

Cell therapy: cGMP facilities and manufacturing

**Raffaella Giancola,
Tiziana Bonfini,
Antonio Iacone**

Department of Transfusion Medicine, Pescara Civil Hospital, Italy

Corresponding author:

Antonio Iacone
Department of Transfusion Medicine
Pescara Civil Hospital, Italy
e-mail: antonio.iacone@ausl.pe.it;
antonioiacone@virgilio.it

Summary

Advanced therapies constitute one of the most complex, organizational, and regulatory areas currently approached by clinical researchers in order to explore new therapeutic applications. Basic scientists and clinicians trying to implement cell therapies into clinical practice, may feel overwhelmed by the apparently endless regulatory requirements that apply. However, regulatory agencies have primary responsibility on patient safety and law enforcement are, and should be, their main considerations. Cell- and tissue-based therapies have the potential to treat many conditions, where present conventional treatments are inadequate. The current approach to cell- and tissue-based therapy development requires using good manufacturing production facilities through master and working cell banks. Facilities need to be purpose-designed and accredited by their national medicinal regulatory body and production scientists need to work in close tandem with quality assurances and ethics committees to absolutely ensure the safety of this cellular products.

Key words: MSC, manufacturing, tissue engineering.

Introduction

Recently a great interest has arisen in research in the field of advanced therapies, which may have important applications in tissue engineering, regenerative medicine, cell therapy and gene therapy owing to their great therapeutic potential which may have important applications^{1,2}. Cell therapy is based on transplantation of live cells in order to repair or restore lost or defective functions. Regenerative medicine is in turn a multidisciplinary area aimed

at maintenance, improvement, or restoration of cell, tissue, or organ function using methods mainly related to cell therapy, gene therapy and tissue engineering.

Somatic cell therapy with skin replacement products and cartilage repair using articular chondrocytes have already been a standard of care for a decade³⁻⁴; however, the cells mainly used for such advanced therapies are stem cells because of their ability to differentiate into specific cells required for repairing damaged or defective tissues or cells⁵. There is much to be investigated about the specific characteristics of stem cells and about the efficacy and safety of the new drugs based on this type of cells both embryonic or adult stem cells. In fact recent progress in the transfer of nuclei from human somatic cells, as well as iPSC technology, has allowed availability of lineage of all three germ layer genetically identical to those of the donor patient, which permits safe transplantation of organ-tissue specific adult stem cells with no immune rejection⁶⁻¹¹. The main objective of stem cell therapy is the need for expansion of stem cell characteristics to maximize stem cell efficacy (proper selection of a stem cell) and the efficacy and safety of delivered drugs. The general objectives in the area of cell- and tissue-based therapies for the next few years are related to the identification of therapeutic targets and potential therapeutic tests, studies of cell differentiation and physiological mechanisms, culture conditions of pluripotent stem cells and safety tests for cell based drugs or procedures to be performed both in animal models and human in the corresponding clinical trials.

c-GMP facilities and quality control requirements of cell therapy products

Cell-based Medicinal Products (CBMPs) or Advanced Therapy Products (ATPs) include cell therapy products and tissue engineered products. These products are manufactured from viable autologous, allogeneic or xenogeneic cells and they can also contain non cellular components (chemical/biological compounds, matrices, scaffold etc.) either as a raw material or as a part of the active substance. Cell therapy medicinal products are intended for treatment or prevention of diseases or to make a diagnosis, via a pharmacological, immunological or metabolic mode of action of the cells. Tissue engineering products (TEPs) are developed for structural and functional repair of tissue/organ defects and their mode of action is to repair, restore or replace tissue structure/function¹².

