細胞產品的品質管控、放行規格及相關法規介紹

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93年-97年度人體試驗案件

Product	Clinical indication	Manufacturer
Autologous natural killer cells	Late stage lung cancer and hepatocellular carcinoma	Biotech company
Autologous bone marrow MN cells	Ischemic end-stage coronary artery disease	Hospital laboratory
Autologous tumor lysate-pulsed dendrtic cells	Malignant glioma	Hospital laboratory
Autologous oral mucosal epithelium cultured on allogeneic amniotic membrane	Ocular surface disease	Hospital laboratory
Allogeneic umbilical cord blood stem cells	Allogeneic umbilical cord blood stem cell transplantation	Hospital laboratory
Autologous serum artificial tear	Ocular surface health and severe dry eye patients	Hospital laboratory
Autologous anti-CD3 antibody-activated T lymphocytes	Advanced hepatocellular carcinoma	Hospital laboratory
G-CSF mobilized autologous peripheral blood stem cells ($CD34^+$)	Chronic stroke	Hospital laboratory
Autologous bone marrow mesenchymal stem cells	Cerebral infarct	Hospital laboratory
Autologous chondrocytes	Repair of cartilaginous defect of femoral condyles	Hospital laboratory
Autologous melanocytes	Vitiligo	Research centre

93年-97年度人體試驗案件 (cont.)		
Product	**Clinical Indication**	**Manufacturer**
Autologous dendritic cells	WHO grades III and IV gliomas	Hospital laboratory
Autologous CD34+ cells from peripheral blood	Autoimmune disease	Hospital laboratory
Autologous bone marrow mononuclear cells	Peripheral arterial occlusion disease	Hospital laboratory
Autologous cornea stem cells cultured on amniotic membrane	Unilateral corneal stem cell insufficiency	Hospital laboratory
Autologous mesenchymal stem cells (derived from bone marrow) embedded in collagen	Production of cartilage-like tissue for repairing cartilage defect	Hospital laboratory
Autologous melanoma cells transduced by viral vectors	Metastatic melanoma	Hospital laboratory
Autologous natural killer cells	Hepatitis C virus related hepatocellular carcinoma (HCC)	Biotech company
Autologous oral mucosal epithelial cells cultivated on amniotic membrane	Ocular surface disorders	Hospital laboratory
Allogeneic umbilical cord derived mesenchymal stem cells (UC-derived MSC)	Transplantation of UC-derived MSC after allogeneic hematopoietic stem cell transplantation	Biotech company/ hospital laboratory
Autologous umbilical cord blood	Cerebral palsy	Hospital laboratory

分級管理

	歐盟	美國 FDA CBER
低風險	非工程化 (not engineered)	PHS ACT 361 最小程度處理 (minimally manipulated)
高風險	工程化	PHS ACT 351 非最小程度處理

