Challenges with cell based medicines in the EU

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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, Advanced 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.

**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.

**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.

**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.

**SPINAL CORD NERVES**
Scientists are investigating 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__
Cell Based Medicinal Products

**Somatic cell therapy medicinal product**
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 of cells or tissues that are *not intended to be used for the same essential function(s)* in the recipient and the donor, and
- used with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues.

**Tissue engineered product**
contains or consists of engineered cells or tissues; = *subject to substantial manipulation*, so that their original biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement, are altered. / *heterologous use* / combined products
– used with a view to, *regenerating, repairing or replacing a human tissue*;

**Genetically modified cell gene therapy – GENE THERAPY**
cells modified with recombinant nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es).

- Risk based approach to construct the dossier
- Cells as starting materials collected and donor tested according to Directives 2004/23/EC and 2006/17/EC
- Traceability of all raw, starting materials and active substance
- TSE minimization and viral safety of active substance considering all raw and starting materials

  • Scaffolds, cells, vectors, bank to produce vector = starting materials
  • Bioactive molecules and cell medium not part of AS = raw materials

- Characterisation include identity, purity, viability, potency, kariology, tumourigenicity, genetic stability

- Genetically modified cells = gene therapy + cell therapy requirements
- Preclinical studies with homologous models
- Biocompatibility of matrix, scaffold, cells, excipients, substances in final product

- Risk management plan include long term follow-up of safety and efficacy
Supervision of tissues and cell collection procurement + donor testing
by EU transplantation authority

Manufacturer using cells imported and exported from and into the EU have
to ensure compliance with the procurement and testing as defined in Directives
2004/23/EC and 2016/17/EC

Viable human cells not possible in Medical Devices – Regulation 2017/745, Art 1. 6.
Human cells not possible in Cosmetics - Regulation 1223/2009 Annex 2 substances Prohibited (416)
GMP for ATMP

• GMP mandatory for all products entering clinical trials
• GMP or equivalent quality system for Hospital Exemption
• Many trials from academic / hospital investigators

- Specific GMP framework for ATMP
- GMP guide for ATIMP and ATMP

• Other quality systems in use in tissue banks acceptable for non-manipulated cell preparations for heterologous use
For CBIMP the following aspects should be considered as applicable:

− A clear definition of a production batch from cell sourcing to labelling of final container should be provided (i.e. size, information on intermediate cell-banking, number of cell passages/cell population doublings, pooling strategies, batch numbering system).

− a description of the manipulation steps after sourcing. This should include a description of any selection/separation equipment used.

− The type of manipulation(s) required for cell processing shall be described.

− Manufacture of combined medicinal products consisting of cells and matrices/devices/scaffolds require additional consideration regarding cell-matrix/scaffold interactions and associated quality issues. Attention should be given to biodegradable materials, which may effect environmental changes (e.g. raising pH) for the cells during the manufacture.

− Information on procedures used to transport material during the manufacturing process of the product, including transportation and storage conditions and holding times, should be provided.

− Microbiological process control and quality evaluation of all cell preparations and should be thoroughly described and justified.
REMARKS ON CELL QUALITY ISSUES throughout development

• Complexity requires **product definition** and a target profile
• The product is as good as the **quality of the starting and raw materials**
• The **manufacturing process** to be characterised, progressively qualified and validated at the commercial scale
• The **analytical methods** matter - qualified later validated
• Potency assays are essential for consistency, stability, comparability
• Any manipulation and changes in cell may have an impact on cell characteristics, fate, persistence, engraftment and overall efficacy
• **Comparability** should be measurable
• **Combined / genetically modified cells** - added complexity – consider the cell component *per se* and as part of the whole product

Market Authorisation Applications CAT 2009-2020

APPROVED AND LATER WITHDRAWN:

**ChondroCelect** - for cartilage repair, 2009 *(withdrawn 06/2016)*

**MACI** - for cartilage repair, 2012 *(closure of EU manufacturing site 09/2014)*

**Provenge** - advanced prostate cancer, 2013 *(withdrawn 05/2015)*

**Glybera** - LPL deficiency, 2013 ..... withdrawn 10/2017

APPROVED:

