Progress in Gene Therapy, Volume 2

pioneering stem cell / gene therapy trials

Editors

Roger Bertolotti, Keiya Ozawa and H. Kirk Hammond

CRC Press
Taylor & Francis Group

Progress in Gene Therapy, Volume 2
pioneering stem cell / gene therapy trials

PROGRESS IN GENE THERAPY

VOLUME 2

pioneering stem cell / gene therapy trials

EDITORS:
Roger Bertolotti, Keiya Ozawa and H. Kirk Hammond

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

First published 2004 by VSP Publishing

Published 2018 by CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

ISBN 13: 978-90-6764-394-8 (hbk)

CONTENTS

PREFACE

The first unequivocal success for Gene Therapy was reported in april 2000 for X-SCID patients. In spite of associate leukemia cases due to retroviral vector random-integration, it stands as the very paradigm of life-long therapeutic gene expression mediated by targeted autologous stem cells. Engrafment and selective repopulating ability of *ex vivo* gene-corrected hematopoietic stem cells have been instrumental in the success of this seminal clinical trial. The self-renewing and differentiative capability of stem cell targets provides thus for long-term transgenic cell replacement in SCID patients. Stem cells are also the targets of transient gene therapy protocols where they are mobilized and recruted in a regenerative process such as the formation of new blood vessels. By its transient nature and the safe use of non-integrating DNA, early therapeutic angiogenic gene therapy for critical limb ischemia (1994-1998) has been important in the establishment of gene therapy as an effective medical practice. These angiogenic gene therapy trials drove their authors to 1) the identification of circulating endothelial progenitor cells (1997) and subsequent development of vasculogenic stem cell therapy, and 2) to the synergistic combination of both pioneering approaches. Such a stem cell-gene therapy method is expected to be a breakthrough for tantalazing regenerative medicine, in particular for myocardium regeneration.

Pioneering stem cell/gene therapy clinical trials are thus the focus of the present book. The few definitive clinical gene therapy successes such as the aforementioned X-SCID trial and improved ADA-SCID ones are presented together with pioneering angio/vasculogenic clinical trials mediated either by transient gene therapy or emerging autologous stem cell transplantation. Highlight also includes 1) promises of the breakthrough combination of stem cell- and transient gene-therapy, 2) gene therapy trials for neurodegenerative disease on non-human primates where long-term gene therapy might involve brain stem cells, and 3) the first clinical trial with non-invasive monitoring of therapeutic gene expression as a prospective conclusion.

Roger Bertolotti
Keiya Ozawa
H. Kirk Hammond

Progress in Gene Therapy, Vol. 2: Pioneering stem cell/gene therapy trials, pp. 1-31
R. Bertolotti *et al*, (Eds)

Stem cell gene therapy: a breakthrough combination magnified by therapeutic stem cell homing

Roger Bertolotti*

CNRS, Molecular Genetics, Department of Hepato-Gastroenterology, Faculty of Medicine, University of Nice Sophia Antipolis, 06107 Nice, France

Table of Contents

* E-mail: Roger.Bertolotti@unice.fr

1. STEM CELLS AND LIFE-LONG THERAPEUTIC GENE EXPRESSION PARADIGM

a) Stem cell gene therapy for X-SCID patients: a paradigm trial

The first unequivocal success for gene therapy was reported in April 2000 (Cavazzana-Calvo *et al,*, 2000) for patients with X-linked severe combined immunodeficiency (X-SCID). This gene therapy trial for an inherited disease stands as the very paradigm of life-long therapeutic gene expression mediated by targeted autologous stem cells. Indeed, engrafment and selective repopulating ability of *ex vivo* gene-corrected hematopoietic stem cells (HSCs) have been instrumental in the success of this seminal clinical trial where efficient retroviral transduction of bone marrow HSCs is synergized by the homing ability of these transduced stem cells and the selective growth advantage of their lymphoid progenitor derivatives over mutant X-SCID cognates (Fig. 1). The self-renewing and differentiative capability of these stem cell targets provides thus for long-term transgenic lymphoid cell replacement (Hacein-Bey-Abina *et al,*, 2002; Fischer *et al,*, 2002) and is thus expected to result in life-long cure for this inherited disease.

b) Selective growth of gene-corrected cells: ADA-SCID case

Interestingly enough, the first gene therapy clinical trial was initiated in 1990 (Culver *et al,*, 1991; Blaese *et al,*, 1995) on another form of SCID in which the pathology results from an adenosine deaminase (ADA) deficiency (ADA-SCID). Original protocols have apparently been hampered by the administration of therapeutic amounts of ADA enzyme conjugated to