Today's Topics

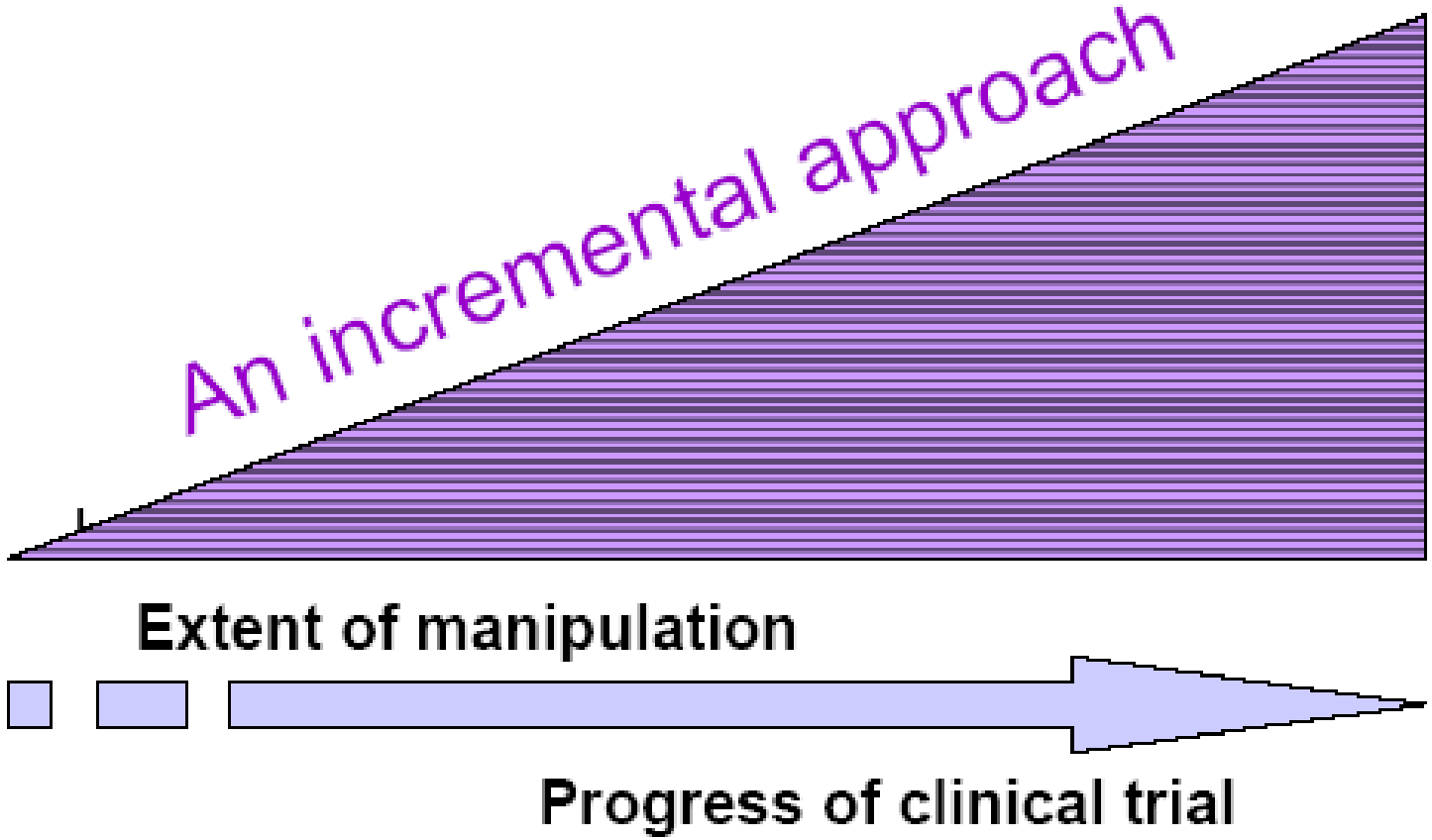
- Cellular Products Manufacturing & Quality Controls
  - So-called Chemistry, Manufacturing and Controls (CMC)
- Product Testing: Potency
- Product Characterization & Release Specifications

Cellular Products Manufacturing & Quality Controls

Principles

- Defined components
- Detailed manufacturing procedures
  - Consistency of production
- Product characterization
  - Specifications
- Adventitious/microbiological agents testing
- Detection for extraneous materials
- Stability

CMC requirement



Defined components

- Cells
- Reagents
- Excipients
- Combination products

Defined components: Cells

- Autologus and/or allogeneic
- Cell source
  e.g. bone marrow mononuclear cells
- Mobilization protocol
  e.g. GCSF-induced mobilization
- Collection method
  e.g. leukapheresis
- Donor eligibility

Defined components: Cells (cont.)

Donor eligibility:

- Autologous
  - 研究單位必須設有檢驗傳染病原 (adventitious agents) 如愛滋病 (HIV) 和肝炎病毒 (hepatitis viruses) 之作業，可以不選擇這種受試者，或對這種特殊受試者事先作保護工作人員及環境之防患措施。
- Allogeneic
  - Medical history screening e.g. TB, CJD
  - Testing e.g. HIV, HBV, HCV, Syphilis
  - HLA matching

Defined components: Reagents

- Reagents/materials/separation devices/media that are *not* intended to be present in the final product
  - Excipients (components in the final product) are not AMs
- USP<1043> Ancillary materials (AMs)
- Vendor qualification
  - cGMP, audit/inspection record
  - Quality control testing program
  - Documentation

    e.g. - grade, traceability or country of origin (animal- derived AMs)

    - QC, batch analytical results or COA

Risk classification of AMs
Tier 1

- Low-risk, highly qualified therapeutic drug or biologic, medical device or implantable material
  - Therapeutic grade

    e.g. human serum albumin (HSA), insulin, cytokines
- Certificates of Analysis (COA)
- Assess removal from the final product

Risk classification of AMs
Tier 2

- Low-risk, well-characterized materials with intended use as AMs, produced in compliance with GMPs
  - For use in drugs, biologics or medical devices manufacture

    e.g. - growth factors, proteolytic enzymes, density gradient media

    - exclude most animal- derived materials
- COA
- Assess removal from the final product
- Qualified vendor

Risk classification of AMs
Tier 3

- Moderate-risk materials not intended for use as AMs
  - For *in vitro* diagnostic use or reagent grade materials
    e.g. growth factors, culture media, chemicals
- COA
- Assess removal from the final product
- Qualified vendor
- Confirm critical test results shown in COA
  - Develop internal specifications eventually

