EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

SME Workshop on CMC of Biological Medicinal Products

EMA London 14.-16.4.2015

CMC ISSUES for Cell based ATMP

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THE NEW ERA OF REGENERATIVE MEDICINE

Dozens of biotech companies and university labs are developing ways to replace or regenerate failed body parts. Here are a few of the projects:

BONE

Bone-growth factors or stem cells are inserted into a porous material cut to a specific shape, creating new jaws or limbs. A product that creates shinbones is in clinical trials.

COMPANIES: Creative Biomolecules, Orquest, Sulzer Orthopedics Biologics, Genetics Institute, Osiris Therapeutics, Regeneron.

SKIN

Organogenesis' Apligraf, a human-skin equivalent, is the first engineered body part to win FDA approval, initially for leg ulcers. Other skins are in the works for foot ulcers and burns.

COMPANIES: Organogenesis, Ad-vanced Tissue Sciences, Integra LifeSciences, LifeCell, Ortec International.

PANCREAS

Insulin-manufacturing cells are harvested from pigs, encapsulated in membranes, and injected into the abdomen. The method has been tested in animals and could be in human trials in two years.

COMPANIES: BioHybrid Technologies, Neocrin, Circe Biomedical

HEART VALVES, ARTERIES, AND VEINS

A 10-year initiative to build a heart has just started. Genetically engineered proteins have been successfully used to regrow blood vessels.

COMPANIES: Organogenesis, Advanced Tissue Sciences, Genetech, LifeCell, Reprogenesis.

SALIVA GLANDS

Proteins called aquaporins that allow cells to secrete water are used to recreate saliva glands damaged by disease or radiation. Glands are also being engineered to secrete healing drugs. The technique has proven successful in mice.

COMPANIES: None yet.

URINARY TRACT

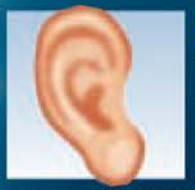
Cartilage cells are taken from the patient, packed into a tiny matrix, and injected into the weakened ureter, where they bulk up the tissue walls to prevent urinary backup and incontinence. The method is in late-phase clinical trials.

COMPANIES: Reprogenesis, Integra LifeSciences.

BLADDER

Doctors at Children's Hospital in Boston have grown bladders from skin cells and implanted them in sheep. They are about to try the same process on a patient

COMPANIES: Reprogenesis.



CARTILAGE

A product is already on the market that regrows knee cartilage. A chest has been grown for a boy and a human ear on a mouse.

COMPANIES: Genzyme Tissue, Biomatrix, Integra LifeSciences, Advanced Tissue Sciences, ReGen Biologics, Osiris Therapeutics

TEETH

Enamel matrix proteins are used to fill cavities. It works in dogs; human trials are a few years away.

COMPANIES: Biora, Atrix Laboratories, Creative BioMolecules.

BREAST

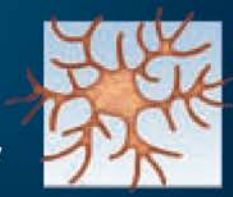
In preclinical studies, several companies have been able to create a cosmetic nipple by inserting a ball of cartilage. Researchers are now trying to grow a whole cosmetic breast.

COMPANIES: Reprogenesis, Integra LifeSciences.

LIVER

A spongy membrane is built up and then seeded with liver cells. Organs the size of a dime have been grown, but a full-size liver could take 10 years due to its complexity.

COMPANIES: Advanced Tissue Sciences, Human Organ Sciences, Organogenesis.



SPINAL CORD NERVES

Scientists are in-vestigating nerve-growth factors, injecting them at the site of damage to encourage regeneration or seeding them along biodegradable filaments and implanting them. Rats have been made to walk again.

COMPANIES: Acorda, Regeneron, CytoTherapeutics, Guilford Pharmaceuticals.

DATA: BUSINESS WEEK, DRUG & MARKET DEVELOPMENT REPORTS

Businessweek July 1998

ADVANCED THERAPY MEDICINAL PRODUCTS - ATMP

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COMMISSION DIRECTIVE 2003/63/EC
of 25 June 2003
amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use

- Somatic cell therapy
- Gene therapy

National authorisations
DE, IT, FR, AU …

TEP not defined

REGULATION (EC) No 1394/2007 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 13 November 2007
on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004

Tissue engineered

Centralised marketing authorisations (MA) from 1/2009

TEP / regenerative medicine
Combined products
Non-substantial manipulation
Long term efficacy follow up
Hospital exemption
...

