Cytotherapy, 2011; 13: 1031–1035

informa
healthcare

PROTOCOLS IN CYTOTHERAPY

Autologous bone marrow mononucleated cell preparation for the clinical treatment of acute myocardial infarction and peripheral arterial disease

GIUSEPPE ASTORI[1], FRANCO BAMBI[2], GIANNI SOLDATI[3], DANIEL SÜRDER[3] & TIZIANO MOCCETTI[3]

[1]*Laboratory of Advanced Cellular Therapies, Department of Hematology, S. Bortolo Hospital, Vicenza, Italy (formerly at Cell Therapy Unit, Cardiocentro Ticino, Lugano, Switzerland),* [2]*Blood Bank and Cell Therapy Unit, Department of Hemato-Oncology, Children's Hospital A. Meyer, Florence, Italy, and* [3]*Cell Therapy Unit, Cardiocentro Ticino, Lugano, Switzerland*

Key Words: *Cellular Therapy, GMP, AMI, PAD, IND, IMP, ATMP*

Introduction

The concept that adult stem/progenitor cells can differentiate into either hematopoietic or non-hematopoietic tissues is supported by a considerable amount of reviewed data (1). Adult mononucleated cells (MNC) containing the stem/progenitor cell fraction can be isolated from mobilized peripheral blood and bone marrow (BM) tissue by density gradients (2). BM MNC also contain mesenchymal stromal cells (MSC) (3), representing less than 0.1% of the density-gradient selected cells and capable of generating non-hematopoietic tissues (4,5). Endothelial progenitor cells (EPC), with their clonogenic potential, are major contributors to angiogenesis (6). As improved cardiac function depends on revascularization, local BM MNC delivery represents a strategy for supplying the reparative effects of EPC in injured hearts (7). Apart from myocardial and vascular regeneration as mechanisms of stem cell action, other models have been proposed where the transplanted cells could release soluble factors that, acting in a paracrine fashion, would contribute to cardiac repair and regeneration (8–10).

Clinical applications and eligibility

Cardiac regeneration

There is now clear evidence that BM MNC engraft, survive and grow within the infarcted myocardium by forming junctional complexes with resident myocytes (11). Several clinical trials have shown that primary percutaneous intracoronary (IC) BM MNC injections can be used to treat patients with acute myocardial infarction (AMI) successfully (12). Other studies have used direct intramyocardial BM MNC injections during coronary artery bypass graft surgery (CABG), mostly in the border zone of the infarcted myocardium (13). The use of electromechanical mapping to identify viable tissue represents a possible approach to improving the efficacy of cells injected directly into the myocardium.

Although promising, these studies represent a heterogeneous group of phase I clinical trials. A systematic review and meta-analysis of the literature suggests that bone marrow cells (BMC) transplantation is safe and associated with modest improvements in left ventricular function, remodeling and scar size in patients with both AMI and ischemic

Correspondence: Dr. **Giuseppe Astori**, Laboratory of Advanced Cellular Therapies, Department of Cell Therapy and Hematology, S. Bortolo Hospital, Contrà S. Francesco 41, VI-36100 ITALY.

(Received 11 April 2011; accepted 6 June 2011)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.605620

cardiomyopathy (ICM), While the benefit seems modest, the results support the need for further large randomized trials (12,14).

Peripheral arterial disease (PAD)

Despite recent advances in surgical and interventional techniques, about 40% of patients with critical limb ischemia (CLI) are not eligible for revascularization because of the anatomical location of the lesions, the extent of the disease or extensive co-morbidity (15,16). In the absence of effective pharmacologic therapy (17,18), amputation is often the only option but peri-operative mortality is 5–20% and a second amputation is required in 30% of cases. Consequently, EPC represent a potentially important new therapeutic strategy. About 700 patients with peripheral arterial disease (PAD) without revascularization options entered into 37 clinical trials are described in a comprehensive meta-analysis (19). Cell therapy significantly improved the ankle-brachial index, transcutaneous oxygen tension, rest pain, pain-free walking distance, ulcer healing and limb salvage. The route of cell administration was mostly intramuscular, intra-arterial or a combination of the two routes.

