Histol Histopathol (2005) 20: 901-906
DOI: 10.14670/HH-20.901

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Labeling of adult stem cells for *in vivo*-application in the human heart

J.M.I. Wiehe[1], O. Zimmermann[1], J. Greiner[2], J.M. Homann[1], M. Wiesneth[3], V. Hombach[1] and J. Torzewski[1]
[1]Department of Internal Medicine II, University of Ulm, Ulm, Germany, [2]Department of Internal Medicine III, University of Ulm, Ulm, Germany and [3]Department for Clinical Transfusion Medicine and Immunogenetics, University of Ulm, Ulm, Germany

Summary. Tissue regeneration with human hematopoietic or mesenchymal stem cells has become a fashionable research topic. In cardiology, intracoronary injection of adult stem cells has already been used for the treatment of human myocardial infarction and ischemic cardiomyopathy. The experimental background of such therapies, however, i.e. the potential of adult stem cells to regenerate myocardium through "transdifferentiation" of hematopoietic or mesenchymal stem cells into cardiomyocytes described in animal models, has recently been challenged by other experimental data. Nonetheless, clinical trials are continuing. This may be due to the fact that, in open-labeled pilot trials, a benefit of intracoronary injection of adult stem cells for the treatment of myocardial infarction has been described. As pilot trials may overemphasize the beneficial effects of intracoronary injection of bone marrow stem cells, controled double-blinded randomised multicenter studies are warranted. Furthermore, a careful characterization of the cells involved in the proposed cardiac repair as well as *in vivo*-monitoring of such cells following intracoronary injection in humans might help to answer many essential questions linked to this important research topic. The latter requires biocompatible labeling. This review focusses on the technologies available for stem cell labeling and summarizes the arguments and contra-arguments to use these labeling technologies for application in humans.

Key words: Hematopoietic stem cells, *In vivo* imaging, Homing, Transdifferentiation

Offprint requests to: Jan Torzewski, MD, M.phil, University of Ulm, Department of Internal Medicine II-Cardiology, Robert Koch Str.8, 89081 Ulm, Germany. e-mail: jan.torzewski@medizin.uni-ulm.de

Introduction

Recently, hematopoietic stem cells and bone marrow derived mesenchymal stem cells have been proposed to participate also in the regeneration of solid tissues and organs (Takahashi et al., 1999; Lagasse et al., 2000; Orlic et al., 2001; Jiang et al., 2002; Toma et al., 2002). This proposed capacity is termed plasticity. In general, the term "plasticity" refers to the proposed ability of adult stem cells to cross over lineage barriers and to adopt the expression profiles and functional phenotypes of cells unique to other tissues. Findings in this novel field, however, are controversially discussed. In the special area this review is placed in, i.e. cardiac regeneration using adult stem cells, the interpretation of data obtained so far ranges from the notion that adult bone marrow stem cells are very able to "transdifferentiate" into cardiomyocytes (Orlic et al., 2001) to the notion that there is no evidence of transdifferentiation at all (Balsam et al., 2004; Murry et al., 2004).

A torrent of studies have been carried out in animal models on how adult bone marrow stem cells might contribute to myocardial regeneration. The results were indeed conflicting. Nonetheless, clinical trials investigating the safety and efficiacy of cell therapy for the treatment of myocardial infarction have begun (Strauer et al., 2001; Assmus et al., 2002; Stamm et al., 2003; Kang et al., 2004; Wollert et al., 2004). In view of the contradictory results obtained from experimental and clinical investigations, identification, localization and follow up observation/fate mapping of autologous stem cells within the human heart is desirable. Technologies to label cells *in vitro* before application, and to consecutively detect them in vivo might provide insight into many essential questions like cell homing, "transdifferentiation", and evaluation of cell-based repair or replacement. Only an understanding of the mechanisms involved in cardiac repair as well as a careful characterization of the cells required may lead to

clinical utility and further improvement of these novel therapies.