COMMISSION DIRECTIVE 2009/120/EC
of 14 September 2009
amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products

- NEW DEFINITIONS for GeneTherapy and Somatic Cell Therapy

ATMP specific Dossier requirements for MA

Directive 2001/83/EC revised

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ATMP REGULATION 1397/2007

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REGULATION (EC) No 1394/2007 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 13 November 2007
on advanced therapy medicinal products and amending Directive 2001/83/EC
and Regulation (EC) No 726/2004

- **Definitions suporting regenerative medicine TEP / Combined - medical devices**
- **Clarfying fronteers - Non-substantial manipulation to separate from transplantation**
- **Centralised MA from Jan 2009 / new Committee CAT**
- **Traceability – flow between cell donation vigilance - pharmacoviglance**
- **national system for hospital exemption for named patient and non routine**
- **Specific GMP requirements**
- **Long term efficacy follow up**
- **hESC - national prohibitions apply**
- **Incentives for SME**
- **Revise Annex 1 of Directive 2001/83/EC to establish new dossier requirements**

Directive 2009/120/EC – specific requirements for MA of ATMP's

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Cell Based Medicinal Products

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(Directive 2009/120/EC + Regulation 1394/2007)

Somatic cell therapy medicinal product

Tissue engineered Products – TEP

Include cells or tissues subject to **substantial manipulation**

"cells or tissues subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered "

Or indicated for **heterologous use**

"not intended to be used for the same essential function(s)"

- used to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues. = **somatic cell therapy**
- used with a view to, regenerating, repairing or replacing a human tissue = **TEP**

hierarchy of guidelines !

European Medicines Agency

London, 21 May 2008
Doc. Ref. EMEA/CHMP/410869/2006

COMMITTEE FOR MEDICINAL PRODUCT FOR HUMAN USE (CHMP)	
GUIDELINE ON HUMAN CELL-BASED MEDICINAL PRODUCTS	
DATE FOR COMING INTO EFFECT	1 September 2008

European Medicines Agency
Evaluation of Medicines for Human Use

London, 10 October 2007
Doc. Ref. EMEA/CHMP/BWP/271475/2006

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)	
GUIDELINE ON POTENCY TESTING OF CELL BASED IMMUNOTHERAPY MEDICINAL PRODUCTS FOR THE TREATMENT OF CANCER	
DATE FOR COMING INTO EFFECT	15 May 2008

14 January 2011
EMA/CAT/571134/2009
Committee for Advanced Therapies (CAT)

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Reflection paper on stem cell-based medicinal products

Adoption by CAT	14 January 2011

London, 08 April 2010
EMA/CAT/CPWP/568181/2009
Committee For Advanced Therapies (CAT)

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SCIENCE MEDICINES HEALTH

Reflection paper on *in-vitro* cultured chondrocyte containing products for cartilage repair of the knee

Final

Adoption by CAT	16 April 2010

Human cells = starting materials

L 102/48 EN Official Journal of the European Union 7.4.2004

DIRECTIVE 2004/23/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 31 March 2004

on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells

authorisation of tissues and cells procurement + donor testing

by transplantation authority

authorisation of collection and testing and tissue establishments (TE) for banking

Export / Import activities in the EU by authorised TE

✓ TE ensures that **imported cells from 3rd countries** allows traceability to donor and collection and testing under equivalent standards as Directive

✓ **Cells exported from EU** comply with this Directive

Not possible in **Medical Devices** – Directive 93/42/EEC, article 1 point 5.
Not possible in **Cosmetics** - Regulation 1223/2009 Annex 2 substances Prohibited (416)

APLIGRAF – Approved as medical device in the USA since 1998

Replacement / repair

bilayer of allogeneic human fibroblasts and keratinocytes isolated from boys' foreskin on a bovine collagen matrix

Submitted in the EU in 2001 - application withdrawn

NOT substantial = NOT medicinal product :

Regulation 1394/2007

- – cutting;
- – grinding;
- – shaping;
- – centrifugation;
- – soaking in antibiotic or antimicrobial solutions;
- – sterilization;
- – irradiation;
- – cell separation, concentration or purification;
- – filtering;
- – lyophilization;
- – freezing;
- – cryopreservation;
- – vitrification;
- …

“heterologous use” = medicinal product

BORDER LINE PRODUCTS - under ATMP Regulation or T&C Directive or none!