Regulatory issues

In the USA, publications regulating the use of cell therapy products are codified within the Code of Federal Regulations in the following sections: IND regulations (21 CFR 312), http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart = 312&showFR = 1, biologics regulations (21 CFR 600), http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart = 600, and cGMP (21 CFR 211), http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart = 211 (accessed date: 24 may 2011). In particular, the USA federal regulation on cellular therapy is divided into two sections of the Public Health Service Act, referred as '361 products' and '351 products', http://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/RegulationofTissues/ucm150485.htm. Traditional blood and bone marrow progenitor cells as well as other tissues for transplantation fall into 361 product definitions and are codified under the Good Tissue Practice (GTP).

The European Union (EU) regulation (1394/2007) on advanced therapy medicinal products (ATMP), http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = OJ:L:2007:324:0121:0137:en:PDF, came into force in December 2008. This regulation makes reference to and is in accordance with the 2004/23/EC directive on donation, procurement and testing of human cells and tissues, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = OJ:L:2004:102:0048:0058:en:PDF, and with directive 2002/98/EC on human blood and blood components, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = OJ:L:2003:033:0030:0040:EN:PDF. This means that any use of human cells has to be in compliance with the quality requirements therein described. Both EU and USA regulations are clear on requiring that cells have to be prepared according to good manufacturing practice (GMP), http://ec.europa.eu/health/documents/eudralex/vol-4/index_en.htm.

Table I. Proposed acceptance criteria for fresh BM.

Test	Acceptance criteria
Cell viability	≥ 80%
Phenotypic analysis of cell markers CD45, CD34 and CD133	FIO
MNC cell count	FIO
Microbiologic control of cellular products (Eu.Ph. 2.6.27)	Sterile

FIO: For information only; EuPh: European Pharmacopoeia.

Methods

The manufacture of cells as medicinal products requires compliance with all aspects of GMP. The entire manufacturing process should be validated with the aim of demonstrating that each step of the manufacturing process is well controlled.

Qualified medical professionals should collect BM MNC; cells should be sent to the production facility under controlled temperatures; the storage time limit should be determined based on stability testing. Cell viability, counting, phenotypic analysis and microbiologic control on the initial BM sample should be performed, and acceptance criteria defined. Certain parameters (e.g. the percentage of certain cell markers) may be reported as 'for information only' (Table I). Complete traceability of the ancillary reagents should be available. MNC cells are separated from erythrocytes and granulocytes after density-gradient centrifugation (1.073–1.077 g/mL); a sample of the final product should be retained for quality control (QC) analysis. If the BM MNC are used for cardiac regeneration by IC administration, cells are typically resuspended in 10 mL of an injectable media. The syringe is defined as the primary container and should be labeled as indicated by GMP for an investigational new drug (IND)/investigational medicinal product (IMP).

Table II. Validation of analytical methods.

Test	Validated parameters	Guideline
Cell viability	Precision Accuracy	ICHQ2 (R1)
Mononucleated cell count	Precision Accuracy	ICHQ2 (R1) Eu.Ph. 2.7.29
Microbiologic control of cellular products	Sensitivity Specificity Repeatability	Eu.Ph. 2.6.27
Sterility	Sensitivity Specificity Repeatability	Eu.Ph. 2.6.1 USP<71> 21 CFR 610.12
Endotoxin content	Sensitivity Specificity Repeatability	 Eu.Ph. 2.6.14 USP<85>

ICH: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; Q: the Code of the guideline; USP: US Pharmacopoeia; CFR: Code of Federal Regulations.

Product

The cell product must be subjected to release testing. Based on pre-clinical and/or clinical observations, an acceptance cell viability range should be specified (20) and the minimum and maximum cell content should be declared based on safety considerations related to the site and route of implantation. For the cell population of interest, the percentage of cell purity should be specified as well as the limit acceptance for undesired populations (red blood cells (RBC), platelets (PLT), granulocytes, etc.). The endotoxin content quantified by using the methods described in the EU (Eu.Ph. 2.6.14, Bacterial Endotoxins) or USA (Text for<85>Bacterial Endotoxins Test, USP 33 Reissue, published April 2010 and official 1 October 2010) pharmacopeia constitutes release criteria (21,22). Cell container should be assessed visually for integrity, correct labeling and absence of macro-aggregates. Analytical methods should be validated according to specific pharmacopoeia chapters and The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (Table II), http://private.ich.org/LOB/media/MEDIA417.pdf (accessed date: 5 June 2011).

