Good Manufacturing Practice Production of Human Stem Cells for Somatic Cell and Gene Therapy

ROLAND BOSSE, MONIKA SINGHOFER-WOWRA, FELICIA ROSENTHAL, GREGOR SCHULZ

CellGenix Technologie Transfer AG, Freiburg, Germany

Key Words. *Good manufacturing practice · Peripheral blood stem cell · Quality control · Somatic cell therapy · Gene therapy · Transplantation*

ABSTRACT

Peripheral blood stem cells (PBSC) are used for transplantation to reconstitute the hematopoietic system after high-dose chemotherapy. PBSC are harvested from peripheral blood upon successful mobilization by cytokines and/or chemotherapy. Further in vitro manipulation steps like enrichment of CD34$^+$ PBSC or gene transfer can be performed. To ensure the quality and safety of the final cell preparations intended for transplantation, national and international guidelines and regulations have been issued. Herein the implementation of a quality assurance program including the principles of good manufacturing practice (GMP) and a quality control (QC) system is one major concern. GMP regulations apply to all phases of cell collection, processing and storage as well as documentation, training of personnel, and the laboratory facility. QC measures have to be taken to ensure consistent quality and safety with an emphasis on preventing any deficiencies.

Stem Cells 1997;15(suppl 1):275-280

INTRODUCTION

The clinical use of purified stem cells instead of bone marrow transplantation has continuously increased during the last years. These cells are, besides others like stroma cells or fibroblasts, also tools for somatic gene therapy. Transfection with genes of interest may be induced by using different methods such as lipofection or retroviral vectors. Most of these cell preparations are autologous which means that the patients' cells will be reinjected after preparation and manipulation. The application of naked cDNA is still in an early clinical evaluation.

Production of cell products requires close interaction with hospitals. Ideally, the cell processing laboratory should be in close proximity to those institutions which are involved in therapeutic use. Though the number of preparations is limited compared to standard pharmaceutical production, these manipulated cells are considered drugs. This results in a number of special requirements concerning equipment and safety standard of the cell processing laboratory as well as qualification and training of personnel. As a consequence, good manufacturing practice (GMP) production of somatic and gene-transfected cells will be transferred from clinic laboratories to special institutions which are pharmaceutical manufacturers, though they do not have the size and the turnover of pharmaceutical companies involved in production of standard drugs. In this short review requirements for the scale-up process and the transmission of stem cells and gene-manipulated cells from a clinical research laboratory to production according to GMP and other regulatory directions are described.

Hematopoietic Stem Cells. STEM CELLS **1997;15(suppl 1):275-280**

General

In the recent past the terms quality assurance (QA), quality control (QC) and GMP have become increasingly well-known in the context of the production of blood and blood components [1]. The same is true for the field of somatic cell and gene therapy where a number of guidelines, regulations and federal laws have to be considered (Table 1). In the following, some regulatory aspects and their consequences for the practice of somatic cell and gene therapy such as peripheral blood stem cell (PBSC) processing and the production of gene-transfected cells are outlined.

Table 1. Legal basis for production and distribution of drugs

- ▲ Drug Law (5. AMG, amendment 1995)
- ▲ Regulation for production of drugs (1993)
- ▲ EC-Guidelines
- ▲ Directives (PIC, WHO, FDA)
- ▲ Pharmaceutical recommendations (EC, BGA)
- ▲ DAB 10, EP, USP
- ▲ Standards (VDI, DIN EN ISO)

WHO = World Health Organization; USP = United States Pharmacopeia;
BGA = Bundesgesundheitsamt; EC = European Communities

Different Legal Impacts on PBSC Processing and Manipulation in Germany and the USA

The applicability of the German drug law (Arzneimittelgesetz; AMG) to somatic cell therapy is defined in different paragraphs [2]. Paragraph two defines drugs as substances and substance preparations intended for cure, mitigation, prevention or diagnosis of diseases and injuries. According to paragraph four, blood preparations are defined as medical products prepared from or containing blood or blood components. PBSC collection and preparation is therefore regulated by the German drug law and consecutive guidelines, directives and recommendations.