**Holoclar** - limbal stem cell deficiency, 2015

**Imlygic** - advanced melanoma, 2015

**Strimvelis** - ADA-SCID, 2016

**Zalmoxis** - high-risk haematological malignancies (adjunctive to HSCT), 2016

**Spherox** - for cartilage repair < 10 cm², 2017

**Alofisel** - complex anal fistulas in Crohn’s disease, 2018

**Kymriah** - children + adult <25yo ALL and adult DLBCL, 08/2018

**Yescarta** - adult DLBCL and PMBCL, 08/2018

**Luxturna** - children and adult retinal dystrophy biallelic RPE65 mutations, 09/2018

**Zynteglo** – β Thalassemia - non β0/β0, 03/2019

**Zolgensma** – spinal muscular atrophy (SMA) / bi-allelic mutation in the SMN1 gene, 03/2020
Examples of potential risk factors for CBMP

- Origin of cells (autologous vs allogeneic)
- Ability to proliferate and differentiate
- Ability to initiate an immune response (as target or effector)
- Level of cell manipulation (in vitro / ex vivo expansion/activation, genetic manipulation)
- Manufacturing process including biologically active reagents
- Mode of administration (ex vivo perfusion, local, systemic)
- Duration of exposure (short to permanent)
- ...
Regenerative medicine – stem / stromal cells

http://stemcells.nih.gov/StaticResources/images/figure2_lg.jpg
Main results

Thirty-three RCTs (1765 participants) were eligible for inclusion. Stem/progenitor cell treatment was not associated with statistically significant changes in the incidence of mortality (RR 0.70, 95% CI 0.40 to 1.21) or morbidity (the latter measured by re-infarction, hospital re-admission, restenosis and target vessel revascularisation). A considerably high degree of heterogeneity has been observed among the included trials. In short-term follow up, stem cell treatment was observed to improve left ventricular ejection fraction (LVEF).

Authors’ conclusions

Despite the high degree of heterogeneity observed, the results of this systematic review suggest that moderate improvement in global heart function is significant and sustained long-term. However, because mortality rates after successful revascularization of the culprit arteries are very low, larger number of participants would be required to assess the full clinical effect of this treatment. Standardisation of methodology, cell dosing and cell product formulation, timing of cell transplantation and patient selection may also be required in order to reduce the substantial heterogeneity observed among the included studies.
Cell product definition
Can conclusions be drawn from experience?

- Cardiomyocytes progenitor ? Endothelial progenitor ? Both ?
- Progenitor cells vs cardiomyocytes – differentiation stage ?
- Autologous vs allogeneic - Immunoregulatory properties ?
- Different Sources of progenitor cells – different clinical outcome?
  - Bone marrow – “ gold standard” ?
  - Adipocytes – Peripheral blood – cord blood – placenta ?
  - Characterisation beyond ISCT ?

Table 1. Summary of criteria to identify MSC

<table>
<thead>
<tr>
<th>#</th>
<th>Phenotype</th>
<th>Positive (≥ 95% +)</th>
<th>Negative (≤ 2% +)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adherence to plastic in standard culture conditions</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Phenotype</td>
<td>CD105</td>
<td>CD45</td>
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<td></td>
<td></td>
<td>CD73</td>
<td>CD34</td>
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<td></td>
<td></td>
<td>CD90</td>
<td>CD14 or CD11b</td>
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<td></td>
<td></td>
<td></td>
<td>CD79a or CD19</td>
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<td></td>
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<td>HLA-DR</td>
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</tbody>
</table>

3 In vitro differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of in vitro cell culture)
Q&A for minimally manipulated – EMA July 2017


