Symposium

Raw Materials for the Production of Cell-based and Gene Therapy Products

3 April 2013

Strasbourg, France

Session 2

User, Manufacturer & Academic Viewpoints

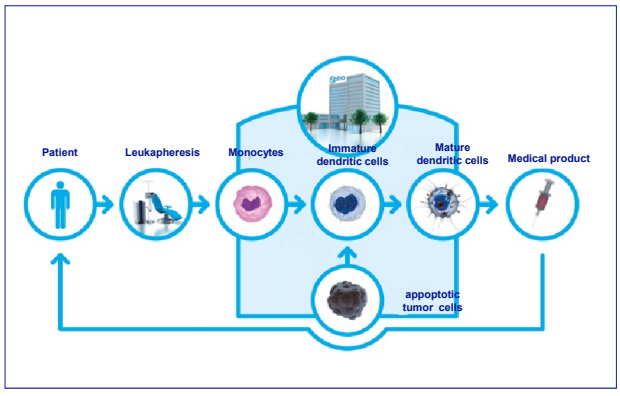
Materials for DCVAC/PCa

Sarka Vosahlikova

SOTIO a.s.

Scheme of manufacture process

- biotechnology company developing a next generation Active Cellular Immunotherapy based on activated dendritic cells
- Prostate Cancer DCVAC/Pca ; Phase III – VIABLE
- Ovarian Cancer DCVAC/OvCa; Phase II - request



starting materials
raw materials

excipients
materials for quality testing

Legislative requirements; regulatory autorities:

Legislative Regulatory autorities	Document
CZ - **SUKL**	**VYR – 26 version 2:** - Guidelines for good manufacturing practice for active pharmaceutical ingredients - Act No 378/2007 Coll.
	VYR – 32 version 3: - Guidelines for Good Manufacturing Practice - Directive 2001/83/ES
Europe - **EMA**	ICH Topic Q 7, Good Manufacturing Practice for Active Pharmaceutical Ingredients
US - **FDA**	Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients
Other	EMA/410/01 rev.3 - guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products

Our materials:

- **A good manufacturing practice** (**GMP**): quality assurance system used in pharmaceutical industry
  - covers both manufacturing and testing of final product
  - requires traceability of raw materials and production follows SOPs
  - Definitions of materials in particular legislatives and guidelines

	CZ Directive	EMA	FDA
API	- API starting materials	- Active Pharmaceutical Ingredient (API) - API Starting Material	- API **starting material** is a **raw material**, intermediate or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API
Raw material	- substances used in the production or extraction of the active ingredient - reagents, culture media, fetal calf serum, additives, and buffers	- **starting materials**, reagents, solvents intended for use in the production of intermediates or APIs	- ingredient intended for use in the production of APIs - include **starting materials**, process aids, solvents, and reagents
Starting material	- starting materials shall mean any substance of biological origin	- API?	- See "API Starting Material"
Excipient	- present in final product	- present in final product	- fillers, diluents, solvents, emulsifiers, preservatives, flavors, absorption enhancers,

DCVAC/PCa: starting materials

Leukapheresis

- Regulation No. 143/2008 Coll
- Cell source, mobilization protocol
- quality control by Flow cytometry

Apoptotic tumor cells

- Ph.Eu 5.2.3
- Cell source, DNA profile, testing frequency
- quality control by Flow cytometry

Master Cell Bank
↓
Stock Cell Bank
↓
Working Cell Bank
↓
Limited passage
↓
Apoptotic tumor cells

- proper requirements for starting materials
- SOPs

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Raw materials: comparison of research and clinical; ideal state

- cell therapy products/ processes often based on academic research environment

Pre-clinical

- *sufficient „In vitro*-use-only“
- Undefined, uncontrolled
- Sera, donor plasma, gelatin
- Xenogeneic
- Home-brewed

Clinical

- Infusible-grade, clinical-grade
- Defined, controlled
- Albumin, culture supplements
- Recombinant or human-derived
- Validated, GMP

Solution:

- research use only grade replace GMP
- find alternative vendor
- qualificatory In-house testing (sterility, activity)

↘

Limitations:

- availability
- cost of raw material
- vendors

DCVAC/PCa: raw materials

- **1 GMP**
- **10 complience with GMP**
- **1 Research Use Only**

- Priority: suitable for clinical use
- Certificate of analysis (CoA)
- Raw materials with components of bovine origin
  - viral safety declaration
  - TSE/BSE Statement (priority: tests for viruses according to EMEA / FDA)
- Raw materials with components of animal origin
  - Certificate of Origin

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DCVAC/PCa: raw materials - qualification

Each delivery	Each batch	Specific tests (in progress)
Identification	Sterility	Verification of the data listed in CoA
Exspiration	Endotoxin	
Control of package	Viral specific tests	
Certificate of Analysis		
Specification		

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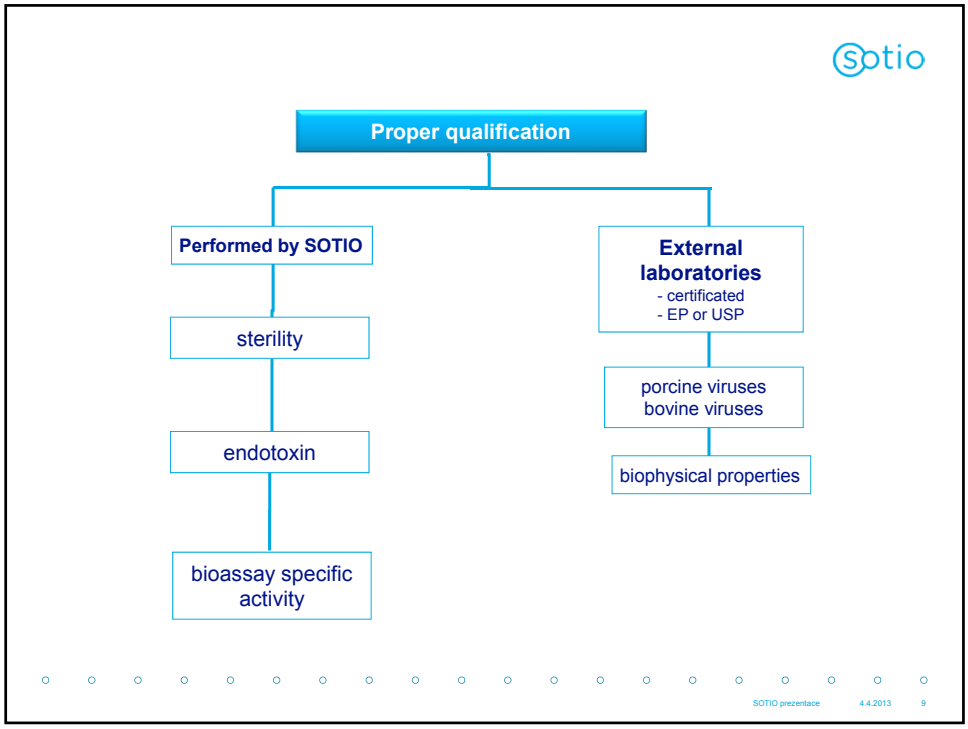

Raw material troubles with „research use only“

System of proper testing

- verify of the identity
- conformity with written specification
- safety and quality of the reagents (qualification program)
- determine „research use only“ components in material
- reliability of the supplier's analyses
- Drug Master File
- audits
- finding of alternative vendor

Example:

- Mature agent: critical material, maturation reagent, single vendor
  - quality agreement (in progress)
  - for every batch: CoA (sterility, endotoxin, activity)
  - BSE/TSE declaration
  - QC in Sotio
  - assessment of residues in DCVAC/PCa

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Material used for quality control of raw materials and DCVAC/PCa

- Material for sterility testing
- Material for endotoxin testing
- Material for bioassay

- Material for flow cytometry
- Material for microscopic evaluation of cell suspension
- Material for assessment of tumor antigens expression
- Material for potency assay
- Material for Mycoplasma testing

What kind of materials are these?????

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Quality control materials

- no exact definitions of requiements for these materials
- no starting material, no raw material
- not include in manufacture process
- but may affect the quality control results of DCVAC/PCa and intermediates
- problem with quality assurance (CoA for batches)
  - posibility: IVD certification (antibodies)
  - not avaible for all these materials

- proper system of qualification
- program for quality control of these materials
- system of alternative vendor, qualification testing

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Quality control materials: example

- end of production CD14 antibody
- vendor provides alternative with other fluorophore
    - problem with results – incomparable result data
    - problem with validation data, affect to other parameters
    - separate measurement
    - no specification parameter, but….