In the U.S. Food and Drug Administration (FDA) draft document concerning the regulations of PBSC for transplantation, a clear differentiation between manipulated and nonmanipulated PBSC is made [3].

Manipulated PBSC products are defined as those obtained after one or more purging or enriching procedures of the starting material. Procedures resulting in manipulated PBSC products are, e.g., centrifugal elutriation, negative or positive cell selection by monoclonal antibodies, and expansion of cell populations in vitro using cytokines or other procedures leading to a somatic cell therapy product [3].

Nonmanipulated or minimally manipulated PBSC are defined as "products that have not been subject to a procedure that selectively removes, enriches, expands or functionally alters specific nucleated cell populations" [3]. This includes ex vivo centrifugation and density gradient separation, lysis of contaminating erythrocytes, addition of medium for cryopreservation, transfer of product from collection devices to storage containers and storage in liquid or frozen state. The depletion of polymorphonuclear leukocytes as well as the mobilization in vivo, e.g., by G-CSF, would not be considered manipulation [3].

Per definition manipulated as well as nonmanipulated PBSC products can be intended for the prevention, treatment, cure, diagnosis or mitigation of diseases or injuries in humans. However, manipulated PBSC intended for transplantation are subject to investigational new drug (IND) regulations during clinical development and as final biological products are subject to licensure, whereas nonmanipulated PBSC IND applications are not requested by the FDA. If the latter are intended for distribution in interstate commerce, licensure of the product (product license application) and establishment (establishment license application; [ELA]) are required. In this case the manufacture of these products has to be performed according to GMP and is subject to FDA inspection. In addition the registration as a blood establishment is necessary [3].

According to the German drug law, all PBSC intended for autologous transplantation are defined as nonfinal medical products and are not subject to new drug application (NDA) or equivalent procedures. The same

is true for those PBSC harvested from definite donors and intended for allogeneic transplantation into predetermined patients. The manufacture of PBSC products, however, requires a production licensure according to paragraph 13 AMG. Manufacture is defined as the acquisition, manipulation, preparation, transfer from collection devices into storage containers, packaging and labeling. The approval is mandatory for the manufacture of medical products from human or animal source for commercial distribution. A commercial distribution is given when the manufacturer is not identical to the user, e.g., the physician at the clinic [2].

One prerequisite for regulatory approval is the appointment of qualified individuals responsible for production, QC, distribution and monitoring of the product's clinical safety. Having equal authority they report separately to the management. In addition to a scientific degree, three year's experience in medical serology or microbiology and one year's experience in transfusion medicine are demanded (paragraph 14 AMG) [2].

According to the national pharmaceutical operation regulation (Pharmabetriebsverordnung), the implementation of a QA system in pharmaceutical companies is required. Pharmaceutical activities included are the manufacture, testing, storage, packaging and distribution of pharmaceutical products. The QA program has to be designed to ensure that manufacturing is consistently performed in such a way as to yield a product of consistent quality. Major parts of the QA program are the GMP and the QC [1, 4]. The pharmaceutical operation regulation is not applicable to physicians testing medical products in clinical trials, which is addressed in paragraphs 40-42 AMG, but it is relevant for all pharmaceutical manufacturing processes, including the preparation of products intended for use in clinical trials.

Processing of PBSC for Transplantation

As mentioned before, the processing of PBSC for transplantation requires product and establishment licenses as well as the implementation of a QA program with GMP and QC as major elements.

What is the major difference between isolating PBSC from a human blood sample for research purposes and the processing of PBSC from a patient's blood according to GMP for later clinical use, e.g., transplantation?

Besides the general requirements of establishment and product-specific approval by regulatory authorities, there are a number of areas that have to be covered to fulfill GMP requirements, some of which will be pointed out in more detail in Table 2 [4].

