CMC ASPECTS OF GENE THERAPY
MEDICINAL PRODUCTS

SME workshop: Focus on chemistry, manufacturing and controls (CMC) regulatory compliance for biopharmaceuticals and advanced therapies

Presented by Matthias Renner on April 16, 2015
Division Medical Biotechnology, Paul-Ehrlich-Institut, Germany
AGENDA

- Definition GTMPs
- Classification
- Critical aspects of GTMP manufacturing and control
- Guidelines
**ex vivo**

**GENE TRANSFER**

**in vivo**

**cell line**

**explantation of target cells**

**gene transfer**

**reinfusion of modified cells (autologous, allogenic, xenogenic)**

**direct application:**

- virus / viral vector
- non-viral vector: naked DNA, RNA complexed
- (non)-replicating recombinant microorganism
In case a medicinal product may fall within TEP or CTMP and GTMP, then GTMP applies.
GENE THERAPY MEDICINAL PRODUCT

means a biological medicinal product which has the following characteristics:

(a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence [AND]

(b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence

Gene therapy medicinal products shall not include vaccines against infectious diseases.
## Classification - Examples

<table>
<thead>
<tr>
<th>INDICATION</th>
<th>PRODUCT</th>
<th>CLASS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended as adjunct treatment in HSC transplantation</td>
<td>Allogeneic T cells encoding an exogenous TK gene</td>
<td>sCTMP</td>
</tr>
<tr>
<td>Intended for prevention and treatment of HPV16 induced pre-malignancies and malignancies</td>
<td>Plasmid encoding a mutation-inactivated E7-E6 fusion protein from Human Papillomavirus 16 linked to the human chemokine hMIP-1a via a dimerization module derived from human IgG3</td>
<td>GTMP</td>
</tr>
<tr>
<td>Intended for prevention and treatment of HCV and HCV-induced hepatocellular carcinoma</td>
<td>Adenoviral vector expressing the non-structural region of hepatitis C virus (HCV) in which a mutation has been introduced</td>
<td>Not an ATMP</td>
</tr>
</tbody>
</table>
CAT Classification

- Is the product classified as ATMP?
- Is it classified as TEP, somatic cell therapy, or gene therapy medicinal product?
- Is it combined or non-combined?
- Classification is
  - voluntary
  - free of charge
  - not legally binding
AGENDA

- Definition GTMPs
- Classification
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Virus vector production principle

AAV genome

- ITR
- rep
- cap

Packaging genes

- P
- rep
- cap

Therapeutic gene expression cassette

Vector genome

Adenovirus helper function

- E1A/E1B
- VA
- E4
- E2A

- transient as virus
- transient/stable as plasmid
Characterisation of vector producer cells

- Adventitious agents/sterility
- Generation of wt-virus
- Cell viability
- Morphology / growth characteristics
- Genetic stability of the cell
- Genetic integrity of the inserts
- Transgene expression
State-of-the-art vector design

Use of non-state-of-the-art vector and packaging cells should be avoided,

• to allow manufacture of consistent and safe product.

Sponsor Statement: “There are molecular strategies by which the generation of RCVs during manufacture can be reduced or potentially eliminated, for example the use of cell lines and vectors which lack overlapping [...] nucleotides, thus preventing homologous recombination. However, such a system is not currently employed by the Sponsor.”

• to avoid later changes in vector design and subsequent ‘comparability’ exercises

Use of non-SIN retroviral vectors, use of WPRE with destroyed X-reading frame
Change in GTMP design

- Change of cell line for vector production could be change in product composition (enveloped viruses)
- Change in nucleotid sequence of therapeutic gene (codon optimisation)
- Change in vector backbone (non-SIN to SIN vector, use of mutated WPRE)

14 December 2011
EMA/CAT/GTWP/44236/2009
Committee for advanced therapies

Reflection paper on design modifications of gene therapy medicinal products during development
Vector design

Information needed on

• history
• genetic manipulation
• establishment and
• characterisation and control of viral vector seed
  • (sequencing data)
GTMP release criteria

Does the agency agree that the tests and acceptance criteria for DS and DP are adequate at the clinical stage of development with the GTMP? Could the Agency provide feedback on further tests that they deem will be necessary to support a Marketing Authorisation Application?
## QC control of virus vector DS/DP

<table>
<thead>
<tr>
<th>Identity</th>
<th>Physical titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Therapeutic gene expression</td>
</tr>
<tr>
<td>Potency</td>
<td>Infectious titer</td>
</tr>
<tr>
<td></td>
<td>Particle to infectivity ratio</td>
</tr>
<tr>
<td></td>
<td>Therapeutic gene expression</td>
</tr>
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<td></td>
<td>Biological activity</td>
</tr>
<tr>
<td>Purity</td>
<td>Process-related impurities: Benzonase, Resins, etc.</td>
</tr>
<tr>
<td></td>
<td>Residual Plasmid DNA (TAT)</td>
</tr>
<tr>
<td></td>
<td>Residual HC-DNA (SV40 T-Ag, E1A)</td>
</tr>
<tr>
<td></td>
<td>Residual HCP</td>
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<tr>
<td>Safety</td>
<td>Sterility, Endotoxin, Mycoplasma</td>
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<tr>
<td></td>
<td>Replication-Competent Virus</td>
</tr>
</tbody>
</table>
Aspects of potency testing of virus vector-based GTMPs

- Infection efficiency one aspect of potency but not sufficient
- Expression of therapeutic gene might be considered acceptable for early clinical trials
- At MAA functional assay based on activity of the therapeutic protein and reflecting clinical efficacy should be in place (if feasible)
Reasons for quality comparability exercise