Strategies for labeling human adult stem cells

The strategies for labeling stem cells have encompassed directly attaching labels to cells as well as methods for a range of genetic modifications. Direct labeling of adult stem cells for clinical application includes magnetic beads and radionuclides. Genetic labeling is based on introduction of genes that are expressed in the implied cell.

The greatest challenge for clinical use certainly is to adopt the procedures to good manufacture practice (GMP)-guidelines. An application of manipulated stem cells into human beings is associated with high legal restraints and requires permisson of regional ethical committees.

Directly attached labels for in vivo-application in humans

Magnetic beads for magnetic resonance imaging (MRI)

MRI is well suited to three-dimensional (3-D) high-resolution whole-body *in vivo*-imaging. Tagging cells with an intracellular magnetic probe would allow non-invasive and repetetive monitoring of cellular biodistribution. Initial attempts to facilitate endogenous cellular uptake of supramagnetic iron oxide particles included targeting the transferrin receptor via monoclonal antibodies or liposomal coating and membrane fusion. Neither, however, resulted in an uptake efficient for in vivo-tracking, and furthermore, significant cellular toxicity or impact on critical cellular characteristics was observed (Bulte et al., 1992, 1993).

Recently, animal models were used to optimize MR tracking of magnetically labeled mammalian stem cells. Bulte and co-workers labeled cells with magnetodendrimers designed specifically to achieve a high degree of intracellular magnetic labeling that is mediated through a nonspecific membrane adsorption process with subsequent intracellular localization in endosomes (Bulte et al., 2001). Magnetically labeled as well as LacZ-transfected control cells could be detected in mice *in vivo* at least as long as six weeks after transplantation, with a high degree of correlation between the obtained MR contrast and staining for ß-galactosidase expression. Importantly, labeled cells were unaffected in their viabililty and proliferation capacity. This labeling may be useful for virtually any mammalian cell, including human stem cells. In other approaches, it was shown that small superparamagnetic iron particles coated with dextran were taken up into cells via endocytosis which allowed dynamic tracking of loaded oligodendrocyte precursors after infusion into the brain (Franklin et al., 1999). This technology, however, was not sophisticated enough for single cell detection. Recently, micron-scale iron oxide magnetic particles have been developed as intracellular contrast agents for uptake into perinuclear endosomes of cells (Hinds et al., 2003). This procedure permits highly efficient, non-toxic tracking with maintenance of biological activity and preservation of colony-forming and differentiation capacity of the cells. Furthermore, cells can be detected at single cell resolution by MRI.

It is important to note that the majority of contrast agents for MRI are not approved for clinical use by national committees as for example the US Food and Drug Administration (FDA). Only ferumoxides, a suspension consisting of dextran-coated superparamagnetic iron oxides and protamine sulfate (the latter being a transfection agent necessary for incorporation of particles within endosomes) are awarded and commercially available (Arbab et al., 2004). Clinical experience with this agent may allow translation of MR labeling from the experimental setting to clinical trials.

Radiolabeling

Radiolabeling of cells has widely been used to monitor the fate and tissue distribution of blood cells. Imaging of leukocyte distribution, for example, is a routine clinical procedure to localize areas of inflammation (Becker and Meller, 2001; Rennen et al., 2004). For this purpose, commercially available and commonly used radiolabels, as for example ^{111}Indium (^{111}In), have been shown to be safe and easy to use. ^{111}In compounds have successfully been applied in various experimental settings to determine the biodistribution, migration, homing characteristics and physiological recirculation of cells as well as the impact of the labeling procedure on cellular function (Wagstaff et al., 1981; Mackensen et al., 1999; Bohnen et al., 2000; Gao et al., 2001). Radiolabeling of transplanted human CD34+ stem cells with ^{111}In is also feasible for monitoring biodistribution of these cells in rats (Brenner et al., 2004), although over a limited time period of 24-48 hrs only.

Nuclear medicine techniques are continuously developing, but it is unlikely they will reach the levels of sensitivity and resolution that are necessary for detection of small cell numbers or localization of these cells anatomically within organs (Adonai et al., 2002). General difficulties in dealing with, for example, ^{111}In are the limited cellular retention on the one hand, and the irradiation of radiolabeled cells resulting in impaired cellular viability or function on the other hand (Brenner et al., 2004). Because of impairment of stem cell proliferation and differentiation, as well as toxicity of the labeling procedure, radiolabeled stem cells do not seem to be suitable for functional analysis of human adult stem cells in myocardial repair.