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Summary

Problems:

- lack of clear definition of starting materials and raw materials
- differences between Europe and USA
- global study - local requirements particular autorities
- alternative critical material with different properties
- lack of definition of quality control materials and their requiements for their quality testing

• no clinical grade of raw material…

Solution:

• adjustment proper system of quality control according actual legislative
•adjustment qualification system for alternative critical material
• adjustment system quality control for other materials (undefined in legislative)
• assesment of risk analysis for each kind of materials
• find and test alternative vendors and suppliers
• try to find new possibilities in manufacturing process

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Thank you for your attention

Miltenyi Biotec

EDQM SYMPOSIUM
RAW MATERIALS FOR THE PRODUCTION OF CELL-BASED AND GENE THERAPY
Raw Materials for GMP-compliant manufacturing of cellular products

Overview – Raw Materials for the Manufacturing of ATMPs

- What is the need for Raw Materials in ATMP and Cell based Processes
- Regulatory background guiding the manufacturing and QC/QA Activities
- Examples for the manufacturing and documentation process
  Cytokines, Peptides and Media
- Interaction with the customers
- Interaction with the Health Authorities
- What would one like to see in terms of harmonisation of raw material quality attributes

Key Facts Miltenyi Biotec

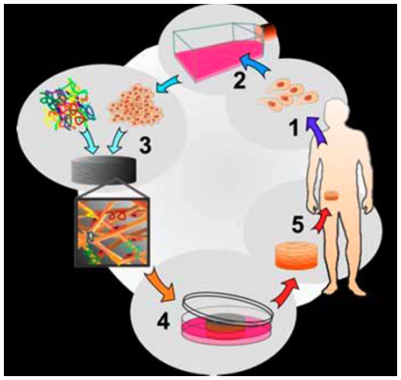
- Founded in 1989
- World market leader in Magnetic Cell Separation (MACS, CliniMACS)
- Germany ‘s largest independent, privately owned Biotech Company
- >1400 employees in 18 countries
- 825 employees in Germany
- > 250 employees in R&D

Product Lines of Miltenyi Biotec

- R&D Products and System Lines for Cells (separation, differentiation , cultivation and Analytics)
- Medical Devices – e.g. CliniMACS® Systems CE marked and/or US Type II Master Filed
- Pharma Products (Investigational drugs, Final Fill and Infusion Solutions)
- **MACS GMP Products – Ancillary Products and US Type II Master Filed = Raw Materials for ATMP and cell based manufacturing**

What is the purpose of an Ancillary - Product?

Faciliate cell manufacturing for research and therapeutic use



1. Remove cells
   Leucapheresis ,GentleMACS
2. Cell Separation
   CliniMACS Product Lines – Medical devices
3. Cultivate, differentiate, expand cells
   MACS GMP Products with Media, Cytokines, Peptide
   Cell Culture Bags
4. Seed onto an appropriate scaffold with suitable growth factors & cytokines
   Media, Cytokines, Peptides, Cell Culture Bags
5. Re-implant engineered tissue repair damaged site.

What are the customer needs of an Ancillary - Product?

Faciliate cell manufacturing for research and therapeutic use

1. Easy to use products which are avaiable today and in future
2. Quality
   - Animal and virus free
   - Lot to lot consistency
   - Testing to regulatory standard
3. Regulatory Support in writing and interacting with the regulatory bodies
4. Vendor approved by authorties
5. Prices must be competitive

Ancillary - MACS GMP - Product Portfolio of Miltenyi Biotec

Activation and expansion tools

- CD3 pure (Clone: OKT3)
- CD28 pure (Clone: 15E8)
- ExpAct Treg Beads

MACS GMP Media

- DendriMACS
- TexMACS

MACS GMP Cell Culture Bags

- Expansion Bags
- Differentiation Bags

MACS GMP Antigens

- **Recombinant Protein**: HCMV pp65
- **PepTivator (peptide pools):** HCMV pp65, AdV5 Hexon, WT1, NY-ESO1, EBV LMP2A, EBV EBNA-1 and EBV BZLF1
- **Lysate:** Aspergillus Lysate

MACS GMP Cytokines

- GM-CSF, IL-4, IL-1β, IL-6, TNF-α, IFN-γ*
- IL-2, IL-7, IL-15, IL-21
- FGF-2, EGF*
- Flt3-Ligand, SCF, TPO*

Miltenyi Biotec– facilitate GMP manufacturing - Certifications

Our Products are manufactured using highest quality standards:

- Produced in modern GMP certified, full QA System acc. ISO 9001 & ISO 13485
- EU GMP certificates for manufacturing ofmonoclonal antibodies (phase I/II), Infusion solutions and Aseptic Filling
- FDA Inspected for the CliniMACS CD34 System
- Free of animal-derived components

Regulatory background guiding manufacturing and QC/QA

The regulatory requirements for RAW materials in the manufacturing process of cells are partly covered:

- Cell, Gene, and Tissue-Engineered products
  - U.S. Pharmacopeia <1043> on Ancillary Materials
- Advanced Therapy Medicinal Products (ATMP)
  - Requirements of Article 5 of Regulation 1394/2007
  - Align with Directive 2009/120/EC
- ICH Q7 – Manufacturing of API
- CPMP/BWP/41450

Points to Consider on the Manufacture and Quality control of human somatic cell therapy medicinal products

- EU GMP Guidance – Annexes for
  - Aseptic products
  - Biologics

MACS® GMP Cytokines

deliver performance, consistency, and compliance
for cell based therapeutics

MACS GMP Cytokines – Portfolio

Current portfolio:

- Human FGF-2 (25 and 500 µg)
- Human Flt3 Ligand
- Human GM-CSF (25 and 250 µg)
- Human IL-1β
- Human IL-2
- Human IL-3
- Human IL-4 (25 and 250 µg)
- Human IL-6
- Human IL-7
- Human IL-15
- Human IL-21
- Human SCF
- Human TNF-α

Under development:

- Human EGF
- Human TPO
- Human IFN-γ1

MACS GMP Cytokines – Key features

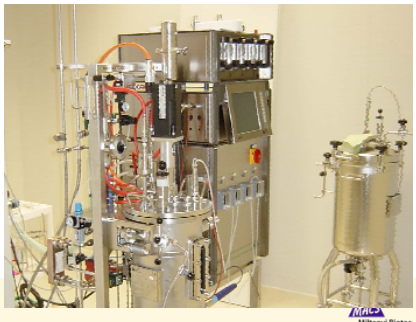
Recombinant cytokines for high reproducibility and safety

- Gene synthesis, MCB, WCB
- Produced in defined media in bacterial cells (*E. coli*)
- Lyophilized without carrier protein or preservatives
- Filled aseptically (Class A) and tested for sterility (Ph. Eur)
- Biological activity (IU/mg):
  - lot-specific
  - normalized with international standards
- Fully-validated gene sequences
- Identity confirmation – Mass spectrometry
- Tested for potential contaminants (endotoxin content, host DNA, host protein)

MACS GMP Cytokines – Key features

Recombinant cytokines for high reproducibility and safety

- Stability Studies
  The first three Lots are under Stability
- Validated analytical tests
- Vendor qualification program for the raw materials of "raw materials"
- Complete Batch and Manufacturing Records
- Design History File
- Change Controls



MACS GMP Cytokines – Biological activity

Determined for each lot after lyophilization

Human GM-CSF

The biological activity of Human GM-CSF is determined by proliferation assay using TF-1 cells.

Normalized with International standard 88/646 provided by the National Institute for Biological Standards and Control (NIBSC)

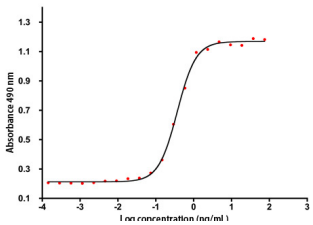
Human TNF-α

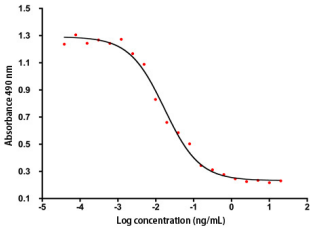
The biological activity of Human IL-4 is determined by growth inhibition assay using L-929 cells.