- Use of "early development batches" for non-clinical analyses and clinical batches when significant changes have been implemented
- Change in manufacturing process during clinical evaluation
- Manufacturing process upscaling
- Change in manufacturing sites, change in analytical procedures
Challenges in manufacturing and control of GTMPs

Example: Genetically modified T-cells/HSCs

Indication:
- inherited monogeneic diseases: ADA-SCID, X-SCID, ALD, β_thalassemia
- tumor, virus infection

modified from Wieczorek and Uharek (2013)
Challenges in manufacturing and control of GTMPs

Is the manufacturing process of the lentiviral vector and the proposed process validation strategy considered acceptable?
COMMISSION DIRECTIVE 2009/120/EC

of 14 September 2009


In the case of genetically modified cells, the starting materials shall be the components used to obtain the genetically modified cells, i.e. the starting materials to produce the vector, the vector and the human or animal cells. The principles of good manufacturing practice shall apply from the bank system used to produce the vector onwards.
QC control of virus vector starting material

- Manufacture compliant to GMP
- Full control of manufacturing process
- Release of starting material equivalent to release of DS/DP except
  - Potency by infectivity and therapeutic gene expression might be sufficient
  - Some process related impurities may be addressed in characterisation studies

of active substance (vector related)

- Transduction rate
- Copy number
- Transgene expression
- Biological activity
Process / batch validation

Is use of cell apharesis material from normal donors for process validation acceptable?

- Mobilisation should be performed in same manner before apharesis
- Consideration of potential differences in
  - Cell type composition before and after expansion
  - Cell growth potential during expansion phase
  - Transduction efficiency and transgene expression
- Challenging to address potency with healthy donor cells in diseases based on mutated gene

Validation strategy based on combination of historical data, process development, characterization and comparability studies, cells from healthy individuals, and a continued process verification on patient samples is acceptable
How to deal with short shelf life of the final product?

A real time release strategy is required for the drug product due to the short shelf life. The intention is to have a two stage release process: Stage 1 being release for infusion based on a subset of the release tests that can be performed prior to infusion and stage 2 being the final product release once all release testing has been completed.

Provided that

- process validation demonstrates robust production
- characterization and validation batches meets reliable release criteria
two-stage release process is acceptable.
<table>
<thead>
<tr>
<th>Topic</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality, preclinical and clinical aspects of GTMPs</td>
<td>04.2001</td>
</tr>
<tr>
<td>Design modifications of GTMPs during development</td>
<td>02.2012</td>
</tr>
<tr>
<td>Risk-based approach according to Annex I, part IV of Directive 2001/83/EC applied to ATMPs</td>
<td>03.2013</td>
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<tr>
<td>CHMP/CAT position statement on Creutzfeldt-Jakob disease and ATMPs</td>
<td>06.2011</td>
</tr>
<tr>
<td>Questions and answers on gene therapy</td>
<td>12.2009</td>
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<tr>
<td>Quality, non-clinical and clinical issues relating specifically to recombinant adeno-associated viral vectors</td>
<td>03.2009</td>
</tr>
<tr>
<td>Quality, preclinical and clinical aspects of medicinal products containing genetically modified cells</td>
<td>05.2012</td>
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<tr>
<td>Development and Manufacture of Lentiviral Vectors</td>
<td>11.2005</td>
</tr>
<tr>
<td>Management of clinical risks deriving from insertional mutagenesis</td>
<td>08.2013</td>
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</tbody>
</table>
5.14. Gene transfer medicinal products for human use

01/2008:51400 corrected 6.0

5.14. GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

Considerations:

Ph. Eur. 5.2.3. Cell substrates for the production of vaccines for human use

<table>
<thead>
<tr>
<th>Test</th>
<th>Cell seed</th>
<th>Master cell bank (NCB)</th>
<th>Working cell bank (WCB)</th>
<th>Cells at or beyond the maximum population doubling level used for production</th>
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<tbody>
<tr>
<td>1. IDENTIFY AND PURITY</td>
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<tr>
<td>Morphology</td>
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<tr>
<td>Identification: nucleic acid fingerprinting and a relevant selection of the following tests: biochemical (e.g. isoenzymes), immunological (e.g. histocompatibility), cytogenetic markers</td>
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<tr>
<td>Karyotype (diploid cell lines)</td>
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<td>Life span (diploid cell lines)</td>
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<tr>
<td>2. EXTRANEOUS AGENTS</td>
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<td>Bacterial and fungal contamination</td>
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<td>Mycoplasma</td>
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<td>Spironucleus (insect cell lines)</td>
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<td>Electron microscopy (insect cell lines)</td>
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<td>Tests for extraneous agents in cell cultures</td>
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<td>Co-cultivation</td>
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<td>Tests in animals and eggs</td>
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<tr>
<td>Specific tests for possible contaminants depending on the origin of the cells</td>
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<td>Retroscreen</td>
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<td>Tumorigenicity</td>
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</tbody>
</table>

Committee for Medicinal Products for Human Use (CHMP)

ICH harmonisation for better health

CHMP/ICH/607698/08

Oncolytic Viruses

Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines

DRAFT
Thank you for your attention

The views expressed in this presentation are in part the personal views of the author and may not be understood or quoted as being made on behalf of or reflecting the position of the Paul-Ehrlich-Institut or the EMA committees or working parties.

Further information

matthias.renner@pei.de
innovation@pei.de
Paul-Ehrlich-Institut, Germany

European Medicines Agency
30 Churchill Place • Canary Wharf • London E14 5EU • United Kingdom
Telephone +44 (0)20 3660 6000 Facsimile +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

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