One major area of GMP is standard operating procedures (SOP) that have to exist for all manufacturing procedures as well as for documentation, in-process controls, cleaning, training, product release, etc. [4]. Methods and procedures have to be described precisely in the SOP and personnel have to strictly follow these descriptions. Every change of a method, a common occurence in a research lab, has to be approved after specific validation experiments have been performed, and the new method can only be implemented after the SOP is updated and signed. This particularly complex procedure represents a major difference compared to basic research.

Another difference is the place of cell processing. For research purposes this will usually happen in a normal cell culture lab under non-GMP conditions. In contrast, patient cells intended for clinical use are processed in a clean room facility according to the EC guideline of GMP for medicinal products for human use and the supplementary guideline for the manufacture of sterile drugs [4, 5]. Within the clean room facility, the air is filtered to meet the criteria for a certain air cleanliness level. The clean room facility should fulfill the air cleanliness classification between classes C and B (10.000 to 100 according to U.S. federal standard No. 209B) and

Table 2. Principles and guidelines of good manufacturing practice

- Quality management
- Personnel
- Premises and equipment
- Documentation
- Production
- Quality control
- Contract manufacture
- Complaints and product recall
- Self-inspection

of class A (100 according to 209B) under the sterile working bench, respectively [5]. Before entering the clean room facility, clothes have to be changed and masks and gloves must be worn. In a general sense, the personnel have to be trained to work in a clean room facility. The aim is to avoid contamination and to ensure sterility and safety of the product. Furthermore, the implementation of a detailed hygiene plan for each area within the establishment is a must. Finally, GMP regulations also define that within the clean room production unit, only one production process is allowed at a time to avoid cross-contamination among different samples. This would present a major obstacle in a common research lab with limited space and with many people working on different projects simultaneously.

To ensure the continued safety of the cell products and prevent the release of unsuitable PBSC preparations, a number of in-process control assays have to be performed. These include routine testing for sterility, determination of cell number and viability, a fluorescence-activated cell sorter analysis to determine the amount of $CD34^+$ stem cells in the preparation and a colony-forming unit assay to investigate the potential of the cells to differentiate and to proliferate in the presence of cytokines in vitro. Based on the results of these assays, the release of the final cell product is decided (Fig. 1).

Additional activities to comply with GMP regulations include: validation of all methods, survey of room surfaces and air to monitor accurate cleaning procedures and the fulfillment of air cleanliness criteria, release procedures for lab material prior to use and for the final cell product, documentation of all production- and patient-related data, regular training of personnel and regular self-inspections (Table 2) [4]. All raw data (e.g., results of in-process controls, validation studies, volumes, times, harvest and storage) have to be kept and reported in detail. Acceptable range limits for cell numbers and yield have to be specified. In addition incubators, liquid nitrogen tanks and other key equipment should be monitored for adequate function. These results also have to be documented. The material used (e.g., tissue culture flasks, pipettes, media and reagents) should be of GMP grade whenever possible. Otherwise, at a minimum, certificates of analysis for the specific lot used are requested.

Production of Gene-Transfected Hematopoietic Progenitor Cells for in Vivo Use

Somatic gene therapy is a medical intervention based on modification of the genetic material of living cells. Cells may be modified in vitro or ex vivo followed by administration to humans or may be altered in vivo. However, in vivo gene transfer is difficult to control and may affect cells other than the specific target cells.