Normalized with reference standard of NIBSC 88/786

Lot-specific standardized biological activity allows for:

- ✓ Exact unit dosing
- ✓ Comparability





Lot-specific Certificate of Analysis

Use by date

Identity (IEF or MS)

Biological Activity (lot-specific IU/mg)

Endotoxin content and Sterility test acc. to Ph. Eur.

Highly accurate analytical tests to determine purity and potential contaminants

Certificate of Analysis

Product:	**MACS® GMP Recombinant Human GM-CSF**
Order No.:	**170-076-136**
Lot No. Finished Product:	**5130226177**
Use-by Date:	**27. Feb. 2015**
Storage Temperature:	**-20°C or below, avoid repeated freeze-thaw cycles**

Note: To manufacture the Finished Product, the final filled Lot No.: GMCSFF1O-1 was used.

Parameter	Specification	Results
Molecular mass (Mass spectrometry)	conforms to theoretical mass	conforms
Identity (Isoelectric focusing)	conforms to reference	conforms
Specific activity (Cell assay) [1]	≥ 5 x 10^6 IU/mg	1.43 x 10^7 IU/mg
Protein content (Bradford)	≥ 250 µg/vial	326 µg/vial
Purity (Chip electrophoresis)	≥ 97%	conforms
Endotoxin content (Ph. Eur.)	< 20 EU/vial	conforms
E. coli DNA content (qPCR)	< 3 ng/vial	conforms
E. coli host cell protein content (HCP ELISA)	< 1 µg/vial	conforms
Sterility (Ph. Eur.)	sterile	conforms

[1]The specific activity is determined by proliferation assay according to Kitamura *et al.* using TF-1 cells provided by the German Resource Centre for Biological Material (DSMZ). The proliferation assay was calibrated with the international standard for human GM-CSF (NIBSC code 88/646) provided by the National Institute for Biological Standards and Control.

Quality Statement on Certificate of Analysis

Quality statement comprises

Origin statement:

No animal- or human-derived materials were used for manufacture of these products

→ TSE statement is not necessary


Miltenyi Biotec

Certificate of Analysis

Quality Statement:
MACS GMP products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications.

MACS GMP products are manufactured and tested under a certified ISO 9001 quality system and in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials.

No animal- or human-derived materials were used for manufacture of these products.

MACS® GMP Media

deliver performance, consistency, and compliance for cell based therapeutics

MACS GMP Media – Key features

- For standardized and reproducible cell cultivation and *ex vivo* differentiation procedures
- Lot-to-lot consistency
- Serum-free
- Animal-component free
- Lot-specific Certificate of Analysis
- Traceability and qualification of raw materials
- Composition of the media are defined

TexMACS™ and DendriMACS GMP Media

- Developed for the *ex vivo* cultivation and expansion of human T cells and dendritic cells

- w/o phenol red
- 0.1µm filtered
- 2000mL bags

- Customized fillings
  - Bottles
  - Bags (0.5L, 10L, 20L, 100L)
  - Other sizes on request

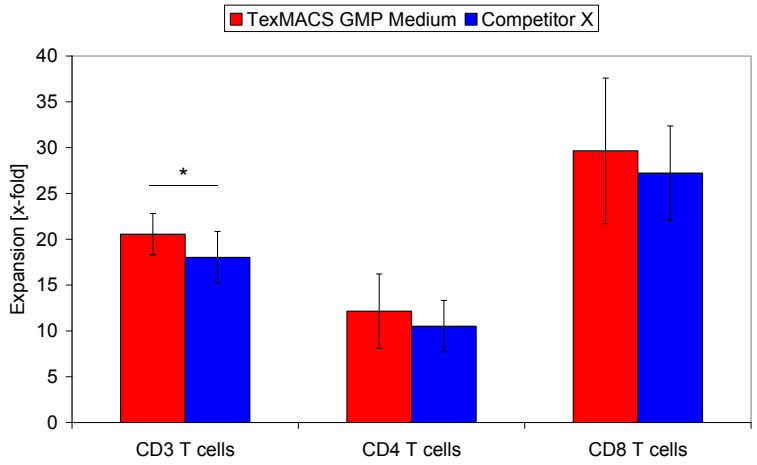
GMP Media - Specifications

- **Sterility:**
  - Sterile (Ph.Eur. / tested on one bag)
- **Endotoxin:**
  - ≤ 0,5 EU/mL (Ph.Eur.)
- **Osmolality:**
  - 280-340 mOsmol kg^{-1}
- **pH:**
  - 6.9-7.3
- **Functionality assay:**
  - *Ex vivo* expansion of primary human T cells
  - Differentiation of CD14+ cells into dendritic cells
- **Storage:**
  - Protected from light at 2-8° C

TexMACS™ GMP Medium – Polyclonal expansion assay with CD2/CD3/CD28 MACSiBead stimulation

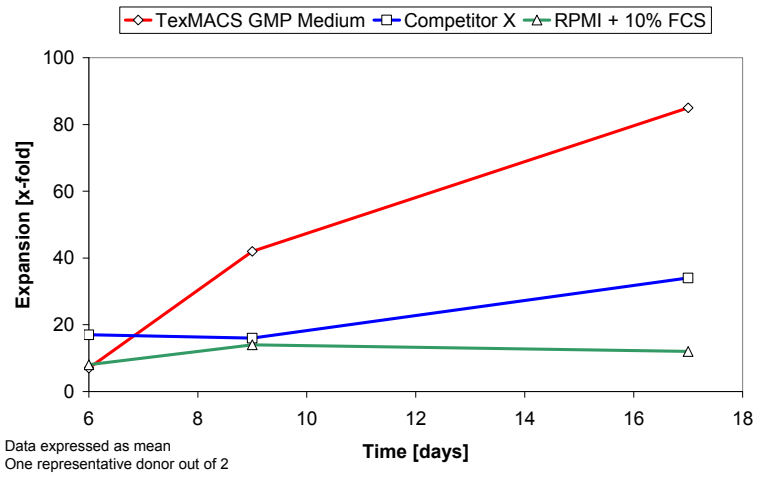
- **Enrichment of Pan T cells ($CD4^+CD8^+$ T cells) from** PBMC
- **Activation of the T cells by CD2/CD3/CD28 MACSiBeads**
- **Cultivation for 14 days with following conditions:**
  - cultured in different media
  - T cell to MACSiBead ratio of 2:1
  - T cell concentration: $2.5x10^6$ cells/mL
  - 200IU/mL Interleukin-2
  - 3 donors in triplicates
- **Analysis of expansion rate via MACSQuant Analyzer cell count**

TexMACS™ GMP Medium – Superior expansion rates of T cells



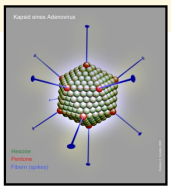
* p<0.05, 2-sided paired t-test

TexMACS™ GMP Medium – Superior expansion rates of T cells in Wilson Wolf chamber

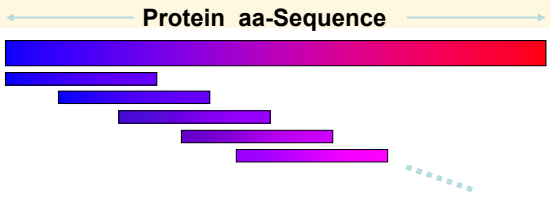


Data expressed as mean
One representative donor out of 2

MACS GMP Peptides - Background



http://de.wikipedia.org/wiki/Adenoviridae

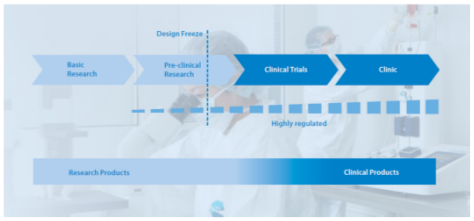


PepTivator:

- Set of overlapping peptides, covering a specific protein sequence
- Format: 15mer peptides with 11 aa overlap
- A PepTivator consists of 50 -250 peptides (antigen-dependent)
- Fully synthetic manufacturing
- Contains individually synthesized and purified peptides

PepTivator – Product formats

Premium grade
Research grade



GMP grade

Antigens:

- CMV, ADV, EBV (misc.)
- Asperg. Fumigatus (misc), Candida albicans
- BKV, JCV, HCV (misc.)
- Tumor antigens (MART, MAGE, Survivin, …)

Antigens:

- Cytomegalovirus (pp65)
- Adenovirus (hexon)
- Epstein-Barr Virus (EBNA1, BZLF1, LMP2a)
- Tumor antigens (WT-1, NY-Eso1)

Peptide - Manufacturing

- Qualified vendors and specified raw materials
- Free of animal-derived components
- Lot-specific documentation of the manufacturing process
- Complete traceability of all materials used
- Stringent QC tests confirm consistent high product quality,
- Comprehensive peptide analysis and identification
- Average peptide purity is > 90 % for 15-mer.