Sexual mismatch

Sexual mismatch-labeling is a special type of

labeling that utilizes native cell properties. Therefore, it does not affect cell function. Usually stem cells from a male donor are transplanted to a female recipient, using the Y-chromosome as a donor-specific marker (Deb et al., 2003). Advantages of male-to-female transplantation for the detection of donor derived cells are, first, that the Y-chromosome is present in virtually every intact donor-derived cell and, secondly, that whole-chromosome paint probes make it relatively easy to image the Y-chromosome. Following orthotopic transplantation of human female hearts into males, recipient derived cells have been found in the myocardium (Quaini et al., 2002). However, the Y-chromosome could not be visualized in all male-derived cells, since thin tissue sections from myocardial biopsies were assessed that only partially sample the nuclei. Furthermore, Y-chromosome staining can cause false positive results because of cell overlay (Rakic, 2002). This is of particular concern in cases when non-hematopoietic cells are incorrectly identified as donor-derived, because marrow-derived small blood cells overlay the cell of interest. Finally, it has to be noted that the sex-mismatch tracking procedure is limited to allogeneic transplantation studies. For clinical trials investigating the fate of autologous stem cells, this technique is invalid.

Genetic labeling

Genetic tracking requires a safe procedure of gene delivery into stem cells, as well as the optimisation of the gene transfer strategy in order to increase its efficiency without reducing the viability and the function of the cells. Several approaches have been used to incorporate foreign DNA into stem cells. Genetically modified stem cells have been engineered to express specific genes that do not exist in the tissue of interest. Genes have been introduced using viral or plasmid vectors. Though the principles seem to be easy, several obstacles have to be overcome to reach the goal of efficient and safe labeling of human stem cells for *in vivo* application.

Labeling with viral vectors

One approach of genetic labeling is based on viral vector systems such as retro- or adenoviral vectors (von Kalle et al., 1994; Baum et al., 1996; Frey et al., 1998). In the case of viral vector based gene delivery, the genetic constructs are designed with the aim of controlled expression of the gene of choice. This is achieved by placing the gene under the control of promoters. Enhancement or repression of gene expression is regulated by transcription factors that are repressed, induced, or stabilized by alterations in the microenvironment. Viral transduction often results in highly efficient and stable gene transfer, thereby providing a basis for effective cell tracking in *in vivo* studies. However, stable genomic integration is associated with the risk of silencing tumor suppressor genes or activating oncogenes and may thus promote tumorigenesis. Furthermore, viral infection as well as viral immunogenicity may cause severe problems. Also, virus-mediated gene incorporation may cause stem cell differentiation and loss of self-renewing capacity (Corrias et al., 1998). For all these reasons, genetic modification using viral transduction is problematic for clinical application. Although gene therapy studies were indeed performed some years ago (Cavazzana-Calvo et al., 2000; Aiuti et al., 2002; Hacein-Bey-Abina et al., 2002), a positive ethical vote for clinical studies using viral transduction will be most difficult to obtain in the future.

