EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Manufacturing of gene therapy products

common issues and advices

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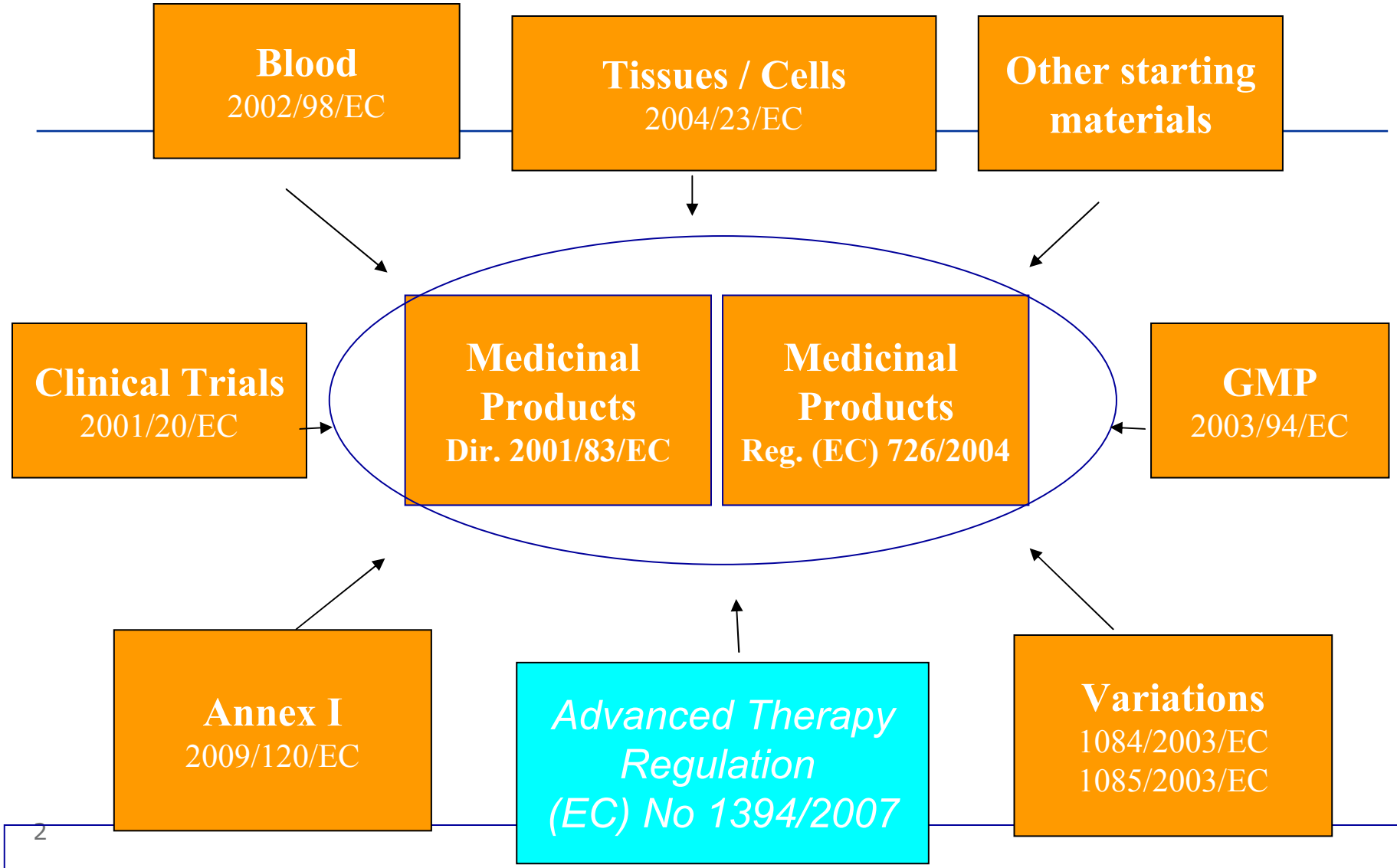
EU REGULATORY FRAMEWORK FOR ATMP