PepTivator - Manufacturing

- Automated aseptic filling, lyophilization, and finishing in a "class A" cleanroom
- Documentation and QC
  - extensive documentation and Lot-spec. release
  - sterility & endotoxin testing according to Ph. Eur
  - low content of residual contaminants (water, org. solvent)

How do we work with our customers

Extensive documentation and service

- Package Insert &Lot-specific certificates of analysis (CoA)
- Regulatory support files
  - USA Type II Master file – Reference Letters to customers
  - EU: Regulatory support file for the authorities

    But: How many authorities are in the EU?
  - Audits – we are a vendor for Raw Materials
  - Long-term perspectives and reliable supply

Interaction with the Health Authorities

- USA Type II Master file – Reference Letters to customers
- EU: Regulatory support file for the authorities
- Upon initiation of clinical studies we and the sponsor discuss and plan the regulatory path for the raw materials
- In general, we do not take part in discussions of sponsor and health authorities
- There is a different approach for industrial or clinical customers

The challenges of producing raw materials for use in this therapy area

- There is not any EU Master File system in place !!!!
  - This is the biggest drawback
  - If one has to interact with 27 country plus local county authorities – this results into a lot of opinion and complicated follow ups upon changes
- No guidelines define the standards for development and production
  - Therefore applicable guidelines for the production for API are used for the production of cytokines, proteins and antibodies
- Determination of the Biological activities of cytokines, when no standard is available
- Often research products are used/allowed in Phase I and II clinical trials
- Complex peptides (e.g. cytokines of the TGF-β1 family) can't be produced in bacterial cells and need to be expressed in mammalian cells.
  - Challenge: viral testing and viral validation is required
- Can Raw Materials of ATMP developed and manufactured economically?

What would one like to see in terms of harmonisation of raw material quality ?

1 What is the applicable standard(s) for development and Production?

Is the e.g. ICH Q7 EU GMP applicable?
Raw materials are not the APIs !
And what will happen to other pharmaceuticals, if one defines standards for ATMP raw materials ?

2 Environments for production (e.g. are clean rooms necessary?)

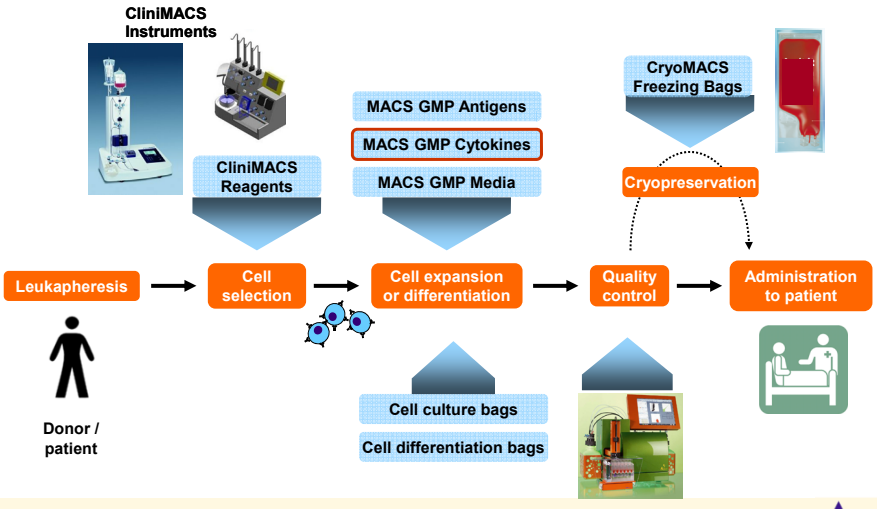
3 Extent of Process validation is a critical point, examples:

Virus removal validation - Allow Generic approaches
Sterile Filtration validation
Consistency
Stability data .-for every products or for product groups?*

4 Preclinical and Toxicological data request

we observe, that many authorities request preclinical safety data on raw materials

Enabling cellular therapy in a regulatory environment



Thank you for attention

Dr. Bernd Leistler
Director Development & Production
CellGenix GmbH

CellGenix

Your Partner for Clinical Cell Therapy

EDQM Symposium on Raw Materials for the Production of Cell-Based and Gene Therapy Products

Strasbourg, 03.04.2013

Cytokines & Growth Factors for Ex Vivo Cell Culture

CellGenix GmbH
Corporate Profile

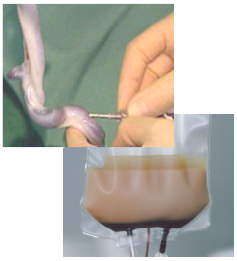
Development, GMP Production & Marketing of High-quality reagents and services for cell therapy enablement

- Founded 1994 in Freiburg, D
- Non-public company, 50 employees
- GMP facility and offices in D, USA, F
- CellGenix™ / CellGro® products distributed worldwide

CellGenix

Products and Services (1995 – 2012)

Cellular and Molecular Therapeutics



GMP Processing of Cell Therapy Products

- 1995: 1st European GMP manufacturing authorization
- 8 GMP manufacturing licenses for cellular products (HSC, cord blood stem cells, DC, chondrocytes, etc.)
- 2009: Marketing authorization for cord blood from PEI
- > 10,000 cell products processed

⇒ > 15 years in-house GMP experience

GMP Manufacturing of Recombinant Protein Pharmaceuticals

- GMP manufacturing licenses for 2 recombinant proteins since 2001
- Recombinant idiotype vaccine for lymphoma patients
- GMP contract manufacturing service for Phase I/II tumor vaccine
- > 140 personalized vaccines produced for > 700 vaccinations

⇒ > 10 years in-house GMP experience

⇒ **Know-how used to establish GMP cytokine manufacturing**

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CellGenix

Products and Services

CELLGRO

>10 years in-house manufactured recombinant GMP products

CellGro® Cytokines

- Hematopoietic stem and progenitor cells
- Dendritic cells
- T and NK cells
- Mesenchymal stromal cells
- ESC, iPSC

CellGro® Serum-free Media

- CellGro SCGM Stem Cell Growth Medium
- CellGro DC Medium
- CellGro MSC Medium
- CellGro ACTive Medium

CellGro® Kit-System, VueLife® and KryoSure® Bags

- Cell culture and freezing vessels for use in cell therapy

CellGro® is a registered trademark of CellGenix in Europe and in several countries worldwide

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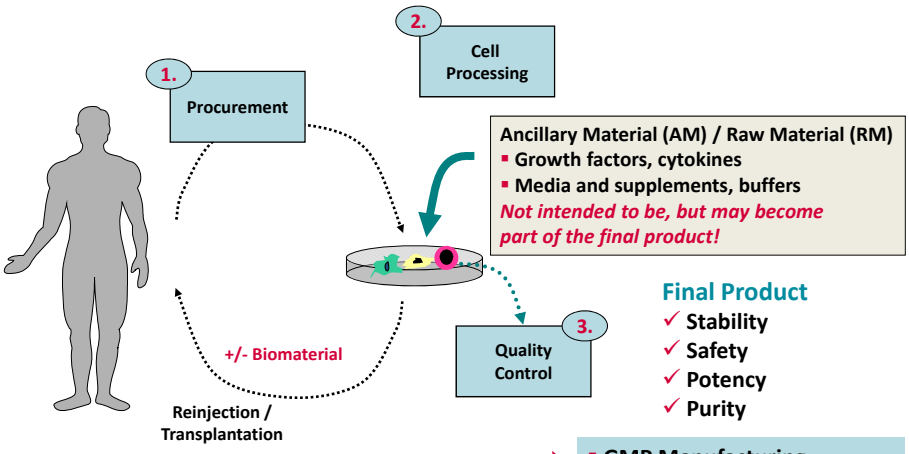
CellGenix

Cell, Gene and Tissue-Engineered Products
Manufacturing Challenges and Quality Aspects



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CellGenix

Cell, Gene and Tissue Engineered Products
Ancillary Materials / Raw Materials

Europe

- **No specific regulations exist for Ancillary Materials**
  - Annex 2 EU GMP Guidelines: *"Special consideration should be given to quality of the starting materials, … , culture media, cytokines, growth factors … used in manufacture"*
  - EU Tissue & Cells Directives:
    parent Directive (2004/23/EC) and two technical directives (2006/17/EC and 2006/86/EC)
  - European Pharmacopeia has no monographs or reference standards
- **AM are handled like raw material**
  - Vendor qualification
  - Raw material qualification & control