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- AUTOLOGOUS NON SUBSTANTIALLY MANIPULATED GENERATED ON THE BED SIDE

Not under T&C / not under ATMP
Adipose derived stromal fraction / Celution

- BANKED CELLS EXPANDED AND CRIOPRESERVED – NOT YET DEDICATED

Under T&C / not ATMP yet but will be when processed
Accept T&C instead of GMP for initial manufacturing and storage?

- HUMAN CELLS IN RESEARCH

  Research excluded from T&C Directives – eg. iPS cells may not be under T&C if developed for research

...

- **Reflection Paper on classification of ATMPs is being updated for more clarity**

TO BE A MEDICINAL PRODUCT not a TRANSPLANT

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- CERTIFIED **GMP** UNDER MUTUAL RECOGNITION WITHIN EU AND WITH OTHER AGREED NON EU MEMBERS
- SUPPLIER QUALIFICATION FOR ALL **RAW MATERIALS** AND TRACEABILITY
- **VALIDATION OF ASSEPTIC MANUFACTURING** PROCESS – GMP ANN1
- EQUIPMENT QUALIFICATION – GMP ANN15
- BATCH RELEASE BASED ON **PRODUCT TESTING BY QP**
- **PRE-ASSESSMENT** FOR PRODUCT QUALITY, SAFETY EFFICACY AND FOLLOW-UP
- **MODE OF ADMINISTRATION** UNDER MANUFACTURER SUVERVISION
- **PHARMACOVIGILANCE** FOR LIFE
- **LIABILITY** ON ALL PARTS OF THE PROCUREMENT / PROCESS / PRODUCT / USE

Volume 4
EU guidelines for
Good Manufacturing Practice for
Medicinal Products for Human and Veterinary Use

Annex 2
Manufacture of Biological active substances and Medicinal Products for Human Use

http://ec.europa.eu/health/files/eudralex/vol-4/vol4-an2__2012-06_en.pdf

Deadline for coming into operation: 31 January 2013

sources	enzymes, hormones	fluid[12]	processing	purification	filling
7. Human and / or animal sources	Gene therapy: genetically modified cells	Donation, procurement and testing of starting tissue / cells[14]	Manufacture vector[13] and cell purification and processing,	Ex-vivo genetic modification of cells, Establish MCB, WCB or cell stock	Formulation, filling
	Somatic cell therapy	Donation, procurement and testing of starting tissue / cells[14]	Establish MCB, WCB or cell stock	Cell isolation, culture purification, combination with non-cellular components	Formulation, combination, fill
	Tissue engineered products	Donation, procurement and testing of starting tissue / cells[14]	Initial processing, isolation and purification, establish MCB, WCB, primary cell stock	Cell isolation, culture, purification, combination with non-cellular components	formulation, combination, fill

Increasing GMP requirements

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- GMP mandatory for all products entering clinical trials
- GMP or equivalent quality system for Hospital Exemption
- Many trials from academic / hospital investigators
- Consideration of other quality systems in use in tissue banks

- **Revision of the GMP framework for ATMP's**
- **GMP specific for ATIMP**

Cell – product definition - diversity

- Autologous or allogeneic
- Toti – pluri – adult starting material
- Separated – enriched - clonal
- Single population – multifactorial complex combination
- Cultured – cell divisions – genetic stability
- Cell bank or cell stock -
- Differentiation to be concluded prior or post administration
- Cell suspension – matrix - combined
- Genetically modified
- ...

CAT CLASSIFICATION PROCEDURE

CARDIAC REPAIR CELL STRATEGIES

heterogenious cell preparations FOR MI or CHF

1ST WAVE →

bone marrow aspirate - autologous
Cell MSC + EPC ?
Minimal manipulation
- Separation / centrifugation

Moderate results / no results

2ND WAVE →

MSC immuneselected / MPC - EPC
Cell expansion
Other sources – PBMC / adipocytes / placenta
allogeneic
Commited cells - Cardiomyocytes

Inconclusive

3RD WAVE ? →

Embryonic stem cells
iPSC

results ?

Product definition

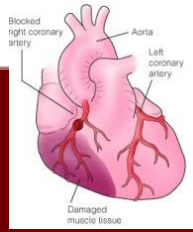
Cells from …

Act where …

Doing what …

Interact with …

Response is …



Autologous MSC's in AMI

Bone marrow MSC's – how pure?