NOTE FOR GUIDANCE ON THE QUALITY, PRECLINICAL AND CLINICAL ASPECTS OF GENE TRANSFER MEDICINAL PRODUCTS

First multidisciplinary guideline

Quality/**N**on **C**linical/**C**linical requirements

Covers all GT medicinal products: plasmids, viral vectors, genetically modified cells

Issued for public consultation in 1999

Adopted in 2001

Presently under revision for updating to new development in the field

GENE THERAPY MEDICINAL PRODUCT

EXPRESSION CASSETTE+VECTOR BACKBONE±CELLS

EC: therapeutic transgene + regulatory sequences

VB: **contains active genes/proteins** needed for production + for in vivo function + for targeting/delivering

Cells: overlap with CBMP

GTMP biological characteristics depend on vector ± cells in addition to therapeutic transgene

biological / toxicological /pharmacological activity needs to be assessed for transgene and vector ± cells

MAIN RISKS FOR GTMP

Germline transduction: unacceptable (dir. 2001/20)

Insertional mutagenesis: oncogenesis

Replicating viral vector: target cell lysis/dissemination/shedding

Oncolytic viruses: ectopic replication

Transgene and/or vector immunegenicity: impairment of clinical efficacy/immunetoxicity

Transgene disregulated expression: toxicity/impairment of clinical efficacy

GENE THERAPY MEDICINAL PRODUCT

Design of GTMP is critical to adequately address risks

A clear understanding of GTMP molecular structure and biological characteristics is essential in order to design (=assess) appropriate Q/NC/C studies

The *ideal* GTMP contains only sequences/proteins needed to achieve the intended clinical goal

The **real** GTMP contains also other sequences/proteins, heritage of early development construct and derived from production system

APPROPRIATE GTMP DESIGN SHOULD BALANCE SAFETY WITH SOUGHT CLINICAL EFFECT

Deletion of sequences responsible for replication ability

Oncolytic Viruses can be useful for killing tumour cells: replication designed and shown to be restricted

Deletion of sequences responsible for integration ability

Integrative vectors are needed to transduce stem/progenitor cells: ex vivo approach, SIN vectors, insulators, cell copy number and MOI as low as transduction efficacy can allow

Minimal vector backbone, to reduce toxicity

GTMP MOLECULAR STRUCTURE

To be justified and described e.g. promoters, insulators, terminators, splicing sites, relevant junction regions, any intended site-specific mutation, deletion etc.

Genes for resistance to antibiotics of clinical use should be avoided

Biological characteristics of parental virus/ packaging cell line of viral vectors are critical:

- to reduce risk or RCR generation, structural or functional genes should be independently expressed from different constructs, in order to increase the number of recombination events required for RCR generation.

GTMP PRODUCTION

Final product depends also on production process

Risk of contamination by extraneous viruses

Quality of starting materials is critical: plasmids, viruses, packaging cell line, bacterial cells, reagents

Robust control of the production process: validation, i.p.c., stability

Characterisation and QC: appropriate mixture of molecular and biological testing methods

QC

Identity: transgene and vector/cell

Integrity: no deletion, no rearrangement

Sequence: at least transgene

Purity: replication competent vector, production cell/virus residues

Viral vectors: infectivity/particle ratio

Plasmids: molecular forms i.e. proportion of supercoils

Genetically modified cells: % transduction, gene copy number

Bioactivity/potency: at least gene expression, quantitative potency assay reflective of bioactivity *in vivo*

GENETICALLY MODIFIED CELLS

Cells with replicating potential transduced with γRV or LV:

- number/location of integration sites
- oncogene activation or tumour suppressing genes inactivation
- adjacent gene identity and function: characterisation where feasible

RCV

For replication-deficient viral vector:

- a test to detect RCV below an acceptable level is required on virus banks, each production run and batch of product
- the upper limits set should be demonstrated to be safe in appropriate animal and/or human studies

For AAV, RV, LV specification is **absent**

RCV TESTING METHODS

Based on biological or molecular systems: LOD/LOQ critical

Test validation should show LOQ on same vector amount as specification limit or clinical dose

eg. limit 100 RCV/dose, clinical dose 10^{12} pfu:

- result 1 RCV when testing 10^{10} pfu: acceptable
- result 1 RCV when testing 10^{9} pfu: **unacceptable**

For LOD same approach: a test method with LOD 100 vp will allow a batch to pass ("below LOD") with up to 100 vp of RCV

GTMP QUALITY CAN HAVE AN IMPACT ON: ISSUES FOR NON CLINICAL STUDIES

Test material characterisation/comparability to clinical lots

Specie-specificity of bioactivity

Animal model selection

Transgenic/knockout/homologous animal models: relevance to human disease

Pleiotropic activity

Immunogenicity of human proteins in animals

Administration: route, frequency, dose, exposure

Developmental aspects for paediatric indication

GTMP QUALITY CAN HAVE AN IMPACT ON: ASSESSING THE RISK AT CLINICAL LEVEL

chromosomal integration, mobilisation, duration of gene expression, bio-distribution to target and non target sites, other treatments (e.g. chemotherapy, radiotherapy, immune-suppressants)

type of product (e.g. genetically modified cells or vector)

type of transfer vector (e.g. integrating or non integrating, replicative or non replicative)

patient disease and age

concomitant diseases

CHANGES DURING GTMP DEVELOPMENT

To obtain improved product characteristics and maximise the efficacy/safety profile

Changes to production process: e.g. packaging cell line

- Comparability as for other products

Changes to GTMP **design**: e.g. change of promoter, of viral vector serotype, introduction of tissue specific enhancers, SIN vector

- additional non-clinical studies and possibly further clinical trials

Extent of additional studies depend also on stage of development

OTHER GTMP GUIDELINES

Lentiviral vectors manufacturing (CHMP/BWP/2458/03) **Q**
AAV reflection paper (CHMP/GTWP/587488/07) **Q/NC/C**
Genetically modified cells (CHMP/GTWP/671639/2008) **Q/NC/C**
Non-clinical testing for inadvertent germ line transmission (CHMP/SWP/273974/2005) **NC**
Non clinical studies required before first clinical use of gene therapy medicinal products (CHMP /GTWP /125459 /2006) **NC**
Clinical monitoring and follow-up (CHMP/GTWP/58311/2007) **C**
Environmental risk assessment (CHMP/GTWP/125491/2006)

THANK YOU FOR YOUR ATTENTION!

GMP ISSUES: PREVENTION OF CROSS CONTAMINATION DURING PRODUCTION

Segregated areas and dedicated equipment for viral vectors

Cell-based GTMP: unidirectional flow of non-genetically modified cells to gene-manipulation areas

multiple LFH stations in one room: use for more than one lot/product at a time is not recommended (small rooms are better than large ones)

cleaning procedures used at changeover: validation to molecular level (e.g. detect transgene and/or vector)

GMP ISSUES: CONTROL OVER MANUFACTURING PROCESS

In some cases of autologous cell-based GTMP, for clinical reasons cell donation can be obtained only once from a patient (e.g. infants) or only at large time intervals (e.g. severe diseases):

rejecting a product lot can create an ethical problem because product cannot be prepared again, unless starting cells are in stock (*with classical drugs it is often an economical issue*)

control over the production process should be maximised -also relevant for investigational GTMP

quality assurance is paramount

BACK UP SLIDES

GMP ANNEX 2

Develop and validate rapid test methods (*incl. microbiological*)

Where allowed in the MA, a modified testing and sample retention strategy may be developed and documented (*also in Eu.Ph. 5.14*)

A suitable control strategy must be in place, built on enhanced understanding of product and process performance (*process validation to be carried out prospectively and more frequently- not possible retrospectively*)

SOP describing the entire release procedure

Continuous assessment of the effectiveness of the quality assurance system

GMP ANNEX 2

Stepwise batch release procedure (before and after QC test results are available):

1) assessment of batch records and results from environmental monitoring, all deviations and the available analytical results, review and conditional certification by QP
2) assessment of the final results, final product certification by QP

SOP with measures to be taken (including liaison with clinical staff) where unsatisfactory test results are obtained after product dispatch

Such events should be fully investigated, corrective and preventative actions taken to prevent recurrence