Any procedure related to CBMPs requires a strict control in c-GMP facilities. A cGMP facility is a production facility for the manufacturing of pharmaceutical or cellular products. It includes the manufacturing space, the storage warehouse for raw and finished product and support laboratory areas¹³. Facilities should be designed and organ-

ized according to *Good Manufacturing Practice for Pharmaceutical Manufactures*¹⁴ including *quality control and quality assurance* programs, which established a *Quality System* approach to control collection, processing, storage and release of cell therapy products and also address the following elements: a) facilities (design, access and maintenance); b) equipment (purchase, use and maintenance); c) materials (specifications, purchase, storage and use); d) quality assurance (quality control, validation, qualification and document control). It builds up on the multidisciplinary team expertise: the *Quality Assurance*, while founded on the following elements: quality program, organization and personnel, standard operating procedures, environmental control, equipment monitoring, supplies and reagents, process controls, process changes, process validation, labeling design and control, storage requirements, records, tracking, non conformances and complaints management, risk assessment, reporting and reviewing. In areas where Quality Assurance interacts with hospital departments and infrastructures, such as housekeeping, engineering, information technology, and supply departments, the aim is to establish relevant service level agreements in order to ensure adequate level of regulatory compliance¹⁵⁻¹⁶. The Quality System approach is *risk-based*. For the facilities it is of paramount importance to prevent potential contamination, both microbiological and by endotoxins due to defects in environmental conditions, handlers, culture containers, or raw materials, or crossed contamination with other products prepared in the same production plant. Care should be taken with methods for container sterilization and control of raw materials and auxiliary reagents, use of High Efficiency Particulate Absorbing (HEPA) filter to prevent airborne cross-contamination, separate handling of materials from different patients, etc. For these reasons, the most important rooms of these facilities include the so-called *clean rooms*, which are classified in four classes (A-D) depending on air purity, based on the number of particles of two sizes ($\geq 0.5 \mu\text{m}$, $\geq 5 \mu\text{m}$). Other parameters such as temperature, humidity, and pressure should be taken into account and monitored because of their potential impact on particle generation and microorganism proliferation¹⁷. Materials and staff flows should be separated and be unidirectional to minimize cross contamination, and documentation of all activities is necessary. The technical staff should be especially trained in basic hygiene measures required for manipulation in clean rooms. Technical staff should have adequate qualification for both the conduct and surveillance of all activities.

Production and distribution of CBMPs or ATPs are controlled by the relevant local and national authorities based on the *International Conference on Harmonization of Pharmaceuticals for Human Use* which standardizes the potential interpretations and applications of the corresponding national recommendations¹⁸.

Manufacturing and regulatory requirements for cell therapy products

A *risk based approach* can be used also to define the amount of scientific and clinical data for each cell therapy

product. The initial risk analysis performed by developers identify the risks related to the product, its production and clinical use, and the evaluation covering the whole product development. The initial risk evaluation also serves as a basis for the preparation of a *risk management plan*¹⁹. The *Good Clinical Practice* are applied to develop clinically these products.

The legal requirement for CBMPs are set in Regulation 1394/2007/EEC²⁰ and in revised Annex I Directive 2001/83/EEC²¹. Likewise the national medicinal regulatory body should be aware of the initiation of the production before it starts.

Product characterization includes testing for *identity, purity/impurity, potency, viability and cell number*. Additionally, *tumorigenicity and biocompatibility* testing should be performed where appropriate. The issues to be considered include cell origin (autologous versus allogeneic), ability to proliferate/differentiate, ability to initiate an immune response, level of cell manipulation, route of administration, duration of exposure, use of combination products etc. In compliance with official standard books such as the *European Pharmacopoeia* (EurPh)²² or *The United States Pharmacopoeia* (USP)²³, each batch of a CBMPs or ATPs should pass a very strict and specific test control depending of the characteristics of the cell therapy product. The major risks related to a cell based product are microbiological contamination, dedifferentiation/loss of cell function, cell transformation malignancies, immunogenicity and ectopic engraftment of cells to non target-tissues. Whenever there are limited possibilities for batch release testing, the missing information are complemented through proper product characterization and process validation data. Definition and characterization of the product are of utmost importance, as these data provides the tools for proper process validation, in process testing and release testing. Tissues and cells, used as starting material for cell therapy products should comply with the requirement set in the Directive 2004/23/EEC²⁴ and the technical directives drawn from it^{25,26}.