Labeling with plasmid vectors

The goal of genetic tracking is to label stem cells with a specific marker gene *in vitro* before transplanting them into the tissue of interest. The selection of an appropriate marker gene is of major importance for several reasons: (1) Introduction of genetic elements into cells can be accompanied by an immune response of the recipient, which may neutralize the marker protein or even eliminate the transgenic cell (Verzeletti et al., 1998; Lutzko et al., 1999), (2) Detection of labeled cells in the tissue of interest is possible only in cases where the transgene is not expressed in other cell types in this tissue. (3) The transgene must not have any toxic influence on cell viability. (4) Detection of the marker protein has to be easy and efficient. (5) The transgene has to be approved by the responsible committee. In humans, there are hitherto two marker genes that were applied for clinical in vivo-studies: the truncated form of the low affinity nerve growth factor receptor (deltaLNGFR) and the neomycin-phosphotransferase gene (NEO) (Mavilio et al., 1994; Bonini et al., 1997, 2003; Ruggieri et al., 1997; Verzeletti et al., 1998; Li et al., 2002). Other markers, commonly used for animal *in vivo*-studies, are green fluorescent protein (GFP) or LacZ. These genes, however, are immunogeneic antigens that might induce immunological reactions. Since there are no adequate antibodies to detect NEO in tissue slides, labeling with deltaLNGFR seems to be the method of choice. LNGFR is a cell surface growth factor receptor expressed in neurons (Hefti et al., 1986). The cytoplasmatic domain of deltaLNGFR is truncated, which inhibits signal transduction. DeltaLNGFR has already been used in clinical trials and has been considered as an eligible marker gene. Recently, Bonini et al. provided a summary of all studies, human as well as animal, utilizing deltaLNGFR (Bonini et al., 2003). This report evaluates deltaLNGFR labeling as non-toxic and safe.

Another challenge is the technology used to implement the marker gene. Conventional transfection techniques like electroporation or lipofection fail to meet the requirements. Lipofection is not efficient enough and also, cells get exposed to serum free conditions during

the procedure, which may lead to differentiation.

Furthermore, the necessary reagents are not GMP adopted. Although electroporation does not encounter problems like contamination or immunogenicity, the procedure results in high viability loss due to "electroporation toxicity" (Li et al., 2001). Thus, it seems not applicable for clinical trials. A novel non-viral transfection method is the nucleofection technology (Greiner et al., 2004). Nucleofection is an electroporation-based method: a combination of specific buffer solution and electric parameters achieves delivery of plasmid DNA directly into the cell nucleus resulting in a markedly enhanced gene expression. The essential advantages of nucleofection are the following: (1) In contrast to viral transduction this transfection method is transient and thus, does not lead to genomic integration of the transgene. (2) In contrast to other transfection methods, nucleofection is highly efficient. (3) Cell viability and differentiation capacity are not considerably affected, and cells maintain their physiological properties (Greiner et al., 2004; Lakshmipathy et al., 2004). (4) The procedure is currently being adopted to GMP-guidelines and to the demands of regional ethics committees. Taken together, nucleofection is an appropriate method to achieve high transfection efficiency for adult human stem cells and retains stem cell properties. Therefore, nucleofection may be superior to other currently available technologies.

In summary, labeling of adult stem cells with deltaLNGFR using nucleofection may be the most practicable of the methods available for in vivo-tracking in humans today. Immunohistochemistry represents an established tool for detection of deltaLNGFR labeled cells in *ex vivo* tissue specimen (as obtained, for example, by myocardial biopsy). For future perspective, MR imaging techniques may represent the most promising approach for non-invasive detection of labeled stem cells. Improvement of resolution for single cell detection and development of contrast agents suitable for *in vivo* application, however, remain substantial challenges.

References

Adonai N., Nguyen K.N., Walsh J., Iyer M., Toyokuni T., Phelps M.E., McCarthy T., McCarthy D.W. and Gambhir S.S. (2002). Ex vivo cell labeling with 64Cu-pyruvaldehyde-bis(N4-methylthiosemicarbazone) for imaging cell trafficking in mice with positron-emission tomography. Proc. Natl. Acad. Sci. USA 99, 3030-3035.

Aiuti A., Slavin S., Aker M., Ficara F., Deola S., Mortellaro A., Morecki S., Andolfi G., Tabucchi A., Carlucci F., Marinello E., Cattaneo F., Vai S., Servida P., Miniero R., Roncarolo M.G. and Bordignon C. (2002). Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science 296, 2410-2413.

Arbab A.S., Yocum G.T., Kalish H., Jordan E.K., Anderson S.A., Khakoo A.Y., Read E.J. and Frank J.A. (2004). Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI. Blood 104, 1217-1223.

Assmus B., Schachinger V., Teupe C., Britten M., Lehmann R., Dobert N., Grunwald F., Aicher A., Urbich C., Martin H., Hoelzer D., Dimmeler S. and Zeiher A.M. (2002). Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 106, 3009-3017.