Recently, various innovative therapies involving ex vivo manipulation and subsequent reintroduction of somatic cells into humans have been used or proposed. The in vitro attempt offers some important advantages—cells of interest can be modified, followed by selection, cloning steps and control of function in vitro. Cells of desired phenotype and/or function can be expanded in vitro under controlled

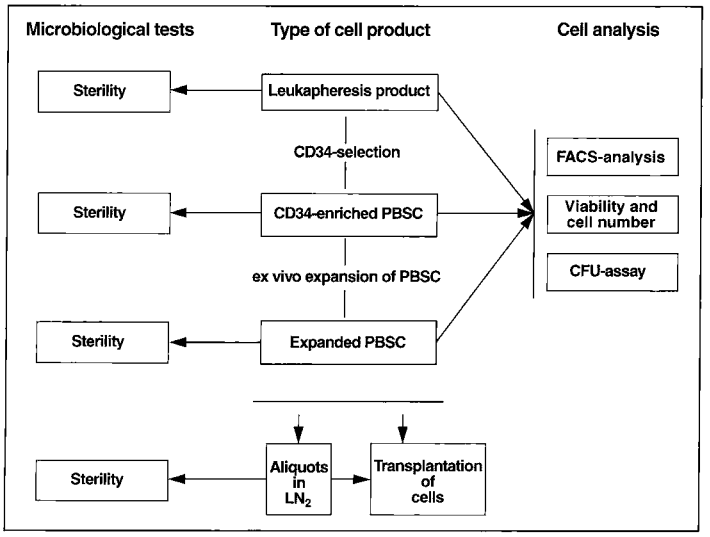

Figure 1. Quality control of ex vivo generated stem cell products. FACS = fluorescence-activated cell sorter; CFU = colony forming unit; PBSC = peripheral blood stem cell.

Table 3. GMP-production of retroviral vector for gene transfer into human PBSC

Production step	Preliminary work	Generation of master cell bank	Pilot production of vector supernatant	Clinical supernatant production	Ex vivo transduction of human PBSC
	GLP	GMP	GLP	GMP	GMP
Working process	• Description and characterization of vector and cloned gene: —history of vector —sequencing of vecor/gene • Description and characterization of packaging cell line	• Production of cell stock in clean room facility • Storage of aliquots in liquid nitrogen • Use of GMP-material • Documentation	• Optimization of growth curves, seeding, density and harvest vector supernatant • Documentation	• Production of retroviral vector-containing supernatant for clinical use in clean room facility	• Transduction of peripheral blood stem cells in clean room facility
Quality control	• Safety testing of packaging cell line (sterility, identity, mycoplasma, viruses) • Identity of insert and vector • Restriction mapping	• Safety testing (sterility, identity, mycoplasma, RCR and other viruses) • Analysis of insert	• Testing for titer, purity, identity	• Safety testing on clinical lot and end of production cells: identity, purity, potency, sterility, mycoplasma, endotoxin, RCR and other viruses, vector and insert stability	• Safety testing of transduced cells (sterility, endotoxin, mycoplasma, RCR etc.) • Monitoring of patients for RCR

GMP = good manufacturing practice; GLP = good laboratory practice; RCR = replication competent retrovirus.

conditions and the final product can be stored until use.

The final product resulting from in vitro genetic manipulation of cells is regarded as a drug according to German drug law [6]. This implies the requirement of a product license for those drugs produced for third parties. In addition, an ELA has to be filed. All steps of developing, testing and manufacturing of the product have to be performed in accordance with the GMP guidelines for medicinal products for human use (Table 2) [4]. In addition, any commercially manufactured gene therapy product will require marketing authorization by the European Medicines Evaluation Agency through the centralized concertation procedure [7].

In general for the production of gene-transfected cells in full compliance with GMP regulations, all procedures outlined above for PBSC processing are applicable. However, the genetic modification of the cells of choice intended for therapeutic use raises novel concerns about safety and efficacy. The gene construct has to be characterized concerning sequence, insert stability as well as biological function. QC of the manufacturing process including cell banking, key intermediates and the final product is necessary since poor control can lead to the introduction of adventitious agents or other contaminants, or to inadvertent changes in the properties or stability of the biological product. Detailed requirements for characterization and testing of cell lines intended for use in the manufacture of biologics, as well as for somatic cell and gene therapy, are defined by the FDA [8-10].