USA

- **USP Chapters on Ancillary Materials for Biological Products**
  - <1043> „AM for Cell, Gene and Tissue-Engineered Products"
  - <92> „Growth Factors & Cytokines Used in Cell Therapy Manufacturing"
    (1st issue and reference standard provided by CellGenix)

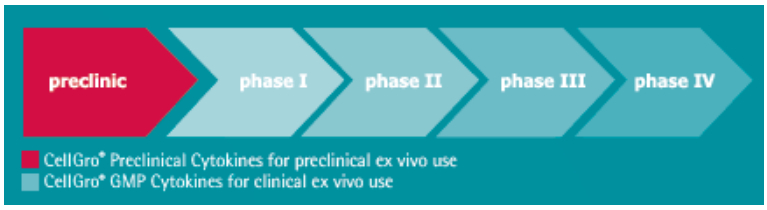
6

CellGenix

CellGro® Cytokines

Quality Policies and GMP Production

CellGro® Preclinical Cytokines



CellGro® GMP Cytokines

Preclinical Grade: Major product characteristics of the GMP product (expression system, production steps)

GMP Grade: manufactured, tested and released in compliance with the relevant GMP-guidelines

Highest Quality for all clinical phases

ADCF ⇒ Safety

GMP ⇒ Purity, Potency, Consistency, Stability

7

CellGenix

CellGro® Products

Major Quality Attributes

- **ADCF policy and Serum-free policy**
  ⇒Safety, Purity
- **GMP production**
  ⇒Safety , Purity, Potency, Consistency
- **Regulatory compliance & support**
  ⇒Qualified Supplier

8

CellGenix

Major Quality Attributes

Animal-Derived-Component-Free (ADCF) Policy (1)

- **Level 1 (cytokine product):**
No ADC are part of any cytokine product.

- **Level 2 (cytokine production process):**
For products designated "ADCF" no ADC used throughout production process. Including all material in contact with product during production.

- **Level 3 (raw materials for production):**
Whenever available, only raw material certified to be free of ADC used in manufacture of GMP products.

Major Quality Attributes

Animal-Derived-Component-Free (ADCF) Policy (2)

- Animal- or human-derived material is only accepted if it is safe, compliant with TSE guidelines (EMEA/410/01), and represent no health hazard. Typically, it is pharmaceutical grade.

- All material used in production is formally approved by QM. Its origin and impurity profile is assessed (raw material control), it is procured from reliable manufacturers and suppliers.

Major Quality Attributes

Manufacturing and QC in compliance with GMP

QA system established for CellGro® GMP Products

- Comprehensive, ISO 9001:2008 certified QA system (incl. change control, OOS procedure, etc.)
- Manufacturing and QC according to SOPs
- Qualified and trained personnel
- GMP clean room facility and qualified equipment
- Starting material and vendor qualification (ADCF & SF Policy)
- Validated processes (manufacturing, QC, cleaning)
- Monitoring of product quality by in-process controls
- Product release by QA/QC according to specifications

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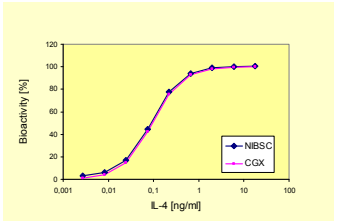
Major Quality Attributes

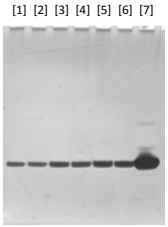
Manufacturing in compliance with GMP

GMP Grade: manufactured, tested and released in compliance with the relevant GMP-guidelines, including:

- Characterized MCB/WCB and plasmid banks
- Validation of analytical methods
- Validation of manufacturing process
  - Reproducibility, consistency (3 consistency batches)
  - Depletion of critical impurities (DNA, Endotoxin, HCP)
- Stability studies (stress, accelerated, real-time)
- QC release specifications

⇒ **>10 years production of recombinant protein pharmaceuticals under GMP license**





Sample: IL-4, #1403HK14 non-reduced

[1] 1% reference (80 ng)
[2] 1% reference (80 ng)
[3] 2% reference (160 ng)
[4] 2% reference (160 ng)
[5] 3% reference (240 ng)
[6] 3% reference (240 ng)
[7] sample (8000 ng)

12

Major Quality Attributes
Regulatory Compliance & Support

CELLGRO

CoA and release specifications

- Identity confirmed (2 methods)
- Purity (2 methods)
- Product variance
  (e.g. oligomers, oxidation products)
- Specific, standardized activity
- Endotoxin content (Ph. Eur.)
- Host-cell DNA
- Protein content
- Sterility (Ph. Eur.)

CELLGRO CellGenix

Certificate of Analysis

Product:	GMP Recombinant Human Interleukin-4 (rh IL-4) USP grade
Intended use:	For clinical ex vivo use
Order Number:	1003-050 Lot: 10033D12
Production:	07/2011 Expiry: 07/2013
Source:	*E. coli*
Formulation:	Lyophilized from a 0.2 µm -filtered solution in PBS
Mass per vial:	50 µg ± 15%

Release Testing:	Specification	Lot Result
Specific Activity:	$5 \times 10^6 - 2 \times 10^7$ USP units/mg	1.0×10^7 USP units/mg[1]
Purity:	≥ 97%	> 98 %
Identity	complies	complies
Sterility:	sterile	complies
Endotoxin level:	< 50 EU/mg	< 25 EU/mg
Host-cell DNA	≤ 1 ng/mg	complies

[1] USP units are equivalent to International Units per mg (IU/mg)

Activity was determined using a TF-1 cell proliferation assay
Purity was determined by SDS PAGE and silver stain
Identity was confirmed by Western Blot, end-of-production DNA and N-terminal protein sequencing
Sterility test of vialed product was performed according to Eur Pharm. (inoculation method)
Endotoxin was determined using the gel clot assay according to Eur Pharm.
Host-cell DNA was determined using the Threshold® assay

Handling Instructions:

Reconstitution: Recommended in 200 µl of sterile H_2O to a final concentration of 250 µg/ml.
Dilution: Recommended in CellGro® / CellGenix™ serum-free media. For dilution with PBS or protein free medium, a carrier protein (0.1-1% albumin or 1-10 % appropriate serum) has to be included. Failure to dilute product according to these instructions will result in loss of activity.
Storage and Stability: Store material at -20°C or below. Store diluted material at -20°C or below. Avoid multiple freeze/thaw cycles.

Quality Statement:

This product is manufactured, tested and released in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. This product complies with USP chapter <92> "growth factors and cytokines used in cell therapy manufacturing". USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered products" has been considered in the design of this product.

Date Quality Manager

Manufacturer

CellGenix GmbH
Am Flughafen 16 | 79108 Freiburg | Germany
Tel.: +49 761 88889-0 | Fax: +49 761 88889-830
www.cellgenix.com | info@cellgenix.com

CellGro® is a registered trade mark of CellGenix GmbH in several European countries and Japan.
In the U.S.A. products are distributed under the name of CellGenix™.

CGX-DOCS-100e

CellGenix

Major Quality Attributes
Regulatory Compliance & Support

CELLGRO

Documents & Support for GMP Cytokines

- Drug Master Files: cross-references (>10 DMFs submitted)
- Product information
  - Data Sheets (visit *www.cellgenix.com*)
  - *Production sheets (please inquire)*
  - *MSDS*
- USP <1043> and <92> compliance (further chapters in progress)
- Technical and regulatory support (on demand)
- Customer audits welcome
- Pro-active information about changes
- Regulatory office and customer service
  Please do not hesitate to call us!