Risk classification of AMs
Tier 4

- High-risk materials
  - Toxins, most animal-derived materials
    e.g. feeder cells, ascites-derived antibodies, cholera toxin, FBS
- COA
- Assess removal from the final product
- Qualified vendor
- Confirm critical test results shown in COA
  - Develop internal specifications eventually

AMs:
Removal from the final product

- AMs are not intended to be part of the final product
- Consider AMs removal in early stages of product development
  - By washing with calculated dilution factors
    - Maybe sufficient for early clinical trials
  - Measurement of residual AMs

    e.g. - immunoassay for BSA

    - PCR for residual DNA from feeder cells
  - AM clearance by process (validation)

Defined components: Excipients

- Components to be part of the final product

  e.g. HSA

- Amount/concentration in the final product
- In compliance with pharmacopoeial specifications
- Novel excipients
  - Previous human experience?
  - Safety concerns?

Defined components: Combination products

- Device components
  e.g. polymeric scaffolds
- Drug components
  - Marketed in Taiwan?
  - Pharmacokinetics (PK)
  - Pharmacodynamics (PD)

Detailed manufacturing procedures

- Preparation procedures
  - Cell collection/processing/culture conditions
  - Irradiation
  - Final harvest
- Process timing and intermediate storage
  - Timing for each step
  - In-process testing
  - Cryopreservation, stability studies initiated
- Final formulation
  - Excipients
  - Cell density

Product testing

- Microbiological testing
  - Bacterial and fungal testing

    e.g. USP<71>
  - Mycoplasma

    e.g. PCR base methods
  - Viral testing (when cell lines are used)
- Identity

  e.g. - physical or chemical characteristics

  - visual examination/microscopic methods

  - immunological tests e.g. surface markers

Product testing (cont.)

- Purity
  - Endotoxin
    - <5 EU/kg body weight/hour (parenteral drugs)
    - <0.2 EU/kg body weight/hour (intrathecally-administered drugs)
  - Residual AMs
- Viability
  - Acceptable viability >70%
  - <70%, address safety concerns of dead cells and cell debris
- Potency
  - Progressive assay design/implementation/validation

Product stability

- In-process stability testing
  - Stable during the period of cryopreservation
- Final product stability testing
  - Stable between the time of product formulation to administration

Product testing:
Potency

Product testing:
Potency

Definition of potency (ICH Q6B)

The measure of the biological activity using a suitably *quantitative* biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties.

Product testing: Potency

Purpose

- A potency measurement is used to correlate with the product's clinical efficacy and hence can be used for lot release, stability protocols and/or comparability studies.

- Used to develop "in house" reference materials in the event that a universal standard or reference material is not available.

Challenges to Potency Assay Development for CGT products:	Examples:
Inherent variability of starting materials	• Autologous and allogeneic donor variability • Cell line heterogeneity • Error-prone replicating viruses
Limited lot size and limited material for testing	• Single dose therapy using autologous cells suspended in a small volume
Limited stability	• Viability of cellular products
Lack of appropriate reference standards	• Autologous cellular material • Novel gene therapy vectors
Multiple active ingredients	• Multiple cell lines combined in final product • Heterogeneous mixtures of peptide pulsed tumor and/or immune-modulatory cells • Multiple vectors used in combination
The potential for interference or synergy between active ingredients	• Multiple genes expressed by the same vector • Multiple cell types in autologous/allogeneic cell preparations
Complex mechanism of action(s)	• Multiple potential effector functions of cells • Multiple steps required for function such as infection, integration, and expression of a transgene • Vectors containing multiple genes
In vivo fate of product	• Migration from site of administration • Cellular differentiation into the desired cell type • Viral or cellular replication • Viral vector infection, uncoating, and transgene expression

Potency tests for cellular and gene therapy (CGT) products, draft guidance, FDA, 2008

Potency methods

- Biological assays
  - Measurement in a “living biological system”

    e.g. *in vivo* animal studies, *in vitro* organs, tissues, cell cultures
- Non-biological analytical assays
  - A surrogate of biological activity

    e.g. quantitative flow cytometry, ELISA, enzymatic reactions
- Multiple assays (assay matrix)
  - Biological or analytical
  - Quantitative readout (e.g. unit of activity) or qualitative readout (e.g. pass/fail)

Example:
Human haematopoietic stem cells

This monograph (Ph Eur 01/2008:2323) applies to haematopoietic stem cells that have not undergone expansion or genetic modification…..

Background

- Haematopoietic stem cells are recognised by their ability to reconstitute human haematopoiesis *in vivo*.
- They also have the capacity to differentiate into colony-forming cells (CFC).
- The membrane marker CD34 is commonly used or the successful isolation/purification of haematopoietic stem cells from crude preparations and as an indicator of haematopoietic stem cell content in routine quality control.