As an example, the retroviral gene transfer into human hematopoietic

progenitor cells for clinical use is described in more detail in the following. In general vector and insert have to be characterized thoroughly, and this should be done according to good laboratory practice (GLP) (Table 3). In the example a previously described vector carrying the gene of interest is used. Initially a master cell bank (MCB) of the producer cell line is generated under GMP conditions. All materials used are of GMP grade if available. To minimize the risk of contamination, all cell culture steps take place in a clean room facility. Aliquots of the MCB are then tested for fungal, bacterial, viral or mycoplasma contamination and isoenzyme, and caryotype analysis is performed to characterize and identify the cell line prior to further use as demanded by the European Communities and FDA [7, 10]. In contrast to the in-house assays performed for QC of untransfected PBSC products, the safety testing of vectors, parental, producer or transfected cell lines, and their products, e.g., vector supernatant, is usually outsourced to a contract laboratory approved by national or international regulatory authorities. The safety testing is performed in a nonclean room facility but GLP regulations are applied. Aliquots of producer as well as end-of-production cells are also tested for adventitious agents. In addition, identity, integrity and vector retention have to be demonstrated. The inserted gene should be sequenced post-production.

During process development, a production pilot run is made to optimize cell density and growth curves with the intention of recovering high titer retroviral vector containing supernatant (Table 3). Thereafter the clinical lot is produced under GMP conditions. An important issue is the testing for replication-competent retroviruses (RCR), e.g., by using the S+L- assay. Testing is recommended at multiple stages in production including MCB/WCB, the pilot run, the final clinical grade supernatant and the end-of-production cells. In addition, an aliquot of the ex vivo transduced cells of each patient has to be screened for RCR. In the course of the clinical protocol, periodic patient monitoring for evidence of RCR is also requested [10].

Conclusion

Cell processing for clinical application will become an important tool for new therapeutic strategies. Besides preparation of hematopoietic cells and gene-modulated cells, the generation of other cells like fibroblasts, keratinocytes, chondroblasts, etc., will be required for a variety of indications. With an increasing number of these new products, the production in a clinical research laboratory has to be transferred to GMP facilities in big hospitals or closely associated institutions. This will enable physicians in clinical disciplines to focus on research projects and treatment of their patients and no longer be busy with routine cell processing. On the other hand, synergies in this field of production of biologics can be used which will lead to cost savings. Somatic cell and gene therapy will provide new opportunities for patients with inherited diseases or malignancies, however, strict regulations during cell processing have to be followed in order to guarantee the highest quality and safety for the patients.

References

1 Guideline for quality assurance in blood establishments. 1995. Docket No. 91N-0450. CBER/FDA.

2 Gesetz über den Verkehr mit Arzneimitteln (AMG). 1994. BGBl. I:2071.

3 Draft document concerning the regulation of peripheral blood hematopoietic stem cell products intended for transplantation or further manufacture into injectable products. 1996. CBER/FDA.

4 Principles and guidelines of good manufacturing practice for medicinal products for human use (91/356/EEC). 1991. European Communities.

5 Supplementary guideline for the manufacture of sterile drugs. 1992. European Communities.

6 Lindemann A, Rosenthal FM, Hase S et al. Guidelines for the design and implementation of clinical studies in somatic cell therapy and gene therapy. J Mol Med 1995;73:207-211.

7 Gene therapy products—quality, safety and efficacy aspects in the production of vectors and genetically modified somatic cells (Draft). 1994. CPMP: Ad hoc working party on Biotechnology/Pharmacy of the European Communities.

8 Points to consider in the characterization of cell lines used to produce biologicals. 1993. CBER/FDA.

9 Points to consider in human somatic cell therapy and gene therapy. 1991. CBER / FDA.

10 Addendum to the points to consider in human somatic cell and gene therapy (1991). Draft-1/96. CBER/FDA.