such as sequence of ITR-CMV promoter, promoter, promoter-expression cassette, and sequence of expression cassette.

Identification of AAV based products

structure schematic diagram of single strand DNA of AAV-2/FIX
PCR result of rAAV-2/FIX ITR-CMV
Lane 1  DNA Marker DL 2000
Lane 2  PCR negative control
Lane 3  rAAV-2/FIX sample
Lane 4  pSNAV-1/ FIX plasmid

PCR result of rAAV-2/FIX CMV
Lane 1  DNA Marker DL 2000
Lane 2  PCR negative control
Lane 3  rAAV-2/FIX sample
Lane 4  rAAV-2/FIX sample
Lane 5  rAAV-2/FIX sample
Lane 6  pSNAV-1/FIX plasmid
Comparison of physicochemical characters identification of Adv, AAV based products

<table>
<thead>
<tr>
<th></th>
<th>Adv vector</th>
<th>AAV vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>capsid identification</td>
<td>neutralization</td>
<td>SDS-PAGE</td>
</tr>
<tr>
<td></td>
<td>UV absorbance</td>
<td>electrophoresis</td>
</tr>
<tr>
<td>characteristic gene and expression cassette</td>
<td>Restriction map</td>
<td>ssDNA fragment</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>PCR</td>
</tr>
<tr>
<td>virus particle purity</td>
<td>Anion HPLC</td>
<td>Cation HPLC</td>
</tr>
<tr>
<td>virus particles numbers/ titer</td>
<td>absorbance TCID50</td>
<td>Dot blotting</td>
</tr>
</tbody>
</table>
B. Bioassay

recombinant Adv – hIL-2

- recombinant Adv - hIL-2 infect cells in vitro, then assay IL-2 bioactivity by MTT method and IL-2 expression level by ELISA;

- this method not only could be used to assay the bioactivity and expression level of target protein in micro-concentration, but also could test recombinant virus infect ability.
IL-2 expression level and bioassay in the supernatant of Adv/hIL-2 infected cells

ELISA for IL-2 content

Bioassay for IL-2
--- bioactivity of in vivo: IX factor gene – knock out mouse model

--- Expression level in vitro: determined by ELISA after infect cells in vitro.

This specification also reflects the virus infectivity.
rAAV – 2/ hFIX bioactivity *in vitro* and *in vivo*

Factor IX expression level of AAV-2/FIX *in vitro*

Bioactivity of AAV-2/FIX in Factor IX gene knock-out mouse
C. Impurities

Assay of residual virus

- **AAV helper virus**
  - AAV is replication defective virus, need helper virus (Adv or HSV), so residual helper viruses should be controlled.
  - control at nuclear acid level: PCR, sensitivity <10 copy
  - control at virus level: CPE, to ensure that there is no integrate and infective helper viruses particle existing in final products
Assay of residual virus

wild type AAV

- more than 80% adults carrying wild type AAV, it is one important containment for Adv, AAV-based products

- sensitivity < 10 copies/test, primers are specific for wild type AAV
1: Negative control;
2 - 7: Positive plasmid control: $1,10,10^2,10^3,10^4,10^5$ copies wtAAV
8: DNA Marker DL 2000;
9: $10^0$ rAAV-2/FIX
10: $10^{-1}$ rAAV-2/FIX
11: $10^{-2}$ rAAV-2/FIX
D. Safety test

1) Bioburden test
2) Pyrogen test
3) Abnormal toxicity test
4) Viral adventitious agents
5) Other related impurities
Conclusion

- The most important consideration for NICPBP is to ensure the safety and product quality of cell and gene therapy.
- NICPBP has established a strong platform to perform all assessments on quality control of gene therapy product.
- There are sophisticated and advancing development programs regarding different gene therapy products in different stages in China.
- Ethical principle and protection of trial subject for all studies on cell and gene therapy both in preclinical and clinical are regulated and supervised strictly by the Chinese Drug Regulatory Authority.
Thanks For Your Attention