CellGenix

CellGro® Cytokines and Serum-free Media

Current Product Portfolio

Hematopoietic stem cells (HSC)
- TPO, SCF, Flt-3L, IL3, IL6
- SCGM Medium

Chondrocytes
- FGF2, PDGF-BB
- ACTive Medium, Gelatin

Dendritic cells (DC)
- IL1ß, IL4, IL6, IL10, GM-CSF, TNFα
- DC Medium

Mesenchymal stromal cells (MSC)
- FGF2, EGF, PDGF-BB
- MSC Medium, Gelatin

T cells
- IL2, IL7, IL10, IL15, IL21
- DC Medium

ESC, iPS cells
- EGF, FGF2
- Media for expansion and differentiation in development

NK cells
- IL2, IL7, IL15
- SCGM Medium

… more in Pipeline

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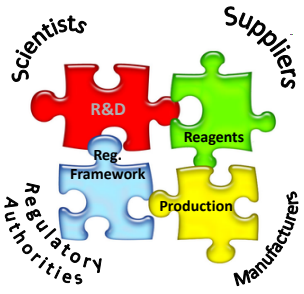
CellGenix

Cell, Gene and Tissue Engineered Products

Chances & Challenges



- **Harmonization of requirements for AM quality:** - purity/impurities, stability studies …
- **Industry-wide definition of ADCF/"Animal-free"**
- **Residual level assessment and removal of AM: AM may become part of the final product!**
- **Guidelines and monographs for cells as AM in the future**
- **"DMF" submission also in EU**
- **Certification of AM-manufacturing process possible for vendor?**

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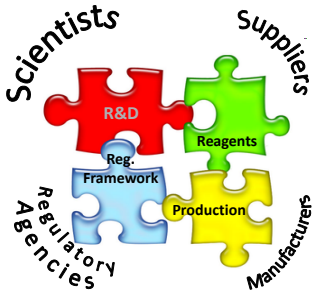
CellGenix

CellGenix GmbH

Competent Partner for Cell Therapy Enablement



Our Contribution as Supplier of RM

- **Highest product quality: safety, purity, potency, stability and lot-to-lot consistency**
- **Complete sets of reagents for relevant cell populations**
- **Two quality grades for seamless transition from preclinical to clinical phase**
- **Performance demonstrated in clinical trials world-wide by leading experts**
- **Supply availability**
- **Regulatory support**
- **Reciprocal customer relationship: continuous, research-driven product development**

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CellGenix

Thank You For Your Attention

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CellGenix

Developing ATMPs and other cellular therapies in Europe

UCL

Need for qualified reagents

Dr Mark Lowdell
Director of Cellular Therapy
Royal Free Hampstead NHS Trust
&
UCL Medical School

Issues to address

UCL

- What raw materials you use in the production of therapy products.
- The challenges of sourcing raw materials that meet regulatory requirements
- What quality attributes you look for in the raw materials used
- How you work with raw material manufacturers
- What you would like to see in terms of harmonisation of raw material quality attributes
- What substances would be most important to be covered by the text and what quality attributes apply to them

Cell therapy production issues

- Starting material has very high intra-donor variability
- Pre-clinical models rarely informative or even dangerously uninformative
- Validation materials ethically difficult to obtain (no paid donors in UK)
- Quantitation and qualification of final product difficult or impossible
- Dose calculation difficult – e.g. equipment for cell counting cannot be validated to GMP
- Sterility testing difficult to EuPh due to tiny batch sizes – blood culture systems are the “industry standard”
- Release criteria difficult to define

ATMP Cell therapy trials at RFH

- CMV-specific immune regeneration post HSCT - PhI/II and PhIII (three different products)
- AdV-specific immune regeneration post HSCT - PhI/II
- DC-Vax in glioma – PhI
- HuESC retinal epithelia in Stargardt’s Macular Dystrophy
- Allogeneic MSC infusions for severe GvHD – PhI/II
- Allogeneic NK cell therapy for AML – PhI/II
- Autologous stem cell seeded cadaveric tracheal transplants – F-I-M
- Autologous stem cell-derived cell seeded biocompatible tissue structure for tracheal transplants – F-I-M
- Autologous stem cell-derived cell seeded biocompatible tissue structure for nasal reconstruction – F-I-M
- Autologous stem cell-derived cell seeded cadaveric larynx transplants – Ph I/II

Reagents used in GMP ATMP manufacture

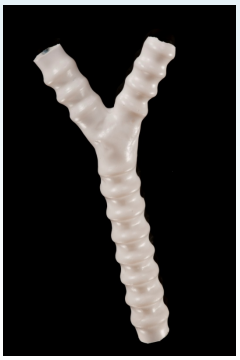
Non-compendium starting materials:

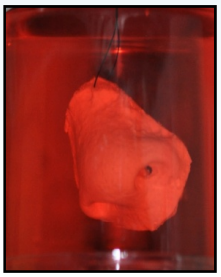
1. Culture media –
    1. Specialised media difficult/impossible to get precise COA
    2. Traceability of reagents
    3. Reluctance to supply for GMP manufacture – perceived litigation risk
2. Monoclonal antibodies –
    1. Mostly only available as “for research use only”
    2. Unknown maximum amount permitted as residual component
    3. Reluctance to supply for GMP manufacture – perceived litigation risk
3. Trypsin –
    1. Non-animal derived available but less active
    2. TrypLE excellent but not GMP compliant
4. Cytokines –
    1. Mostly only available as “for research use only”
    2. Unknown maximum amount permitted as residual component
    3. Reluctance to supply for GMP manufacture – perceived litigation risk

How do we qualify non-com materials?

1. Critical supplier questionnaire –
    1. ISO standard?
    2. Traceability of components
    3. Control of suppliers
    4. Assessment of contamination / cross-contamination risk
    5. Exposure to animal-derived products
2. GMP compliant option –
    1. What does manufacturer mean by “GMP compliant”?
3. History of use in other ATMPs –
    1. Network of academic ATMP manufacturers sharing F-I-M data
    2. Published data
4. Pre-clinical experiments
    1. In vitro and animal studies
    2. PD / PE / PV data

3-D Tissue-Engineered ATMPs at UCL





Decellularised – recellularised "autologous" trachea as a "Special"

Risk assessment: "Introduction of a New Product" SOP

1. Donor IDM & traceability
2. Decellularisation procedure
    1. Removal of donor cells?
    2. Residual biomechanical properties?
    3. Contamination risks?
    4. TSE?
    5. Analar grade vs pharmacological vs research grade reagents
3. Recellularisation procedure
    1. BM and tracheal procurement - **SLA**
    2. MSC characterisation & expansion
    3. Epithelial cell characterisation & expansion
    4. Contamination risks – **microbial testing**
    5. Transport and packaging – **DIFFICULT**
    6. Reagent quality and traceability
4. Staff & training
5. Space
6. Equipment
7. Cross contamination

Pre-production risk assessment

Non-compendium starting materials Risk assessment:

1. Allogeneic Donor Trachea
    1. IDM & life style history from N-O-K
    2. Procurement via NHSBT to HTA standards
        1. Labelling
        2. Traceability
        3. TSE risk assessment
        4. Removal of donor cells?

2. Autologous bone marrow and tracheal biopsies
    1. IDM
    2. Aseptic procurement and micro testing
    3. Labelling

3. Mesenchymal stromal cell manufacture reagents

4. Tracheal epithelial cell manufacture reagents

MSC manufacture

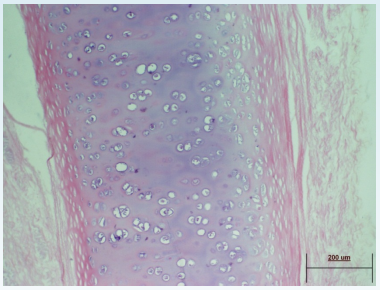
Reagent	Supplier		GMP compliant	Compendium
Antibiotic/antimycotic	Life technologies		N	N
Ascorbic acid	Sigma Aldrich		N	N
αMEM	Life technologies		N	N
Dexamethasone	Hospital pharmacy		Y	Y
Fetal bovine serum	Lonza		EDQM	N
Glutamax/Dipeptiven	Fresenius Kabi		EuPH	Y
HBSS	Life technologies		N	N
HCL	Hospital pharmacy		Y	N
High glucose DMEM	Sigma Aldrich		Y	N
rHuman insulin	Novo Nordisk		Y	Y
rHuman transferrin	Sigma Aldrich		N	N
Sodium pyruvate	Sigma Aldrich		Y	N
TryplE	Life technologies		N	N