Enriched - Complex distribution of various types of cells

What cells relevant for various functions

+ deleterious cells + no effect

Different roles not well assigned – immunesupression - modulating inflamation, paracrine, *engraftment?*

Functionality – multiple

Safety - Tumorigenic ? on target?

Sources of variability in cell therapy

MATERIALS

Donor

Collection

Dynamic starting cell material

Biological raw materials

MANUFACTURE

- **Same process different dynamics**
- **Length of process**
- **different differentiation stages**
- **Complex process – multiple stages**

CONTROL

- **Bioanalytical methods**
- **No standardised reference materials**

ADMINISTRATION

- **Complex system often surgical procedures**
- **Patient response**

Raw materials : reagents

general text in the Eur Phar *(in 2015)*

serum/medium

cytokines / growth factors

enzymes (such as trypsin)

antibodies

individual proteins

buffers

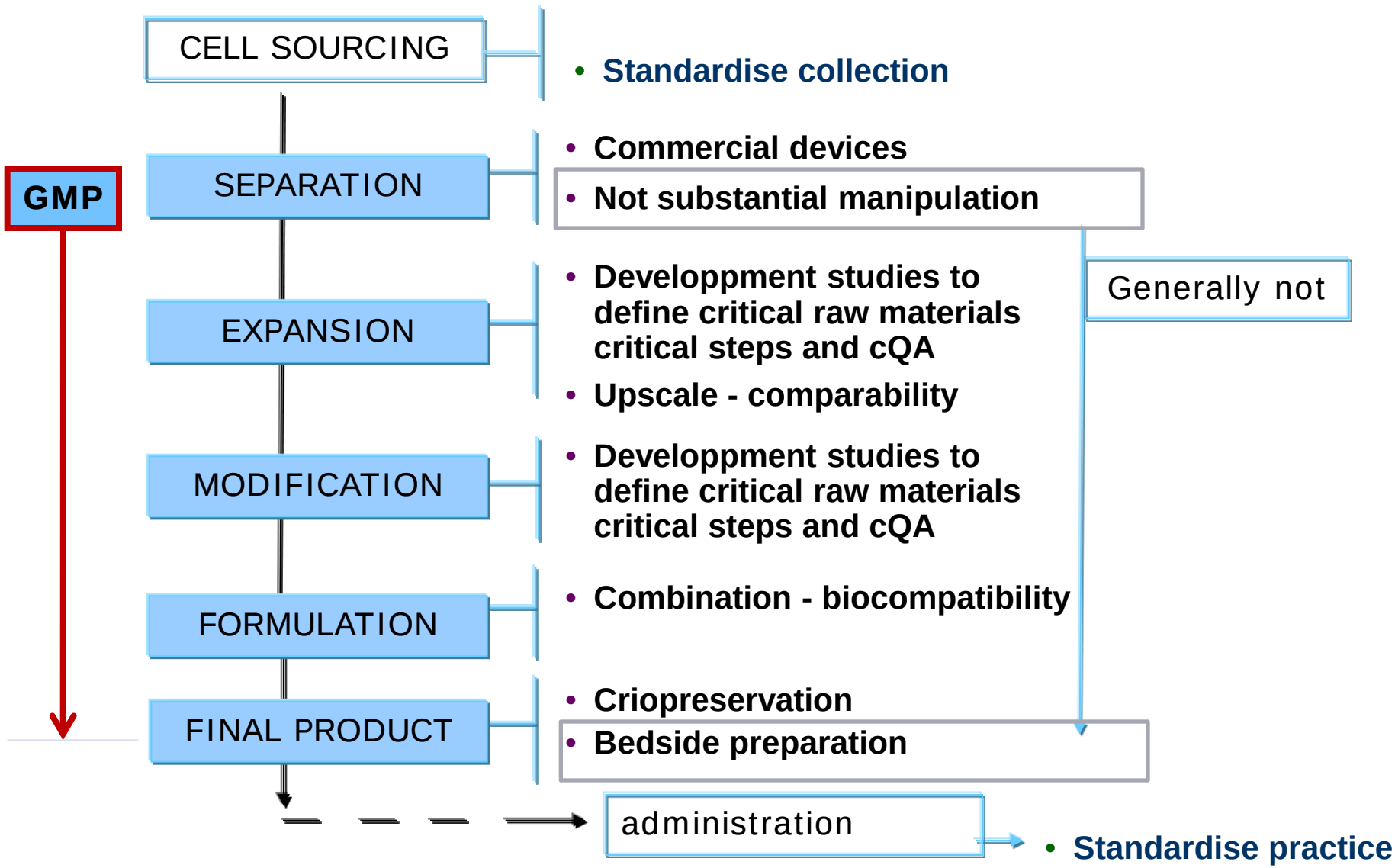
plasmids/ viral vectors

Identity
Purity
Biological Activity / Functionality
Specific Activity
Total Protein content
Impurities, Product-related
Impurities, Process-related
Viral Safety / TSE compliance
Microbial Contamination
Stability / Storage conditions

-GUIDELINE the use of bovine serum in the manufacture of human biological medicinal products (EMA/CHMP/BWP/457920/2012 rev.1) UNDER NEW REVISION

- GUIDELINE the use of porcine trypsin used in the manufacture of human biological medicinal products (EMA/CHMP/BWP/814397/2011) DRAFT

MANUFACTURING PROCESS



STERILITY

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01/2008:20627

2.6.27. MICROBIOLOGICAL CONTROL OF CELLULAR PRODUCTS

This test has been shown to be preferable to the test for sterility (*2.6.1*) for certain cellular products, since it has better sensitivity, has a broader range, and is more rapid. It is applied instead of the test for sterility (*2.6.1*)

G Kielpinski, S Prinzi, J Duguid and G du Moulin. Roadmap to approval: use of an automated sterility test method as a lot release test for Carticel†, autologous cultured chondrocytes. *Cytotherapy* 2005; 7(6): 531-541

Khuu HM, Patel N, Carter CS, Murray PR, Read LJ. Sterility testing of cell therapy products: parallel comparison of automated methods with a CFR-compliant method. *Transfusion* 2006; 46: 2071

European Pharmacopoeia – General Text on Viral Safety (50107)

Integrated strategy:

- **Source of possible infectious agents:**

  - Origin of the cells / tissues

  - reagents in manufacturng process