Manufacture of living cells does not allow terminal sterilization of the product or removal/inactivation of microbial contaminants. Thus, appropriately tested and qualified starting materials and a validated aseptic manufacturing process are the key factors to ensure microbiological purity of the product. Sterility testing for the absence of the bacteria, mycoplasma and fungi should be conducted at released whenever possible. However sterility testing may not be possible for all products, especially when the shelf-life of the product is very short; in such a case, alternative methods with shorter read-out could be used^{27,28}. Cell therapy products are initially tested in non-clinical studies. Non-clinical studies include pharmacology, pharmacokinetics, and toxicological studies. The objectives are to demonstrate the *proof of principle* (specific therapeutic utilization) for the medicinal product and to define the pharmacological and toxicological effect that are predictive of the response in humans. Further objectives comprise the establishment of safe doses for subsequent clinical studies and to support the route of administration of the cell-based product. Moreover, a non clinical study also identifies target organs for toxicity and should allow for the definition of parameters to be monitored in the pa-

tients. It is also necessary to provide safety suitability and biocompatibility data for any additional substances that are administered together or as a part of the cell-based medicinal product, such as cellular components, biomolecules, biomaterials and/or chemical substances. The selection of materials should be justified based on biocompatibility. Especially for scaffolds, the physical, mechanical, biological and chemical properties should be considered, all these aspects influence their interactions with the cells and should be addressed. Finally non-clinical studies should be performed in relevant animal models, meaning that the animals should allow the human response to CBMP to be predicted.

The clinical development of a CBMP follow the same regulatory requirements as established for other medicinal products²⁰ and the existing general specific guidelines available for the clinical conditions to be treated. The initial risk evaluation is used to design the entire clinical development plan that include pharmacodynamic and pharmacokinetic studies, mechanism of action, dose finding studies and randomized pivotal clinical trials. During the explorative phase of clinical studies, special attention should be paid to pharmacodynamic, and pharmacokinetic studies by monitoring of viability, proliferation/differentiation, body distribution/migration during the intended viability of the CBMP. One of the critical features is the *definition of the dose*. Efficacy are established using clinical meaningful endpoints and both structural and functional measurement are considered. As with other medicinal products, a prospective, randomized concurrently pivotal study is expected, unless otherwise justified. In case a single pivotal study approach is used, it should comply with the requirement of the point to consider on application with one pivotal study²⁹. More than one pivotal clinical study should be considered if: a) the mechanism of action is unknown; b) there is a new pharmacological principle; c) phase I and II data are too limited or d) there is a new therapeutic area with a history of failed studies. As it expected that CBMPs do have long-lasting effects, the risks of late events both from an efficacy and safety point of view should be addressed during clinical development and in a risk management plan. Another set of risks emerges from long term use of CBMPs. Considerations should be given in presence of a plato-effect of efficacy (no increase in efficacy despite further time flow dosage increases) and if the product shows unsafe features as well as loss of beneficial effect or late adverse events such as malignancies.

In USA regulatories have been developed using the same approach. FDA defines a regulatory framework that controls Advanced Therapy Products (ATPs) based on three general areas: a) prevention of use of contaminated tissues or cells (e.g. AIDS or hepatitis); b) prevention of inadequate handling or processing that may damage or contaminate those tissues or cells; and c) clinical safety of all tissues or cells that may be processed, used for functions other than normal functions, combined with components other than tissues, or used for metabolic purposes. In fact, the key points of the current regulation for cell therapy products³⁰⁻³¹ include: 1) demonstration of preclinical safety and efficacy; 2) no risk for donors of transmission of infectious or genetic diseases; 3) no risk

for recipients of contamination or other adverse effects of cells or sample processing; 4) specific and detailed determination of the type of cells forming the product and what are their exact purity and potency; 5) “*in vivo*” safety and efficacy of the product. The FDA also provides specific recommendations on how scientists should address the safety and efficacy issues related to this type of therapy^{32,33}.

Regulatory requirements for embryonic and iPSC therapy products

Recently the first clinical trial of a product derived from human embryonic stem cells in acute spinal cord injuries was approved in the USA by the Food and Drug Administration³⁴⁻³⁵, of course, cell therapy with human embryonic stem cells has raised moral and ethical issues^{36,37}. Such considerations refer to donor consent and problems associated to oocyte collection and the issue of destruction of human embryos³⁸. Guidelines ranging from total prohibition to controlled permissiveness, defining what may be permitted in research with pluripotent stem cells, have been issued in countries all over the world³⁹. Such guidelines reflect the different views about when life starts during the human embryonic development as well as regulation of measures to protect oocyte donors and to reduce the probability of human embryo destruction⁴⁰.