MSC manufacture

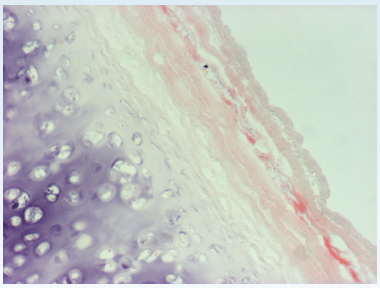
Reagent	Supplier	GMP compliant	Compendium
Linoleic acid	Sigma Aldrich	N	N
Lymphoprep	Axis shield	Y	N
Monaparin	Hospital pharmacy	Y	Y
PBS	Sigma Aldrich	Y	N
Perfusion Solution	Lonza Biowhittaker	Y	N
Protein Solubilizer X-100	A.G. Scientific, Inc.	N	N
Pulmozyme 1000 U/ml	Roche UK	Y	Y
rhTGFβ3	R&D	N	N
RNase I (cloned)	Applied Biosystems	N	N
RPMI	Life Technologies	N	N
Saline	Baxter	Y	Y
Selenous acid	Sigma Aldrich	N	N
Sodium deoxycholate	Sigma Aldrich Aldrich	N	N

Pre-clinical validation of decellularisation

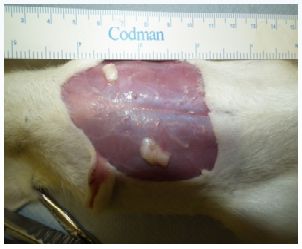
Ansari method – human trachea

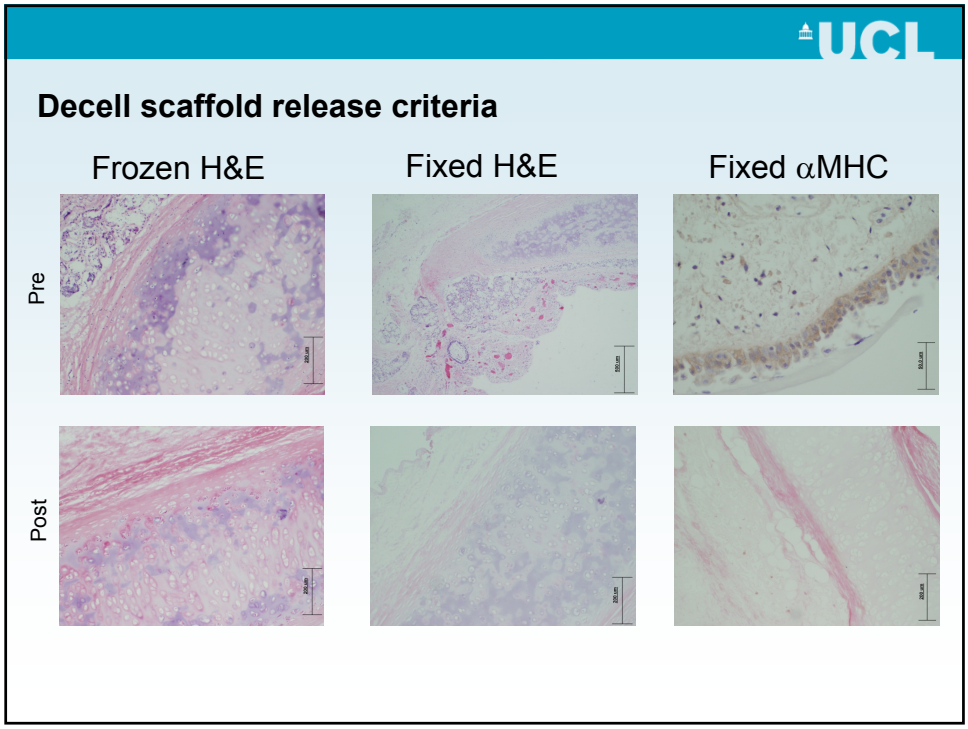


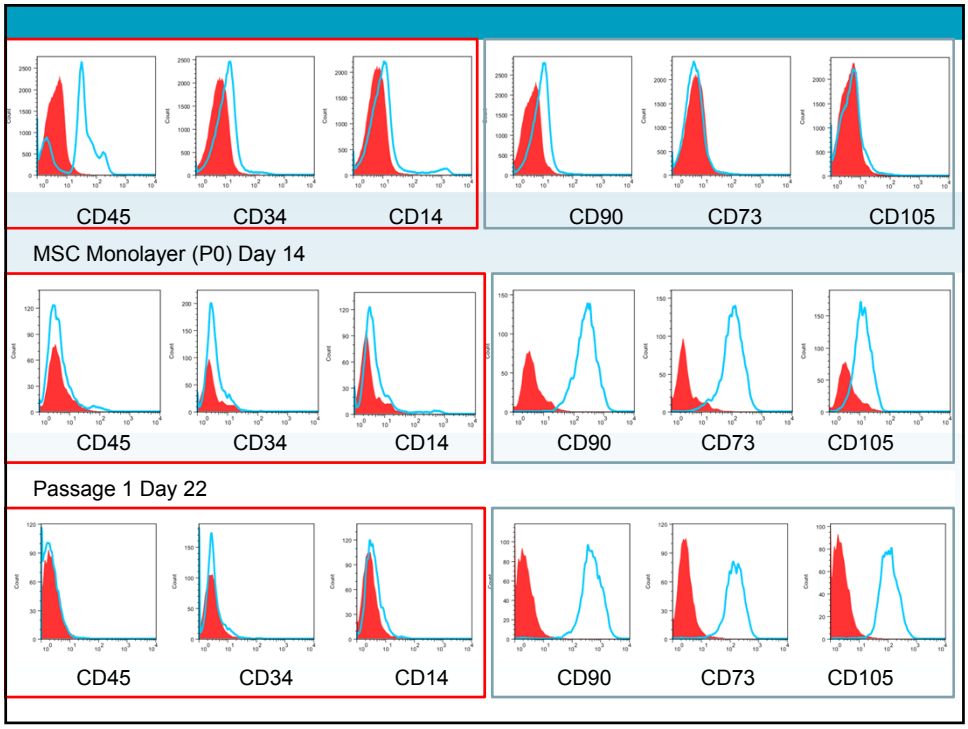
Conconi method – Cairan trachea



Exposed implanted scaffolds at termination




Decell scaffold release criteria
Frozen H&E
Fixed H&E
Fixed αMHC
Pre
Post



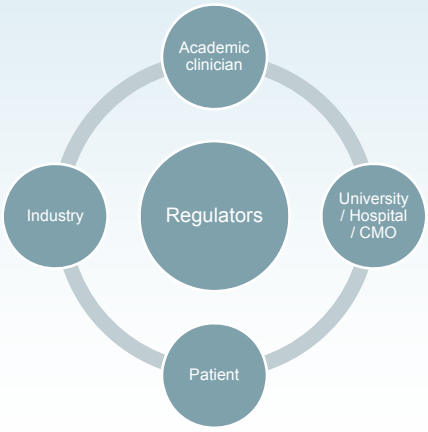
Urgently needed EDQM reagents

UCL

- Recombinant cytokines
- Monoclonal antibodies for “in process” cell selection
- Trypsin
- RNAse
- Specialised media for
  - MSC
  - Epithelia
  - Chondrocytes

The ATMP stakeholders

The virtuous circle



All are interdependent:

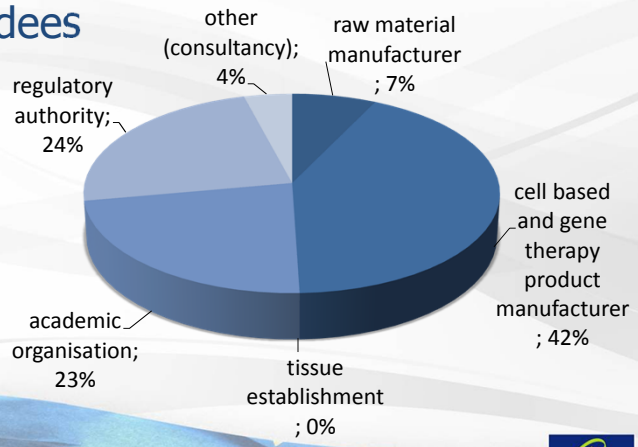
1. Clinician wants to innovate treatments and NEEDS patients
2. University NEEDS high impact publication and IP but not to fund phase III trials for MA
3. Industry NEEDS clinician to innovate and University to support proof-of-principle
4. University NEEDS Industry to buy its IP and commercialise it after clinical trials
5. Regulators NEED to ensure patient safety and maximum availability of novel therapies and commercialisation
6. Patients need access to new, safe and effective treatments from Clinicians AND Industry

Survey results
&
Afternoon Session

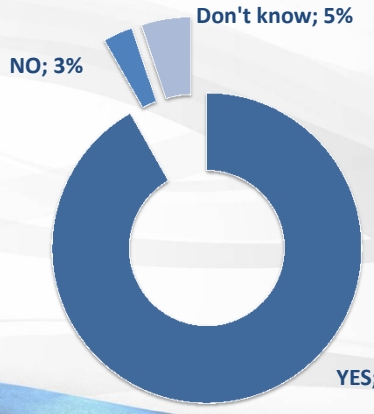
Dr Stephen Wicks, EDQM

2012 Survey Results

- Respondees



Do you agree that harmonisation of quality requirements for raw materials is needed, within Europe?