- **Infectivity and pathogenicity of the infectious agent considering the use and mode of administration**

- **Testing at the level of the donation, biological raw and other starting materials/ and at final product**

- **Removal / inactivation capacity of the manufacturing process**

separation

Listed as non substantial manipulation *(Reg. 1394/2007)*

Often using collection medical devices – CE covers intended use ?

Single manufacturing step – generally for autologous use

Generate heterogenous AS

Large number of runs to establish acceptable specifications

Characterisation highly dependent on phenotypic profile referred to functionality / potency

ensure GMP QC release

Autologous minimally manipulated MSC's

Phenotypic profile – ISCT not sufficient

Adequate for the intended use - Bioassay to support profile

Immunomodulatory – part of the indication - potency

Bioassay sensitive to deleterious cells + no effect

Expansion – variable complexity

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Always considered substantial manipulation

Biological raw materials - CRITICAL – new EP text in prep

Culture conditions - Development studies provide relevant information on cell growth – dedifferentiation – senescence

Population doubling time based on exponential growth phase + passage number – CRITICAL

In-process controls – pH, temperature, oxigen, time,.. - CRITICAL

Extensive characterisation to support target phenotypic and genotypic profile – more than CQA!

Set CQA - phenotypic profile referred to functionality / potency , sterility, mycoplasma, endotoxin - CRITICAL

Allogeneic expanded MSC's

Immunomodulatory – true? allogeneic? After culture? Repeat administration?

modification

Variable complexity - genetic modification, activation, de- and re-differentiation, combination …

Always substantial manipulation - CRITICAL

Viral vectors, matrixes - manufacture for purpose – starting materials – manufacturing process and control - CRITICAL

Biological raw materials - CRITICAL – new EP text in prep

Development studies provide relevant information on efficiency of cell modification, improved function, genetic stability, lateral damage …

Set defined conditions and in-process controls - CRITICAL

Aquired phenotype, genotype assessed by physico-chemical and functional tests - expression studies at the level of the protein

Complex multistep generate intermediates – identify CPP + CQA with specs - CRITICAL

•ACTIVE SUBSTANCE RELEASE SPECS – morphology, purity, phenotypic profile for efficiency, functionality / potency , sterility, mycoplasma, endotoxin