Balsam L.B., Wagers A.J., Christensen J.L., Kofidis T., Weissman I.L. and Robbins R.C. (2004). Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 428, 668-6673.

Baum C., Eckert H.G., Stockschlader M., Just U., Hegewisch-Becker S., Hildinger M., Uhde A., John J. and Ostertag W. (1996). Improved retroviral vectors for hematopoietic stem cell protection and in vivo selection. J. Hematother. 5, 323-329.

Becker W. and Meller J. (2001). The role of nuclear medicine in infection and inflammation. Lancet. Infect. Dis. 1, 326-333.

Bohnen N.I., Charron M., Reyes J., Rubinstein W., Strom S.C., Swanson D. and Towbin R. (2000). Use of indium-111-labeled hepatocytes to determine the biodistribution of transplanted hepatocytes through portal vein infusion. Clin. Nucl. Med. 25, 447-450.

Bonini C., Ferrari G. and Verzeletti S. (1997). HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science 276, 1719-1724.

Bonini C., Grez M., Traversari C., Ciceri F., Marktel S., Ferrari G., Dinauer M., Sadat M., Aiuti A., Deola S., Radrizzani M., Hagenbeek A., Apperley J., Ebeling S., Martens A., Kolb H.J., Weber M., Lotti F., Grande A., Weissinger E., Bueren J.A., Lamana M., Falkenburg J.H., Heemskerk M.H., Austin T., Kornblau S., Marini F., Benati C., Magnani Z., Cazzaniga S., Toma S., Gallo-Stampino C., Introna M., Slavin S., Greenberg P.D., Bregni M., Mavilio F. and Bordignon C. (2003). Safety of retroviral gene marking with a truncated NGF receptor. Nat. Med. 9, 367-369.

Brenner W., Aicher A., Eckey T., Massoudi S., Zuhayra M., Koehl U., Heeschen C., Kampen W.U., Zeiher A.M., Dimmeler S. and Henze E. (2004). ^{111}In-labeled CD34+ hematopoietic progenitor cells in a rat myocardial infarction model. J. Nucl. Med. 45, 512-518.

Bulte J.W., Douglas T., Witwer B., Zhang S.C., Strable E., Lewis B.K., Zywicke H., Miller B., van Gelderen P., Moskowitz B.M., Duncan I.D. and Frank J.A. (2001). Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nat. Biotechnol. 19, 1141-1147.

Bulte J.W., Hoekstra Y., Kamman R.L., Magin R.L., Webb A.G., Briggs R.W., Go K.G., Hulstaert C.E., Miltenyi S. and The T.H. (1992). Specific MR imaging of human lymphocytes by monoclonal antibody-guided dextran-magnetite particles. Magn. Reson. Med. 25, 148-157.

Bulte J.W., Ma L.D., Magin R.L., Kamman R.L., Hulstaert C.E., Go K.G., The T.H. and de Leij L. (1993). Selective MR imaging of labeled human peripheral blood mononuclear cells by liposome mediated incorporation of dextran-magnetite particles. Magn. Reson. Med. 29, 32-37.

Cavazzana-Calvo M., Hacein-Bey S., de Saint Basile G., Gross F., Yvon E., Nusbaum P., Selz F., Hue C., Certain S., Casanova J.L., Bousso P., Deist F.L. and Fischer A. (2000). Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 288, 669-672.

Corrias M.V., Scuderi F., Pasino M., Biglino P., Bocca P., Marotta F., Figini E., Pistoia V. and Mori P.G. (1998). Growth factors increase

retroviral transduction but decrease clonogenic potential of umbilical cord blood CD34+ cells. Haematologica 83, 580-586.

Deb A., Wang S., Skelding K.A., Miller D., Simper D. and Caplice N.M. (2003). Bone marrow-derived cardiomyocytes are present in adult human heart: A study of gender-mismatched bone marrow transplantation patients. Circulation 107, 1247-1249.