<table>
<thead>
<tr>
<th>Risk Factor / Quality</th>
<th>Risk factor / Quality</th>
<th>Tumour formation</th>
<th>Unwanted tissue formation</th>
<th>Unwanted immunogenicity</th>
<th>Disease transmission</th>
<th>Treatment failure Lack of efficacy</th>
<th>Toxicity Safety issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell starting material</td>
<td>Autologous cells, not substantially manipulated, are expected to represent no risk for tumourigenicity</td>
<td>Autologous cells are not expected to trigger immune reactions</td>
<td>For autologous product disease transmission to the recipient is not an issue</td>
<td>Quality and consistency of cells has to be ensured; harmonized procedures for procurement, handling, transport. Acceptance criteria for volume and cell numbers</td>
<td>In principle autologous cells are not expected to be associated with toxicity, but altered environment for cells has to be considered</td>
<td></td>
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</tr>
<tr>
<td>Aspects of the manufacturing process and level of cell manipulation</td>
<td>Autologous cells, not substantially manipulated, are expected to represent no risk for tumourigenicity</td>
<td></td>
<td></td>
<td>The process should not introduce additional variability; the consistency needs to be ensured. Conditions for manipulation/handling before final product administration (including transport) need to be defined.</td>
<td>Safety of the product could be affected by the potential process-related impurities and microbiological contamination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell population, heterogeneity &amp; differentiation potential</td>
<td>Autologous cells, not substantially manipulated, are expected to represent no risk for tumourigenicity</td>
<td>Autologous cells are not expected to trigger immune reactions</td>
<td>For autologous product disease transmission to the recipient is not an issue;</td>
<td>Quality and consistency of cells/mixture has to be ensured and monitored; Though the manufacturing is very limited, the cell selection process has to be validated</td>
<td>In principle autologous cells are not expected to be associated with toxicity, but altered environment for cells has to be considered</td>
<td></td>
<td></td>
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<tr>
<td>Structural / functional integrity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Potency assay needs to be established; functional &amp; viability markers</td>
<td></td>
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</tr>
</tbody>
</table>
Expansion / modification – variable complexity / risk evaluation / ipc

- Always considered **substantial manipulation**
- Biological raw materials – Ph.Eur. text
- Culture conditions - Development studies provide relevant information on conditions for cell growth
- In-process controls – pH, temperature, oxigen, time, critical attributes, safety
- Extensive process characterisation to support target phenotypic and genotypic profile
- Population doubling time based on exponential growth phase + passage number - cumulative population doublings – *dedifferentiation* – *senescence* – *apoptosis* – *genetic stability*
Alofisel
expanded adipose stem cells - eASC

• Darvadstrocel
• Expanded human allogeneic mesenchymal adult stem cells extracted from adipose tissue
• 5 million cells/mL suspension for injection
• Authorised: 23 March 2018
• **Approved indication:** treatment of complex perianal fistulas in adult patients
• Marketing Authorisation Holder: Takeda Pharma A/S
  • original MAH: Tigenix

2.5.4. Tumourigenicity and genomic stability

Undifferentiated and proliferative / pluripotent cells namely iPSCs and hESCs have a relatively high potential risk of tumour formation, which should be carefully addressed during product development. The presence of proliferative and pluripotent cells tolerated in the final product should be limited and justified. Therefore it is essential that stem cell preparations undergoing extensive in vitro manipulation such as prolonged cell culture, as well as those derived from hESCs or iPSCs are evaluated for both their tumourigenicity and chromosomal stability before their initial clinical use. Cytogenetic analysis, telomerase activity, proliferative capacity and senescence could be of relevance.
Risk of Tumourigenicity – Alofisel (eASC)

QUALITY LEVEL
- Genetic stability of AS – release specification

(QUALITY) NON-CLINICAL LEVEL in vitro - eASC expansion process studied beyond clinical population doublings
- negligible telomerase activity
- low c-myc expression with no increase in telomerase activity or c-myc expression during the process of expansion
- eASC preparations showed no anchorage-independent growth in the soft-agar test.
- normal male or female diploid karyotypes

NON-CLINICAL LEVEL in vivo -
- human cells in animal model no tumour formation observed

CLINICAL LEVEL
- Ectopic tissue formation and tumourigenicity included in the RMP to be followed up to 5 yrs with the agreed PASS
5th ATMP authorised in the EU – March 2015

HOLOCLAR

transparent circular sheet of 300,000 to 1,200,000 viable autologous human corneal epithelial cells (79,000-316,000 cells/cm²) expanded in cell culture and including on average 3.5% (0.4% to 10%) limbal stem cells in addition to stem cell-derived transient amplifying and terminally differentiated cells.