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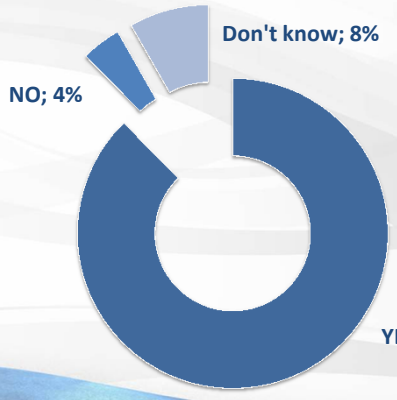
Would you find a Ph. Eur. text useful for the harmonisation of quality requirements for raw materials?



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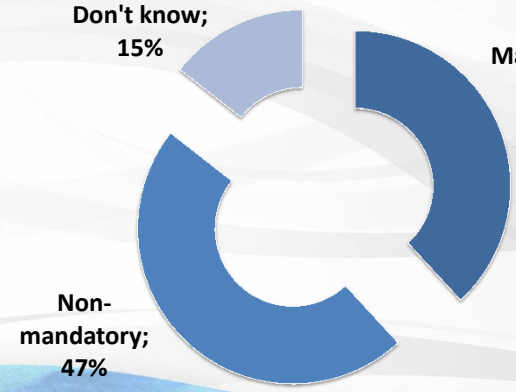
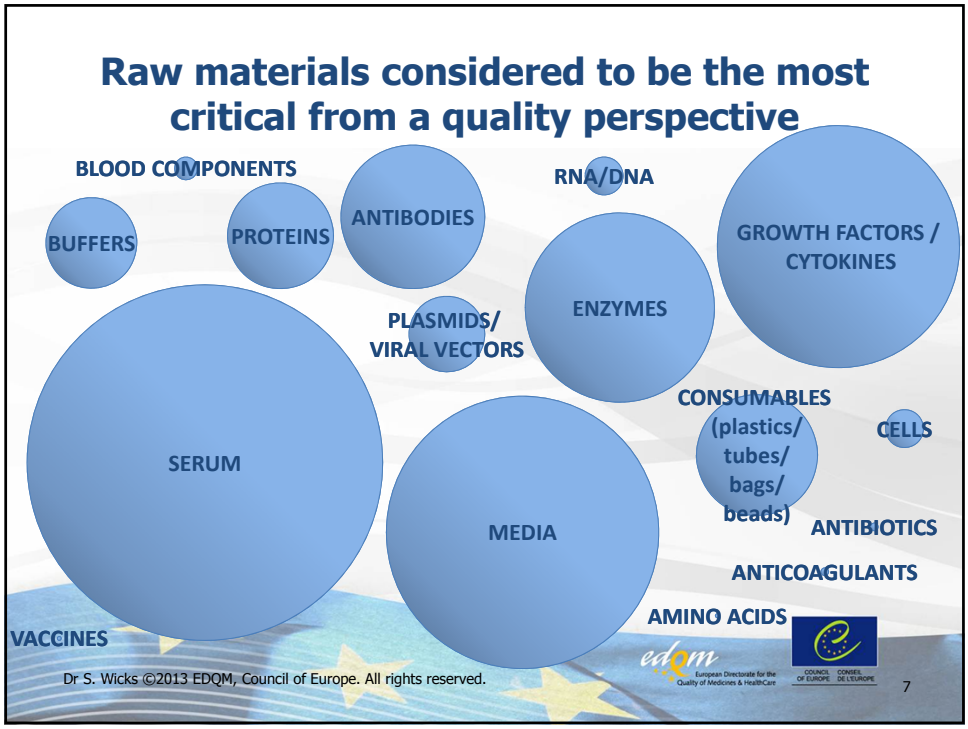
Do you think a Ph. Eur. text on the quality requirements of raw materials should be mandatory or non-mandatory



Raw Materials

- Raw materials in this context are defined as substances of biological origin used for manufacturing or extracting the active substance(s) but from which the active substance is not directly derived, such as antibodies, enzymes, growth factors, serum, etc.


Raw materials considered to be the most critical from a quality perspective
BLOOD COMPONENTS
BUFFERS
PROTEINS
ANTIBODIES
RNA/DNA
GROWTH FACTORS / CYTOKINES
ENZYMES
PLASMIDS/ VIRAL VECTORS
CONSUMABLES (plastics/ tubes/ bags/ beads)
CELLS
SERUM
MEDIA
ANTIBIOTICS
ANTICOAGULANTS
AMINO ACIDS
VACCINES
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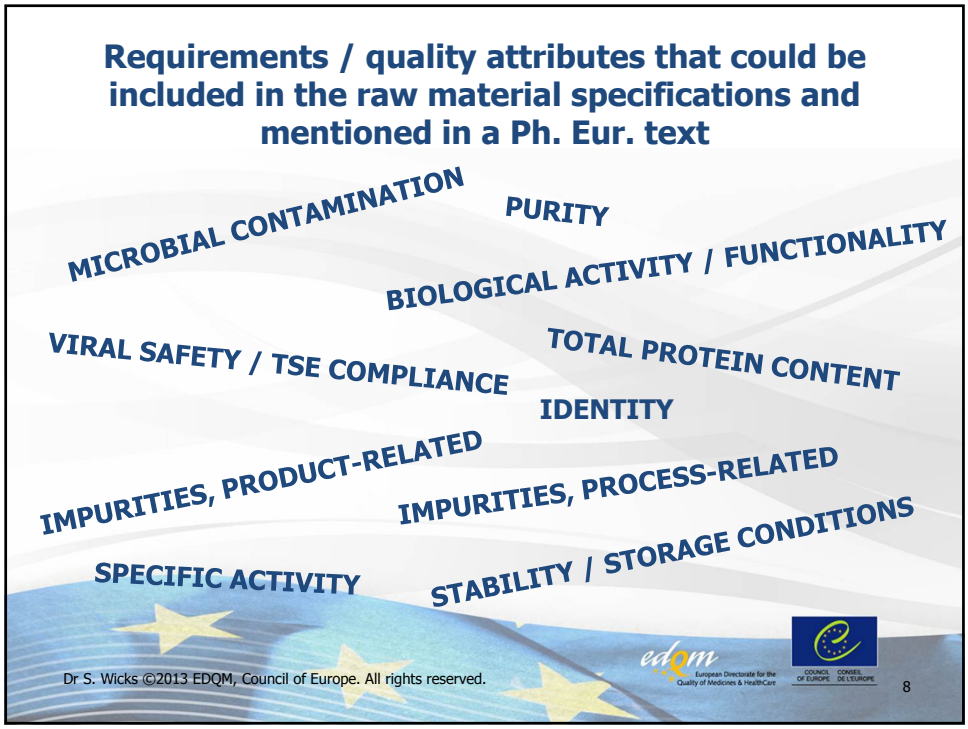
Requirements / quality attributes that could be included in the raw material specifications and mentioned in a Ph. Eur. text
MICROBIAL CONTAMINATION
PURITY
BIOLOGICAL ACTIVITY / FUNCTIONALITY
VIRAL SAFETY / TSE COMPLIANCE
TOTAL PROTEIN CONTENT
IDENTITY
IMPURITIES, PRODUCT-RELATED
IMPURITIES, PROCESS-RELATED
SPECIFIC ACTIVITY
STABILITY / STORAGE CONDITIONS
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Out of scope of this symposium

- Manufacturing requirements under GMP
- More complex raw materials (such as feeder cells)
- A certification system
- International harmonisation (need to establish what is required in Europe before considering any wider harmonisation)

Afternoon session

- Delegates to split into 4 parallel sessions
  - Lists in your pack and outside
- 1h30 to discuss raw materials and quality attributes
- We want to hear your views
- At the end of the session we will feedback to all delegates

Discussion ground rules

- Please give delegates airspace
- Respect all views
- We welcome all comments
- Time is limited
  - May not have time to discuss all your views
  - Please submit them to us via form/email

Please be at your discussion room at 14:30