Franklin R.J., Blaschuk K.L., Bearchell M.C., Prestoz L.L., Setzu A., Brindle K.M. and French-Constant C. (1999). Magnetic resonance imaging of transplanted oligodendrocyte precursors in the rat brain. Neuroreport 10, 3961-3965.

Frey B.M., Hackett N.R., Bergelson J.M., Finberg R., Crystal R.G., Moore M.A. and Rafii S. (1998). High-efficiency gene transfer into ex vivo expanded human hematopoietic progenitors and precursor cells by adenovirus vectors. Blood 91, 2781-2792.

Gao J., Dennis J.E., Muzic R.F., Lundberg M. and Caplan A.I. (2001). The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs 169, 12-20.

Greiner J., Wiehe J., Wiesneth M., Zwaka T.P., Prill T., Schwarz K., Bienek-Ziolkowski M., Schmitt M., Döhner H., Hombach V. and Torzewski J. (2004). Transient genetic labeling of human CD34 positive hematopoietic stem cells using nucleofection. Transfus. Med. Hemother. 31, 136-141.

Hacein-Bey-Abina A., Le Deist F., Carlier F., Bouneaud C., Hue C., De Villartay J.P., Thrasher A.J., Wulffraat N., Sorensen R., Dupuis-Girod S., Fischer A., Davies E.G., Kuis W., Leiva L. and Cavazzana-Calvo M. (2002). Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N. Engl. J. Med. 346, 1185-1193.

Hefti E., Hartikka J., Salvatierra A., Weiner W.J. and Mash D.C. (1986). Localization of nerve growth factor receptors in cholinergic neurons of the human basal forebrain. Neurosci. Lett. 69, 37-41.

Hinds K.A., Hill J.M., Shapiro E.M., Laukkanen M.O., Silva A.C., Combs C.A., Varney T.R., Balaban R.S., Koretsky A.P. and Dunbar C.E. (2003). Highly efficient endosomal labeling of progenitor and stem cells with large magnetic particles allows magnetic resonance imaging of single cells. Blood 102, 867-872.

Jiang Y., Jahagirdar B.N., Reinhardt R.L., Schwartz R.E., Keene C.D., Ortiz-Gonzalez X.R., Reyes M., Lenvik T., Lund T., Blackstad M., Du J., Aldrich S., Lisberg A., Low W.C., Largaespada D.A. and Verfaillie C.M. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418, 41-49.

Kang H.J., Kim H.S., Zhang S.Y., Park K.W., Cho H.J., Koo B.K., Kim Y.J., Soo Lee D., Sohn D.W., Han K.S., Oh B.H., Lee M.M., and Park Y.B. (2004). Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 363, 751-756.

Lagasse E., Connors H., Al-Dhalimy M., Reitsma M., Dohse M., Osborne L., Wang X., Finegold M., Weissman I.L. and Grompe M. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. Nat. Med. 6, 1229-1234.

Lakshmipathy U., Pelacho B., Sudo K., Linehan J.L., Coucouvanis E., Kaufman D.S. and Verfaillie C.M. (2004). Efficient transfection of embryonic and adult stem cells. Stem Cells 22, 531-543.

Li L.H., McCarthy P. and Hui S.W. (2001). High-efficiency electrotransfection of human primary hematopoietic stem cells. FASEB J. 15, 586-588.

Li Z., Dullmann J., Schmidt M., von Kalle C., Meyer J., Forster M., Stocking C., Wahlers A., Frank O., Ostertag W., Kuhlcke K., Eckert H.G. and Baum C. (2002). Murine leukemia induced by retroviral gene marking. Science 296, 497-500.

Lutzko C., Kruth S., Abrams-Ogg A.C., Lau K., Li L., Clark B.R., Ruedy C., Nanji S., Foster R., Kohn D., Shull R. and Dube I.D. (1999). Genetically corrected autologous stem cells engraft, but host immune responses limit their utility in canine alpha-L-iduronidase deficiency. Blood 93, 1895-1905.

Mackensen A., Krause T., Blum U., Uhrmeister P., Mertelsmann R. and Lindemann A. (1999). Homing of intravenously and intralymphatically injected human dendritic cells generated in vitro from CD34+ hematopoietic progenitor cells. Cancer. Immunol. Immunother. 48, 118-122.

