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Clinical grade expansion of MSCs

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ABSTRACT

Producing advanced therapy medicinal products (ATMP) according to Good Manufacturing Practice (GMP) guidelines represents a global challenge for the expansion of cells intended for human use. Mesenchymal stromal cells (MSCs) from different sources are one of the most actively developed cell type for a variety of clinical applications in cellular therapy. Complying with GMP means defining accurately both the production process and the release criteria required for a final safe product. We have here reported our manufacturing experience on 103 consecutive clinical-grade *in vitro* expansions of both bone marrow-derived and umbilical cord-derived mesenchymal stromal cells together with description of methods and reagents utilized in our Cell Factory. The same animal- and serum-free medium, additioned with human platelet lysate, has been used for all the expansions performed. This is the largest experience published so far with this alternative and clinical-grade reagent (compared to the traditional fetal bovine serum) and shows the feasibility and the reproducibility of the method. Indeed, we have been able to produce a sufficient number of MSCs to treat 57 patients so far, enrolled in 7 different experimental phase I/II protocols.

1. Introduction

Mesenchymal stromal cells (MSCs) have been hypothesized to play a major role for the therapy of several severe human conditions, both assuming a role in regenerative medicine (based on their "stemness" and differentiating capacities) [1–3] and also in inflammatory/autoimmune disorders (based on their anti-inflammatory and immunoregulatory activity) [4–10]. Mechanistically, MSCs have been supposed to act both by direct infiltration of the target organs [11–15] but also as producers of soluble factors and exosomes which may act as paracrine at a distance [16–18].

When we entered the "somatic cell therapy" arena, our original aim was to try and find solid therapeutic alternatives for severe clinical conditions still lacking consolidated therapeutic options. As the best example, grade III-IV steroid resistant Graft *versus* Host Disease (GvHD) in hematopoietic stem cells transplanted patients represents a deadly and extremely painful condition which, at the moment, has not found a validated second or third line therapeutic treatment [19–22]. In our Department of Hematology and Bone Marrow Transplantation, approximately 10 patients per year suffer from this clinical complication and that was the main motivation to try and introduce treatment using bone marrow-derived MSC, as originally suggested by Le Blanc et al. [23].

It was immediately clear that, in order to introduce Advanced Therapy Medicinal Products (ATMP) as a clinical option in a General Public Hospital, we needed to comply with a large body of European (European regulation 1394/2007, amending European Directive 2001/83/EC) and Italian laws (only in extreme synthesis summarized in the D.L. 24/04/06 n.219 "Codice Comunitario"), schematically defined as Good Manufacturing Practice (GMP), and to be inspected and approved by the Italian Regulatory Authority AIFA (Agenzia Italiana del Farmaco). Similar regulations are in place in other countries around the world. Many authors [24–27] have provided a thorough summary of these regulatory requirements. Moreover, clinical trials have to be conducted according to Good Clinical Practice (GCP) guidelines (EU Directives 2001/20/CE and 2005/28/CE). Thus, only fully authorized GMP compliant facilities can release cell products for experimental clinical trials, following the presentation of a detailed Investigational Medicinal Product Dossier (IMPD) and consequent approval by the National Health body, Istituto Superiore di Sanità (ISS) and, more recently, also by AIFA (for experimental phase I/II studies), in addition to each local Ethical Committee of the participating centers. Therefore we built a

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Cell Factory containing two class B clean rooms and obtained a first approval on 28/11/2008 (n.aM-189/2008), an authorization which has been confirmed up to the present day.

2. MSCs production processes and reagents

A variety of protocols describing the isolation of MSCs from bone marrow aspirates have used density gradient centrifugation to enrich the mononuclear cell fraction. Importantly, products used for clinical-scale manufacturing have to comply with GMP standards, and a variety of these media are now indeed available [28]. Manual Ficoll gradient centrifugation can now also be replaced by completely automated procedures. An automated cell separation system has been developed and it has been demonstrated to yield significantly enhanced cell recoveries when it was compared to the manual Ficoll separation [29,30]. Nevertheless, to facilitate GMP compliance, it is mandatory to reduce at the minimum all the manipulation necessary to produce any cellular product. Thus, we have established a procedure which allows the direct seeding of whole bone marrow with no density gradient centrifugation step.