Example:
Human haematopoietic stem cells (cont.)

CD34+ cell count (quantitatively analytical assay)

- For peripheral blood stem cells, CD34+ cell count is determined using a validated automated apparatus to analyse cells labelled with anti-CD34 antibodies.

- The method must be sensitive, accurate and reproducible.

Example:
Human haematopoietic stem cells (cont.)

CFC assay (quality biological assay)

- Proliferative capacity is established by a suitable assay. The correlation between the dose of CD34 and the number of CFCs in a given situation (pathology, packaging, mobilisation) is determined.

- The CFC assay is carried out periodically; whenever a change that could affect the quality of CD34+ cells is made to the protocol for packaging or mobilisation.

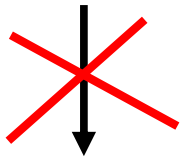
Biological activity vs analytical assays: Establish a correlation

Accumulate knowledge regarding your product from the following:

- Relevant preclinical investigation
- Proof of concept studies
- Available historical experience
- Available reference materials and controls
- Extensive product characterization
- *Early clinical studies*

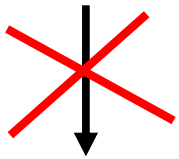
Clinical trial progress: A misconception

Phase I trial



Not necessarily

Phase II trial



Not necessarily

Phase III trial

Clinical trial progress: Establish a correlation

Aim

Analytical assays ↔ Biological activity ↔ Clinical efficacy

Product testing:
Potency

At clinical Phase I or II trials

- Define the biological activity to certain extent
- Assays possibly correlate with clinical efficacy

  e.g. viability, surface markers

- Multiple assays where appropriate to help establish the relevant biological activity of your product

Aim of most Phase I trials: Safety

Product testing:
Potency

At clinical Phase II or III trials

- Quantitative assay preferred (by the end of Phase II)
- Biological activity that can correlate with clinical efficacy
  - Direct assay of the biological activity

    e.g. cytotoxic activity of NK cells
  - Assay(s) correlating with biological activity

    e.g. surface marker analyses correlating with cell proliferative activity

Product testing: Potency

At clinical Phase III trials (pivotal studies)

- A sufficient potency design and acceptance criteria to assure a well-characterized, consistently manufactured product is administered.

- Use well-characterized potency assay(s) with established acceptance criteria in stability protocols to establish expiry dating for licensure.

Product Characterization & Release Specifications

Characterization

- Determination of the following characteristics of a product
  - Physicochemical properties
  - Biological activity
  - Immunochemical properties
  - Purity and impurities

- Allow relevant specifications to be established

Specifications

- Tests

  e.g. tests for safety, purity, potency and identity

- Analytical methods

  e.g. USP<71>, LAL gel clot test

- Acceptance criteria
  - Numerical limits

    e.g. <0.5 EU/ml
  - Ranges

    e.g. pH 6.8-7.4; 85-90% CD34+ cells
  - Others

    e.g. gel like, pass/fail

Release specifications for a clinical Phase I trial

Example 1

Release specification of the final cellular product-

Test	Method	Acceptance criteria
Sterility	USP<71>	Negative
Mycoplasma	Mycoplasma test kit	Negative
Endotoxin	LAL gel clot test	Negative
cyte ratio	Morphology and staining	≧ %
Total viable cell number	Trypan blue staining and hemacytometer	cells
Viability	Trypan blue staining and hemacytometer	≥ %

Release specifications for a clinical Phase I trial

Example 2

Analytical method	Acceptance criteria
Appearance	
Visual examination	Gel-like appearance
Cell number	1 to 1.5 ($\times 10^5$) cells
Microbiological testing	
Bacterial and fungal testing	Negative
Mycoplasma testing	Negative
Identity	
Real-time reverse transcription/polymerase chain reaction for cell specific genes	Detection of 1, 2 and 3 example genes
免疫組織化學染色分析	染色法檢測應為陽性
細胞表面抗原測定	表面抗原1 (+), 表面抗原2 (-)
Impurities	
Endotoxin	< XX EU/product
Residual antibiotics (HPLC)	< 1 ppm
Residual hTGF (ELISA)	< 1 ppm

References

- 體細胞治療人體試驗申請與操作規範，衛生署，2003

  Scope: autologous, allogeneic and xenogeneic cell products
- Content and Review of Chemistry, Manufacturing and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs), FDA, Apr 2008

  Scope: autologous and allogeneic cell products
- Guideline on human cell-based medicinal products, EMEA, May 2008

  Scope: autologous and allogeneic cell products
- Potency tests for cellular and gene therapy products, draft guidance, FDA, Oct 2008

References (cont.)

- ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.
- ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology.
- ICH Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process.

Thank you for your attention.

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