Mavilio F., Ferrari G., Rossini S., Nobili N., Bonini C., Casorati G., Traversari C. and Bordignon C. (1994). Peripheral blood lymphocytes as target cells of retroviral vector-mediated gene transfer. Blood 83, 1988-1997.

Murry C.E., Soonpaa M.H., Reinecke H., Nakajima H., Nakajima H.O., Rubart M., Pasumarthi K.B., Virag J.I., Bartelmez S.H., Poppa V., Bradford G., Dowell J.D., Williams D.A. and Field L.J. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428, 664-668.

Orlic D., Kajstura J., Chimenti S., Jakoniuk I., Anderson S.M., Li B., Pickel J., McKay R., Nadal-Ginard B., Bodine D.M., Leri A. and Anversa P. (2001). Bone marrow cells regenerate infarcted myocardium. Nature 410, 701-705.

Quaini F., Urbanek K., Beltrami A.P., Finato N., Beltrami C.A., Nadal-Ginard B., Kajstura J., Leri A. and Anversa P. (2002). Chimerism of the transplanted heart. N. Engl. J. Med. 346, 5-15.

Rakic P. (2002). Neurogenesis in adult primate neocortex: an evaluation of the evidence. Nat. Rev. Neurosci. 3, 65-71.

Rennen H.J., Frielink C., Brandt E., Zaat S.A., Boerman O.C., Oyen W.J. and Corstens F.H. (2004). Relationship between neutrophil-binding affinity and suitability for infection imaging: comparison of (99m)Tc-labeled NAP-2 (CXCL-7) and 3 C-terminally truncated isoforms. J. Nucl. Med. 45, 1217-1223.

Ruggieri L., Aiuti A., Salomoni M., Zappone E., Ferrari G. and Bordignon C. (1997). Cell-surface marking of CD34+-restricted phenotypes of human hematopoietic progenitor cells by retrovirus-mediated gene transfer. Hum. Gene Ther. 8, 1611-1623.

Stamm C., Westphal B., Kleine H.D., Petzsch M., Kittner C., Klinge H., Schumichen C. and Nienaber C.A, Freund M. and Steinhoff G. (2003). Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 361, 45-46.

Strauer B.E., Brehm M., Zeus T., Gattermann N., Hernandez A., Sorg R.V., Kogler G. and Wernet P. (2001). Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction. Dtsch. Med. Wochenschr. 126, 932-938.

Takahashi T., Kalka C., Masuda H., Chen D., Silver M., Kearney M., Magner M., Isner J.M. and Asahara T. (1999). Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat. Med. 5, 434-438.

Toma C., Pittenger M.F., Cahill K.S., Byrne B.J. and Kessler P.D. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105, 93-98.

Verzeletti S., Bonini C., Marktel S., Nobili N., Ciceri F., Traversari C. and Bordignon C. (1998). Herpes simplex virus thymidine kinase gene transfer for controlled graft-versus-host disease and graft-versus-leukemia: clinical follow-up and improved new vectors. Hum. Gene. Ther. 9, 2243-2251.

von Kalle C., Kiem H.P., Goehle S., Darovsky B., Heimfeld S., Torok-Storb B., Storb R. and Schuening F.G. (1994). Increased gene transfer into human hematopoietic progenitor cells by extended in vitro exposure to a pseudotyped retroviral vector. Blood 84, 2890-2897.

Wagstaff J., Gibson C., Thatcher N., Ford W.L., Sharma H. and Crowther D. (1981). Human lymphocyte traffic assessed by indium-111 oxine labeling: clinical observations. Clin. Exp. Immunol. 43, 443-449.

Wollert K.C., Meyer G.P., Lotz J., Ringes-Lichtenberg S., Lippolt P., Breidenbach C., Fichtner S., Korte T., Hornig B., Messinger D., Arseniev L., Hertenstein B., Ganser A. and Drexler H. (2004). Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 364, 141-148.

Accepted January 14, 2005