In addition MSCs have been historically cultured in basal medium (DMEM or alpha-MEM) with the addition of fetal bovine serum (FBS) as a source of growth factors, cytokines and mitogens [31–35], raising a general concern for public health due to the possible diffusion of communicable diseases, such as prion-transmitted bovine spongiform encephalopathy (BSE) and therefore posing a long-term health-risk. Furthermore, FBS batches may differ from one to another which could deeply impact on the proliferation rate and hamper reproducibility and consistency of the production process. FBS may involve the risk of transferring xenogeneic proteins into the recipient and inducing host immune responses against foreign antigens. Immunogenicity against FBS proteins has demonstrated to compromise the therapeutic benefit of MSCs [36]. Thus although GMP-compliant FBS batches are available and used in clinical grade manufacturing, the regulatory authorities encourage to replace FBS with a non-xenogeneic alternatives. We therefore set out to replacing FBS with the human platelet lysate (PL), a blood-derived products prepared as a clinical grade reagent at the Transfusion Center of the Hospital, as already detailed [37]. PL is known to be a rich source of platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor β1 (TGF-β1) and insulin-like growth factor (IGF), and evidence from the literature suggested that it contains all the components required to expand MSCs [38–40]. In addition to the compliance with GMP standards, we can monitor and trace all the healthy donors who contributed to the platelet lysate batches we use in the productions. In the literature, a vast number of papers have been recently published to support the idea to replace FBS with human PL [38,41–48]. We have already demonstrated that PL has better effects than FBS on proliferation capacity of BM-MSC, therefore providing more efficient expansion, together with significant time saving [37]. Moreover, the *in vitro* immunophenotypic and immune regulatory properties of PL-expanded MSCs resulted to be comparable with those of MSCs cultured in the presence of FBS. Similar results were also confirmed by Bernardo et al. [49]. Recently Ikebe et al. reviewed a total of 47 reports of clinical trials of BM-derived MSC-based therapy published between January 2007 and June 2013, which had described the methods of isolation and expansion of clinical MSCs and reported that more than 10% of the trials used PL [50].

It is worth noting that the majority of current clinical trials have been conducted with cells detached by enzymatic dissociation using porcine-derived trypsin. Similarly to FBS replacement, enzymatic cell treatment can be optimized using recombinant trypsin which is more gentle on cells, does not require neutralization and does not contain xenogeneic proteins.

Furthermore all reagents used in Cell Factory for the manufacturing of cell-based medicinal products should be well-defined and controlled, the suppliers should be validated and the companies have to provide certifications attesting the suitability of their reagents for use in the generation of cell products to be used in humans. Therefore, reducing to a minimum all the manipulation steps and using a totally animal-free clinical grade expansion medium, we have been able to successfully establish a fully GMP-compliant expansion procedure for MSCs [51,52].

3. MSC manufacture in our GMP-facility

We review here the quality data on our own MSCs productions, from 2010 to the present day. We have used two sources of MSCs, bone marrow, in the form of explants or bone marrow filter washouts (as previously detailed [37,52]) and umbilical cord [51]. In general, the starting "raw" material is a critical issue and must comply both with the Directive 2006/86/EC and with the Directive 2006/17/EC on the donation, procurement and testing of human tissues and cells. In particular, donor samples must be screened for HIV, HBV, HCV, Syphilis within 30 days prior to donation and prove to be negative.

The manufacturing procedure and the subtle differences in the expansion methods of MSCs from the different sources are illustrated in Fig. 1. As far as umbilical cord (UC) is concerned, transversal sections of approximately 5 cm are cut into small pieces avoiding the use of any collagenase or other digestive enzymes, directly seeded in Petri dishes until first passage (P1), then expanded in one 5-layers flask (up to second detachment, P2) and subsequently seeded into multiple 5-layers flasks (up to six) up to final product (P3). The multilayer systems provide a large growth surface in limited surface areas together with easy handling and low risk of contamination. Furthermore, manual procedures are reduced to a very few operations [53,54]. At P2 detachment we freeze intermediate vials for future expansions (P2 banking). The total median UC-MSC yields at P1, P2 and P3 are indicated in Fig. 1. By contrast, the bone marrow (BM) is directly seeded in one 5-layers flasks up to first harvesting (P1) and then subsequently cells are expanded in multiple (up to six) 5-layers flasks until final harvesting (P2). In this case, the P1 cells are used for banking in vials for further productions. The total BM-MSC yields at P1 and P2 are indicated in Fig. 1. As mentioned above, bone marrow can be in the form of total explanted bone marrow or can be the material trapped in the filter, after filtration of bone marrow. This material, which potentially contains aggregates and bone fragments, is normally discarded during the procedure of hematopoietic stem cell transplantation (HSCT) and is a rich source of MSCs [52].

A more detailed quantitative analysis of our MSCs production in the last 5 years is shown in Table 1. In this analysis, we show what is the real potential for clinical application of PL-conditioned medium on a large number of procedures performed by the same team, in the same facility, with the same methodology. We have used 33 unmanipulated bone marrow explants to produce 42 frozen bags and 28 have been infused in 28 patients. In this case, the frozen 40 vials produced at P1 have never been used for further expansions, due to the design of the clinical project, which indicated the infusion of a single bag of "autologous" cells. Using filter washouts material, 27 samples have allowed the production of 50 frozen bags (31 have been infused to patients) and 93 frozen vials (at P1). Thirty of such P1 vials have been used subsequently for the production of other 75 bags (58 have been infused to patients). Overall,