Induced pluripotent stem cells (iPSCs) from somatic cells are revolutionizing the field of pluripotent stem cells. iPSCs were obtained by reprogramming somatic stem cells of a patient through the introduction of certain transcription factors⁴¹⁻⁴³, and they can be used to discover new drugs and to establish cell therapy protocols because they show pluripotentiality to differentiate into cells of all three germ layers. The iPSC technology offers the possibility of developing patient-specific cell therapy protocols⁴⁴ because the use of genetically identical cells may prevent immune rejection. In addition, unlike embryonic stem cells, iPSCs do not raise a bioethical debate, and are therefore a “consensus” alternative that does not require use of human oocytes or embryos⁴⁵. Moreover, regulatory issues of iPSCs are included in those of embryonic stem cells⁴⁶⁻⁴⁹.

Conclusion

A regulatory framework will be required to ensure patient accessibility to products and governmental assistance for their regulation and control. Up to now, most cell therapy protocols have not been controversial. The exception is therapy with human embryonic stem cells, which has raised moral and ethical issues. Such considerations refer to donor consent and problems associated to oocyte collection and the issue of destruction of human embryos. Bioethical aspects⁵⁰⁻⁵² will be required related to the scientific and therapeutic relevance and cost of cryopreservation over time, but specially with respect to embryos which may ultimately be used as source of embryonic stem cells, in which case the bioethical conflict may be further aggravated.

Complex legislation makes it difficult, expensive and time-consuming for researchers to approach these issues, but regulatory agencies have primary responsibility on patient safety and law enforcement are their main considerations. However the therapeutic applications are fascinating and cell therapy represents a great promise for the treatment of many diseases.

References

1. Tiede MA Stem Cell: applications and opportunities in drug discovery. *Drug Discov World* 2009;10:9-16.
2. Razvi ES, Oosta GM. Stem Cells for cellular therapy space. *Drug Discov Today* 2010;11:37-40.
3. Mc Neil S. Progress and opportunities for tissue engineering skin. *Nature* 2007;445:974-880.
4. Getgood A, Brooks R Fortier L, Rusthon N. Articular cartilage tissue engineering: today's research tomorrow's practice? *J Bone Joint Surg Br* 2009;91B:565-576.
5. Ahrund-Richter L, De Luca M, Marshak DR et al. Isolation and production of cells suitable for human therapy: challenges ahead. *Cell Stem Cell* 2009;4:20-26.
6. Brivanlou AH, Gage FH, Jaenisch R, Jessell T, Melton D, Rossant J. Stem cells. Setting standards for human embryonic stem cells. *Science* 2003;300(5621):913-916.
7. Rubio D, Garcia-Castro J, Martin MC et al. Spontaneous human adult stem cell transformation. *Cancer Res* 2005;65(8):3035-3039.
8. Suter DM, Preynat-Seauve O, Tirefort D et al. Phenazopyridine induces and synchronizes neuronal differentiation of embryonic stem cells. *J Cell Mol Med* 2009;13(9B):3517-3527.
9. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;318(5858):1917-1920.
10. Lowry WE, Richter L, Yachechko R, Pyle AD et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *PNAS*;105(8):2883-2888.
11. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 2009;324(5928):797-801.
12. EMEA/CHMP 2006. Guideline on human cell-based medicinal products. EMEA/CHMP/410869/2006.
13. Dietz AB, Padley DJ, Gastineau DA. Infrastructure development for human cell therapy translation. *Clin Pharmacol Ther* 2007; 82: 320-324.
14. Good Manufacturing Practice for Pharmaceuticals Manufactures. www.gmp-quality.com.
15. FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing, and Administration (Foundation for the Accreditation of Cellular Therapy (FACT-Joint Accreditation Committee of International Society of Cellular Therapy (ISCT) and European Group of Blood and Marrow transplantation (EBMT) (JACIE)). www.factwebsite.org
16. Cellular and Gene Therapy Products. US Food and Drug Administration. www.fda.gov
17. EU Guidelines: Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Annex 1. Manufacture of Sterile Medicinal Products, 2009 (corrected version).
18. The International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. www.ich.org/cache/compo/276-254-1html.
19. EMEA/CHMP 2005. Guidelines on risk management systems for medicinal products for human use. EMEA/CHMP/96268/2005.
20. Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No726/2004.
21. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use.
22. European Pharmacopoeia (EurPh). www.aemps.es/proffHumana/farmacopea/doscs/index_5-3edic.pdf
23. The United States Pharmacopoeia (USP) www.usp.org/
24. Directive 2004/EC of the European Parliament and of the Council of 31 March 2004 on setting standards quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.
25. Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.
26. Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reaction and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.
27. Ph.Eur.Text 5.1.6. (2008) Alternative methods for control of microbiological quality (01/2008:50106) and General Methods 2.6.27: Microbiological control of cellular products.
28. United States Food and Drug Administration. Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products. Rockville: Guidance for Industry (Draft), 2008.
29. EMEA/CPMP Point to consider on application with one pivotal clinical study (CPMP/EWP/2330/1990).
30. Proposed approach to regulation of cellular and tissue-based products. FDA www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM062601.pdf
31. C.F.R. 1271 (2006). Part 1271-Human Cells, Tissues, And Cellular And Tissue-Based Products www.access.gpo.gov%2Ffara%2Fcftr%2Fwaisidx_06%2F21cfr1271_06.
32. Halme DG, Kessler DA: FDA Regulation of Stem-Cell-Based Therapies. *N Engl J Med* 2006, 355:1730-1735.

