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CELL MANUFACTURE

Design and validation of a consistent and reproducible manufacture process for the production of clinical-grade bone marrow–derived multipotent mesenchymal stromal cells

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Abstract

Background. Multipotent mesenchymal stromal cells (MSC) have achieved a notable prominence in the field of regenerative medicine, despite the lack of common standards in the production processes and suitable quality controls compatible with Good Manufacturing Practice (GMP). Herein we describe the design of a bioprocess for bone marrow (BM)–derived MSC isolation and expansion, its validation and production of 48 consecutive batches for clinical use. *Methods.* BM samples were collected from the iliac crest of patients for autologous therapy. Manufacturing procedures included: (i) isolation of nucleated cells (NC) by automated density-gradient centrifugation and plating; (ii) trypsinization and expansion of secondary cultures; and (iii) harvest and formulation of a suspension containing $40 \pm 10 \times 10^6$ viable cells. Quality controls were defined as: (i) cell count and viability assessment; (ii) immunophenotype; and (iii) sterility tests, *Mycoplasma* detection, endotoxin test and Gram staining. *Results.* A 3-week manufacturing bioprocess was first designed and then validated in 3 consecutive mock productions, prior to producing 48 batches of BM-MSC for clinical use. Validation included the assessment of MSC identity and genetic stability. Regarding production, 139.0 ± 17.8 mL of BM containing $2.53 \pm 0.92 \times 10^9$ viable NC were used as starting material, yielding $38.8 \pm 5.3 \times 10^6$ viable cells in the final product. Surface antigen expression was consistent with the expected phenotype for MSC, displaying high levels of CD73, CD90 and CD105, lack of expression of CD31 and CD45 and low levels of HLA-DR. Tests for sterility, *Mycoplasma*, Gram staining and endotoxin had negative results in all cases. *Discussion.* Herein we demonstrated the establishment of a feasible, consistent and reproducible bioprocess for the production of safe BM-derived MSC for clinical use.

Key Words: *advanced therapy medicinal product, bone marrow, cell culture, clinical-grade manufacture, Good Manufacturing Practice, inactivated human serum, multipotent mesenchymal stromal cell, process validation*

Introduction

Recent scientific advances preclude that regenerative medicine will become an essential component of medical care in the near future [1,2]. The high number and scope of currently active clinical trials will likely make new treatments available to patients in a growing wave of medicinal cell-based products [3,4]. As a consequence of developments in this emerging field, a specific regulatory framework introducing new concepts such as Advanced Therapy Medical Products (ATMP) has developed on methodologies taken from traditional pharmaceutical drugs [5], which are governed in Europe by the Directive 2001/83/EC and Regulation 726/2004, amended by Regulation 1394/2007.

Among all adult somatic cells proposed for clinical application, multipotent mesenchymal stromal cells (MSC) have reached a major prominence. In particular, MSC isolated from bone marrow (BM) display a number of biological properties that qualify them

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for clinical use, either based on their stem or stromal properties [6,7]. These include migratory, homing and differentiation potential toward mesodermal lineages [8–10], as well as a powerful paracrine activity involving regulation of inflammation, immune response and tissue regeneration [11,12]. Such pleiotropic activity has served as rationale in several hundred clinical trials worldwide, mostly in the autologous therapy setting. However, the major weakness is the lack of common standards in cell production processes and their quality controls, thus challenging the reliability of meta-analyses conducted on data from those clinical trials. In this sense, adherence to white papers and compliance with voluntary accreditation schemes and mandatory Good Scientific Practice (GxP) regulations may play a major role in the understanding and comparability of clinical results when using MSC-based medicines [13–16].

In the present work, we described the design and validation of a Good Manufacturing Practice (GMP)-compliant manufacture process within a small academic laboratory and the subsequent production of 48 consecutive GMP-compliant batches of BM-MSC for use in a Phase I/IIa clinical trial for the treatment of chronic osteoarthritis and compassionate treatments [17], thus confirming the feasibility, consistency and reproducibility of the bioprocess, and safety of the ATMP. It includes a description of quality controls, key technical aspects and areas of potential improvement on standardization and quality assurance.

Material and methods

Production of clinical-grade BM-MSC

Within the context of a prospective, open-label, single-dose, single-arm Phase I–IIa clinical trial (Eudra-CT, 2009-016449-24; ClinicalTrials.gov identifier, NCT01227694) conducted by Institut de Teràpia Regenerativa Tissular (ITRT) at the Hospital Quirón Teknon (HQT) from October 2010 to June 2012, and compassionate treatments related to the clinical study, a cell-based therapy product for clinical use was manufactured in GMP-certified facilities (Banc de Sang i Teixits) [18,19]. Appropriate donor informed consent for research use was obtained according to procedures approved by HQT's Ethics Committee and the Spanish Agency of Medicines and Medical Devices (AEMPS).

For the production of the medicinal product, a GMP-compliant bioprocess was designed including a derivation step of MSC from BM aspirates and *ex vivo* expansion in an approximately 21-day protocol.

BM samples were harvested from the posterior iliac crest of patients and introduced in heparinized collection bags (Miltenyi Biotech) for subsequent shipment to the processing facility in temperature-monitored containers (Sarstedt). BM samples were tested on receipt to verify that they fulfilled the required specifications, which included cell count, viability assessment, sterility tests (according to European Pharmacopeia, EuPh, 2.6.27) and detection of the presence of *Mycoplasma* (EuPh 2.6.7) (Tables I and II).

An automated Sepax device (Biosafe) and Ficoll-Paque reagent (GE Healthcare) were used for the isolation of nucleated cells (NC). Then NC were washed and resuspended in Dulbecco's Modified Eagle's Medium-High Glucose (DMEM-HG; Life Technologies) supplemented with 10% human serum (hSer; Banc de Sang i Teixits). Cells were plated at 2×10^5 cells/cm^2 onto cell culture vessels (CellSTACK). All cultures were maintained at 37°C and 5% CO_2 in humidified incubators. The medium was changed every 3–4 days and trypsinization was performed using 0.05% Trypsin/ethylenediaminetetraacetic acid (EDTA; Gibco, Life Technologies), when 70–90% confluence was reached at day 10. Then cells were replated at 1000 cells/cm^2. On day 21, cells were harvested and washed with a saline solution (Plasmalyte; Baxter) and resuspended in saline solution supplemented with 2% human serum albumin (HSA; Albutein, Grifols).

During the process, intermediate products were analyzed to verify compliance with the established criteria (Table II). The finished product consisted of $40 \pm 10 \times 10^6$ viable cells with 24-hour shelf life at room temperature. Final volume varied according to the intended use either in the ankle or the knee (that is approximately 3.5 and 10.5 mL, respectively). Single-use sterile consumable kits were developed in our laboratory specifically for each cell culture manipulation step to ensure a semi-close culture bioprocess. According to GMP regulations, microbiological contamination and environmental particle levels were continuously monitored in critical equipment and clean room facilities during the manufacturing process. Cumulative population doublings (CPD) were determined as CPD = [ln (initial cell density/final cell density)]/ln 2.

Media fill

The Media Fill procedure included the complete manufacturing operations using fertility-tested DMEM-HG/GlutaMAX (Gibco, Invitrogen) culture medium instead of reagents and cells used in the actual bioprocess according to current regulations [20]. At the end of the process, the final containers were filled and incubated in a temperature-controlled incubator as per Pharmaceutical Inspection Convention (PI007-6, January 1, 2011). Media Fill was considered satisfactory when no evidence of turbidity was observed by visual inspection in three consecutive process runs.

To verify the fertility of the media used, no more than 100 colony-forming unit (CFU) of each micro-