Questions

potency, feeder cell safety, risk mitigation for safety (shelve life 36h), stability, container closure transport stability.

Release based on macroscopic and microscopic appearance and results of intermediate control testing.

Tumorigenicity at non-clinical (cells + 3T3 J2 feeder cells) – Karyotype, soft agar, growth factor dependent
Chondrocytes

ChondroCelect® - 1st generation = cell suspension - < 5 cm²
- 2009 - withdrawn

MACI® - 2nd generation = cell in matrix
- 3-20 cm²
- 2012 - withdrawn

Spherox® - 2nd generation = cell spheres
- < 10 cm²
- 2017
CONSIDERATIONS ON QUALITY DATA –
Starting material - biopsy
Manufacturing process – dedifferentiation – fibroblast like / contamination
Potency – hyaline cartilage formation
Biocompatibility with all materials in contact with the cells

CONSIDERATIONS ON NON-CLINICAL DATA
In vitro and small species (rodents / rabbits) for initial proof-of-concept ECFA (ectopic cartilage forming)
Large species (goat, horse, sheep) for pivotal study - to validate MRI
Safety end-points on POC studies

CONSIDERATIONS ON CLINICAL DATA
Pharmacology – Macroscopic, histological, MRI assessment ok for pharmacodynamic
Exploratory trials - Dose reflecting cell engraftment (minimum nº of cells/cm2
Confirmatory trials – primary and secondary end-point s - (eg KOOS)
Trial design and clinical safety
Availability of tocilizumab and site qualification

To minimise the risks associated with the treatment of KYMRIA, the MAH must ensure that hospitals and their associated centres that dispense KYMRIA are specially qualified in accordance with the agreed control distribution program.

The MAH must ensure on-site, immediate access to 4 doses of tocilizumab for each patient as CRS management medication prior to treating patients.

KYMRIA will only be supplied to hospitals and associated centres that are qualified and only if the healthcare professionals involved in the treatment of a patient have completed the educational program.

The availability of tocilizumab at all hospitals and associated centres must be ensured by the MAH until an authorised treatment for CRS is available in the EU.

- Automated manufacturing
- Bed side / decentralised manufacturing
- Exceptional OOS administration
• CE mark not sufficient
• Qualification DQ / IQ /OQ / PQ as ATMP-GMP
• Training / maintenance
• Aseptic process – integrity + cleaning validation
• Closed system in controlled area grade D
  ❖ except single very short procedure at operating theatre clinically justified
• Process validation reduced if used as recommended by the device manufacturer
• Start / stop / critical points defined and controlled
• Batch release - certified by QP
  ❖ except QP release for decentralised manufacturing as defined
A "central site", which should be established in the EU, should be identified. The central site is responsible for the oversight of the decentralised sites.

To this end, the central site assumes, as a minimum, the following tasks:

(i) ensuring that those involved in the batch certification and release process are adequately qualified and trained for their tasks, and

(ii) performing audits to confirm that the batch certification and release process (as described in SOP) is complied with.
In exceptional circumstances only the administration of a cell/tissue-based ATMP that does not comply with the specifications set out in the marketing authorisation may be considered to avoid an immediate significant hazard to the patient.

The MAH/manufacturer/importer should immediately inform the treating physician and to conduct an evaluation of risks

– Physician confirms the need
– Patients may be informed but they are not part of the decision

The MAH/manufacturer/importer should inform EMA within 48h after administration that a oos batch was used

Pharmacovigilance reporting obligations or specific additional obligations to follow-up patients treated with the ATMP (e.g. registry) continue to apply in respect of OOS batches
Common issues with CBMP

PRODUCT DEFINITION – COMPOSITION / MODE OF ACTION / POTENCY

CELL PROCUREMENT AND DONOR TESTING -

CELL EXPANSION – POPULATION DOUBLINGS / GENETIC STABILITY / DEDIFFERENTIATION

CHANGES IN DONOR / PROCESS – COMPARABILITY

SHORT SHELF LIFE AND STAGGERED RELEASE

LOGISTICS / TRANSPORT / ACCEPTANCE CRITERIA

PREPARATION / RECONSTITUTION / RELEASE AT BEDSIDE
Thank you!