The maintenance of cell integrity and product stability should be ensured: a shelf life, based on a stability testing, should be assigned to the cell product, http://private.ich.org/LOB/media/MEDIA419.pdf. Once released, a container able to maintain the declared temperature for the maximum interval of time established should be used.

The identity of the cells should be defined in terms of their phenotypic profile. MNC cells should be characterized at least in terms of expression of CD45, CD34 and CD133. While these markers do not relate strictly to the regenerative actions of BM MNC, they represent surrogate markers linked to a function already validated by published data. The cell product should be free from adventitious microbial agents: testing for aerobes, anaerobes and fungi must be performed by using the methods described in the EU (Text 5.1.6: Alternative Methods for Control of Microbiologic Quality 01/2008:50106 and General Method 2.6.27: Microbiologic Control of Cellular Products) and USA (USP<71>, Sterility Testing and Guidance for Industry 'Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products, draft', http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm078696.pdf) pharmacopeias.

Cell potency is the quantitative measure of biologic activity based on the attribute of the product and its relevant biologic properties. The importance of characterizing the function of injected cells has been pointed out (23,24). The *in vivo* invasion capacity of BM MNC has been proposed as a potency testing based on the observation that it predicts functional improvement after cell transplantation in ischemic tissue (25,26).

Concerning tumorigenicity, a number of clinical trials have evaluated the safety of selected and unselected BM MNC. For cardiac regeneration or PAD, as cells are autologous and minimally manipulated, the risk could be considered low. An example of release criteria for BM MNC used for IC infusion is reported in Table III. Specific guidelines have been issued by European Medicine Agency (EMA)/ Committee for Medicinal Products for Human Use (CHMP), http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003898.pdf,

Table III. Proposed release criteria for BM MNC obtained after density-gradient centrifugation of 50 mL fresh BM.

Test	Acceptance criteria
Cell viability	$\geq 80\%$
Phenotypic analysis of cell markers CD45, CD34 and CD133	FIO
MNC concentration	From 5.5×10^6 to 55×10^6/mL
Lymphocytes	$\geq 25\%$
Monocytes	$\geq 4\%$
Granulocytes	$\leq 60\%$
Platelets	$\leq 22 \times 10^7$/mL
Hematocrit	$\leq 3\%$
Bacterial endotoxins	<5.00 EU/mL
Visual appearance	Clear absence of micro-aggregates/colorless to intensely hematic
Microbiologic control of cellular products (Eu.Ph. 2.6.27)	Sterile

EU: Endotoxin Unit; FIO: For information only.

and FDA, http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm081670.pdf.

Discussion

With the new EU regulation in force, although the isolation of BM MNC represents a minimal manipulation, by virtue of its non-homologous use reinfusion is not allowed without an investigational medicinal product dossier (IMPD). In the USA, reinfusion is allowed when it is performed during the same clinical procedure and for an autologous use.

BM MNC represent an important cell source for cell-based therapies: the ease of collection, combined with minimal manipulation, has meant that many clinical trials have been carried out, revealing both the safety and efficacy of its use in many conditions. Concerning the cell processing described here, the need to process a patient's cellular sample immediately upon receipt, together with an immediate product delivery and reinfusion, requires foreknowledge of when and where the cells will be reinfused. This implies that scheduling the sample collection, processing and release/delivery is critical, requiring a well-designed manufacturing system.

A crucial aspect is the quality of the starting material: there is variability in the number of BM MNC obtained in the final product, linked to the quality of the BM collection (presence of clots and peripheral blood (PB) in the sample) and underlying the importance of a physician's training. Despite the fact that devices that can isolate BM MNC in a closed system are available, most BM MNC isolation remains a manual process and is therefore subject to operator variability, demanding a rigorous operator training program. Regarding QC, the volume of the sample to be collected pre- and post-processing for viability, cytofluorimetric analysis, sterility, Limulus amebocyte lysate (LAL) testing and counter sampling, requires a volume of product representing a significant proportion of the total cell volume. Finally, as the cells are reinfused shortly after processing, the results of sterility testing may not be available at the time of reinfusion: a solid validation of the aseptic manufacturing process is mandatory.