TARGET PROFILE

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Directive 2009/120/EC – SPECIFICATIONS required for CB-ATMP

- identity,
- purity,
- viability,
- potency,
- kariology, tumourigenicity, genetic stability
- Genetically modified cells = gene therapy + cell therapy requirements

EXTENSIVE CHARACTERISATION
Based on previous knowledge + development relevant for
CONSISTENCY - PROCESS VALIDATION - CQA COMPARABILITY

PURITY = phenotypic and genotypic profile

= Phenotype + Quantify / viable total (flow cytometry)

- **Relevant cells**
- **Inert cells**
- **Deleterious cells**
- **Apoptotic cells**
- **Viable cells**
- **Cell debris**
- **Exosomes**

= Genotype – (sequencing – PCR – STR)

- **separated / purified populations**
- **clonal cells**
- **differentiation stage**
- **genetically modified vs non GM**

Biological CHARACTERISATION

Assessing Biological activity = qualitative or quantitative

Bioassays have intrinsic variability – standardisation - validation

Bioassays – to reflect relevant molecular/structural interactions related to the mode of action

Quantitative bioassays = potency assays - allow for reference active substance / product

Biological characterisation can overcome the lack of structural homogeneity focusing on relevant interactions

potency assay – considerations

- Potency has to be quantitative and related to relevant biological properties = drawn from characterisation (and preclinical studies)
- Potency may evolve during development – define reference preparations
- Potency assay required for consistency, comparability, stability – validated for MA
- Potency of active substance if final product not possible
- Functional assay might not be quantitative but can serve to cross validate potency measurement of surrogate markers – mixed approaches possible and often necessary
- Quantification of mRNA should be complemented with the expressed protein
- cells and induced differentiation / engraftment / *ex vivo* use might need kinetic studies to validate relevant biological activity(ies)

Comparability

Starting point:

ICH Q5E – comparability guideline applies to protein / peptide based products but excludes from the scope advanced therapies. Only general concepts may apply to CBMP

Specific CBMP guideline has little guidance on comparablility
manufacturers should ***consider the critical parameters*** *drawn from the characterisation of their product to establish the analytical tools necessary for the required comparability studies throughout development* ***and start as early as possible***

Comparability of CBMP

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Critical steps identified during development – needed for comparability

analytical tools for comparability exercise should be established through product development.

Develop comparability tools as early as possible

During the pivotal clinical studies changes should not be introduced to the manufacturing process and the final product.

CBMP comparability

extended characterisation

identity (phenotypic analysis using FACS, karyology or isoenzyme, morphology),

dose (viable cell concentration),

potency (*in vitro* and/or *in vivo* assay),

purity (% viability, % with product related substances)

Impurities to be kept to the minimum – not part of comparability

? **Cell based impurities – kept to a minimum or comparable?**
nutrient requirements / metabolic products / dynamic equilibrium

CBMP scenario:

periodic introduction of new cell stock

Cell master often limited in time - not covering the entire life cycle of the product

✓Validated process from various cell stocks

✓Establish a predefined comparability program applied to various lots originated from several stocks

✓Validate the multiplicity of stocks by using in clinical trials the various lots generated that were comparable

ATMPs on the market and the future

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5 ATMPs approved so far, 4 other under review,

> 3 expected to start in next 12 mo

- **ChondroCelect** - TEP / Approved on 5 October 2009
- **Glybera** - GTMP / Approved on 25 October 2012
- **MACI** – TEP, combined ATMP / Approved on 27 June 2013
- **Provenge** – CBMP / Approved on 6 September 2013
- **Holoclar** – CBMP / Pending EC Approval on 6 December2014

1st stem cell approved

moderate to severe limbal stem-cell deficiency due to ocular burns

CONDITIONAL

✓ 4 applications withdrawn prior to approval

❖ **4 new approval procedures on going:** 2 GTMP, 2 CBMP

- ophtalmology, haematology, oncology, metabolic disorders

REMARKS ON QUALTY ISSUES

- Complexity requires definition and a target profile
- Your product is as good as the quality of the starting and raw materials
- The fully established manufacturing process has to be controlled and validated at the commercial scale
- The analytical methods matter - qualified later validated
- Potency assays are essential
- Manipulation and changes in cell characteristics may have an impact on cell fate, persistence, engraftment and overall efficacy of a CBMP
- Is Comparability measurable
- Combined products … not mentioned but add complexity