33. Fink DW. FDA Regulation of Stem-Cell-Based Products. *Science* 2009; 324:1662-1663.
34. Rowland JW, Hawryluk GW, Kwon B, Fehlings MG: Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. *Neurosurg Focus* 2008, 25:E2
35. A Catalyst for Cures: Embryonic Stem Cell Research. The Coalition for the Advancement of Medical Research http://camradvocacy.org/resources/camr_wp.pdf.
36. Rao M, Condic ML. Alternative sources of pluripotent stem cells: scientific solutions to an ethical dilemma. *Stem Cells Dev* 2008;17:1-10.
37. Hyun I. The bioethics of stem cell research and therapy. *J Clin Invest* 2010, 120:71-75.
38. Hyun I, Lindvall O, Ahrlund-Richter L, Cattaneo E et al. New ISSCR guidelines underscore major principles for responsible translational stem cell research. *Cell Stem Cell* 2008, 3:607-609.
39. International Consortium of Stem Cell Networks: Global Regulation of Human Embryonic Stem Cell Research and Oocyte Donation www.stemcellcentre.edu.au/.
40. Rao M, Condic ML. Musings on genome medicine: is there hope for ethical and safe stem cell therapeutics? *Genome Med* 2009, 1:70.
41. Sommer CA, Mostoslavsky G. Experimental approaches for the generation of induced pluripotent stem cells. *Stem Cell Res Ther* 2010,1:26.
42. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-676.
43. Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;318:1917-1920.
44. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* 2007;1:39-49.
45. 2008 Amendments to the National Academies' guidelines for human embryonic stem cell research National Research Council and Institute of Medicine www.nap.edu/openbook.php?record_id=12260&page=1
46. Canadian Updated Guidelines for Human Pluripotent Stem Cell Research, June 30, 2010 Please note: these Updated Guidelines for Human Pluripotent Stem Cell Research supersede the June 2007 version. www.cihr-isrc.gc.ca/
47. UK guidelines Human Fertilisation and Embryology Act 1990 and Human Fertilisation and Embryology Act 2004 www.legislation.gov.uk/ukxi/2004/1511/
48. UK guidelines SCAG (02/08) 04 Human Fertilisation and Embryology Act 2008 www.dh.gov.uk/en/.../Legislation/Actsandbills/DH_080211
49. Zarzeczny A, Scott C, Hyun I, Bennett J, Chandler J et al. iPS cells: mapping the policy issues. *Cell* 2009 Dec 11;139(6):1032-1037.
50. Global Summit of National Bioethics Advisory Bodies www.who.int/ethics/globalsummit
51. European Conference of National Ethics Committees www.coe.int/t/dg3/healthbioethic/
52. International Bioethics Committee www.unesco.org/new/en/social-and-human-sciences/themes/bioethics/international-bioethics-committee/

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