Acknowledgments

We are grateful to the 'Fondazione Progetto Ematologia, Vicenza, Italy' for its kind support of this paper.

Declaration of interest: The authors declare no competing financial interests.

Disclaimer: The information in this article is intended for educational purposes only and should not be considered as a standard of care or recommended treatment for any particular patient.

References

1. Kuci S, Kuci Z, Latifi-Pupovci H, Niethammer D, Handgretinger R, Schumm M, et al. Adult stem cells as an alternative source of multipotential (pluripotential) cells in regenerative medicine. Curr Stem Cell Res Ther. 2009;4:107–17.
2. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest. 1968; 97(Suppl):77–89.
3. Fibbe WE, Noort WA. Mesenchymal stem cells and hematopoietic stem cell transplantation. Ann NY Acad Sci. 2003; 996:235–44.
4. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7.
5. Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci USA. 2000;97:3213–8.
6. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circ Res. 2004;95:343–53.
7. Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. Circ Res. 2004;95:354–63.
8. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res. 2008;103: 1204–19.
9. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005;11:367–8.
10. Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006;20:661–9.
11. Rota M, Kajstura J, Hosoda T, Bearzi C, Vitale S, Esposito G, et al. Bone marrow cells adopt the cardiomyogenic fate in vivo. Proc Natl Acad Sci USA. 2007;104:17783–8.
12. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med. 2007;167:989–97.
13. Stamm C, Kleine HD, Choi YH, Dunkelmann S, Lauffs JA, Lorenzen B, et al. Intramyocardial delivery of $CD133^+$ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. J Thorac Cardiovasc Surg. 2007;133:717–25.
14. Sürder DSJ, Moccetti T, Astori G, Rufibach K, Plein S, Lo Cicero V, et al. Cell-based therapy for myocardial repair in patients with acute myocardial infarction: rationale and study design of the SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction (SWISS-AMI). Am Heart J. 2010;160:58–64.

15. Standards of Practice Committee of the Society of Cardiovascular and Interventional Radiology. Guidelines for percutaneous transluminal angioplasty. Radiology. 1990;177:619–26.
16. Valentine RJ, Myers SI, Inman MH, Roberts JR, Clagett GP. Late outcome of amputees with premature atherosclerosis. Surgery. 1996;119:487–93.
17. Schainfeld RM, Isner JM. Critical limb ischemia: nothing to give at the office? Ann Intern Med. 1999;130:442–4.
18. Hiatt WR. Medical treatment of peripheral arterial disease and claudication. N Engl J Med. 2001;344:1608–21.
19. Fadini GP, Agostini C, Avogaro A. Autologous stem cell therapy for peripheral arterial disease meta-analysis and systematic review of the literature. Atherosclerosis. 2010;209:10–7.
20. U. S. Department of Health and Human Services, Food and Drug Administration: Center for Biologics Evaluation and Research, Rockville, MD. April 2008.
21. European Pharmacopoeia, 7th edition, 2010. EDQM, Strasbourg, France and US Pharmacopoeia, 2011 Rockville, Maryland, USA.
22. Soncin S, Lo Cicero V, Astori G, Soldati G, Gola M, Surder D, et al. A practical approach for the validation of sterility, endotoxin and potency testing of bone marrow mononucleated cells used in cardiac regeneration in compliance with good manufacturing practice. J Transl Med. 2009;7:78.
23. Dawn B, Bolli R. Bone marrow for cardiac repair: the importance of characterizing the phenotype and function of injected cells. Eur Heart J. 2007;28:651–2.
24. Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S. Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. Eur Heart J. 2007;28:766–72.
25. Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation. 2004;109:1615–22.
26. Britten MB, Abolmaali ND, Assmus B, Lehmann R, Honold J, Schmitt J, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation. 2003;108:2212–8.