Meet regulators - the earlier the better

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Molecular Therapy vol. 20 no. 3 march 2012

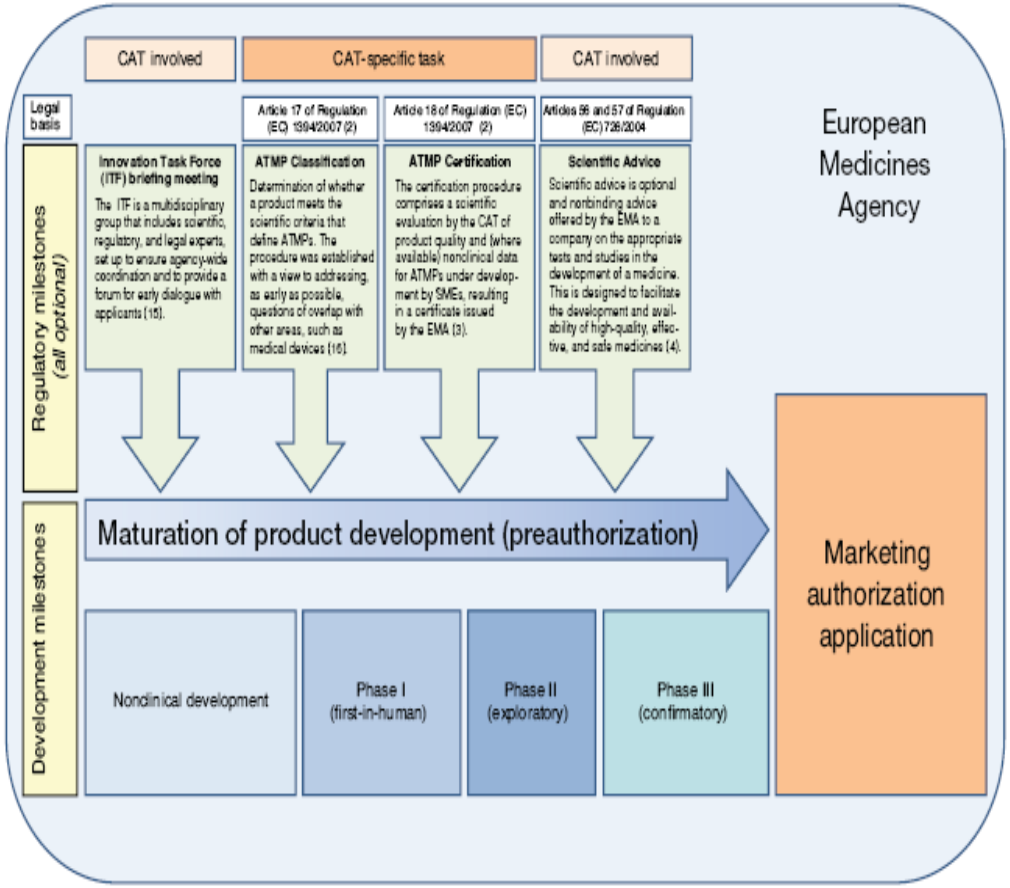

Figure 1 Regulatory pathways for ATMPs in Europe. The usual sequence in which procedures are requested by applicants. Note that all procedures can be requested at any time during development. ATMP, advanced therapy medicinal product; CAT, Committee for Advanced Therapies; EMA, European Medicines Agency; SME, small and medium-sized enterprise.

References:

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- Barkholt L, Flory E, Jekerle V et al. Risk of tumorigenicity in mesenchymal stromal cell-based therapies – bridging scientific observations and regulatory viewpoints. ***Cytotherapy*** (2013) 15(7), 753–759.
- Bravery, C, Carmen, J, Fong, T et al. Potency assay development for cellular therapy products: an ISCT review of the requirements and experiences in the industry. ***Cytotherapy*** 15(1), 9–19 (2013).
- Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells: the International Society for Cellular Therapy position statement. ***Cytotherapy*** 8(4), 315–317 (2006).

Guidelines at EMA : http://www.ema.europa.eu/
Home -> Human -> regulatory -> Scientific guidelines -> Multidisciplinary -> Cell therapy and tissue engineering

Guidelines and legislation at EC: http://ec.europa.eu/health/human-use/index_en.htm
Pharma legislation / Clinical Trials / Advanced Therapies

THANK YOU!

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