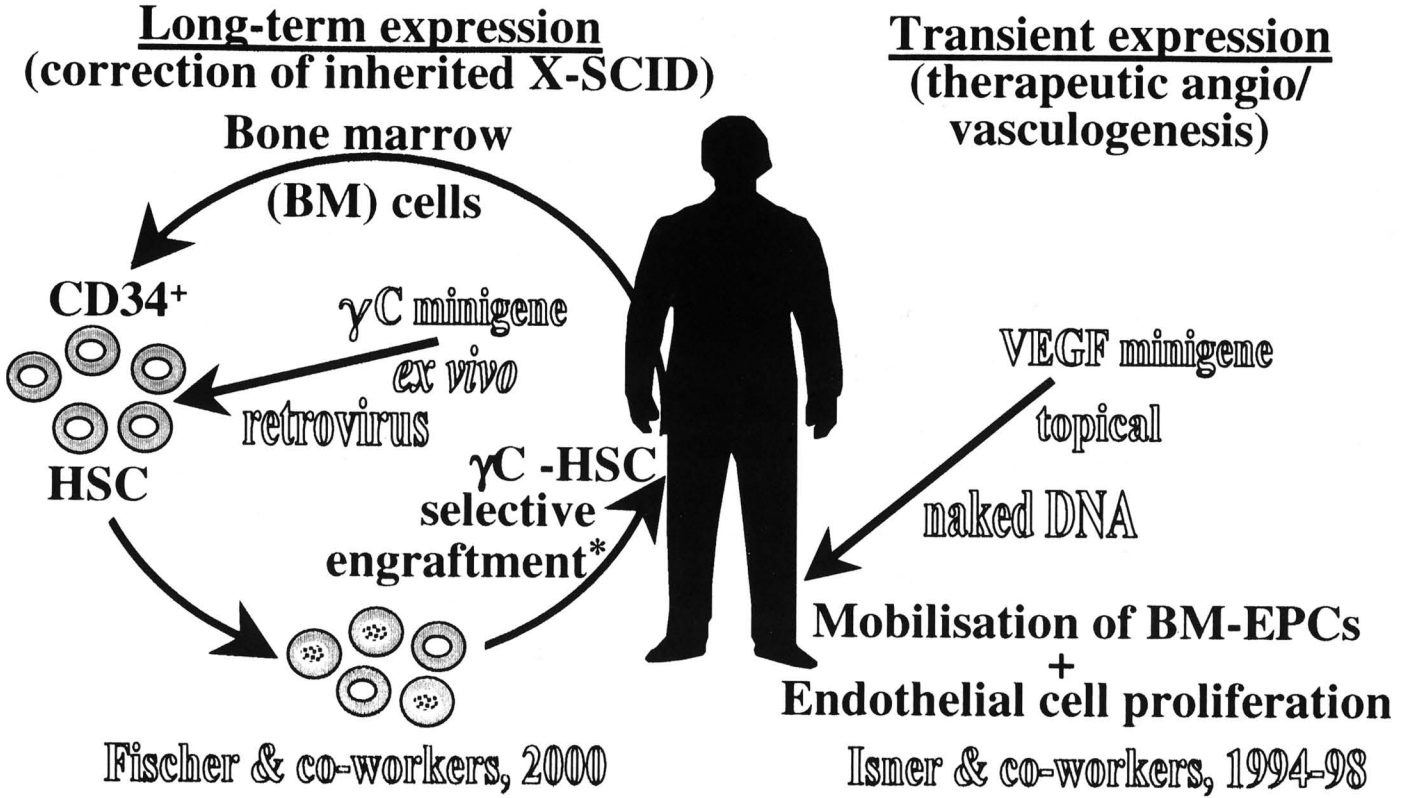


Figure 1. Stem cells as targets of both long-term and transient gene therapy protocols. For X-SCID patients (Fischer *et al.*), purified autologous bone marrow (BM) CD34+ cells containing hematopoietic stem cells (HSCs) are retrovirally transduced *ex vivo* with a γC minigene encoding the common cytokine-receptor γ-chain and returned to the patient where they engraft and reconstitute an active lymphoid cell population thanks to the selective growth advantage of their lymphoid progenitor derivatives. For therapeutic angio/vasculogenesis (Isner *et al.*), transient topical expression of a vascular endothelial growth factor (VEGF) minigene into ischemic limb muscle promotes mobilization and recruitment of BM-derived endothelial progenitor cells (EPCs) into foci of neovascularization.

polyethylene glycol (PEG) which appears to abolish the selective growth advantage of gene-corrected lymphoid cells (Blaese *et al,*, 1995; Bordignon *et al,*, 1995; Kohn *et al,*, 1995). Omission of PEG-ADA and protocol improvements including light bone marrow conditioning with busulfan have now cleared the way (Aiuti *et al,*, 2003; see Candotti, this Volume).

c) Potential universality of life-long stem cell gene therapy

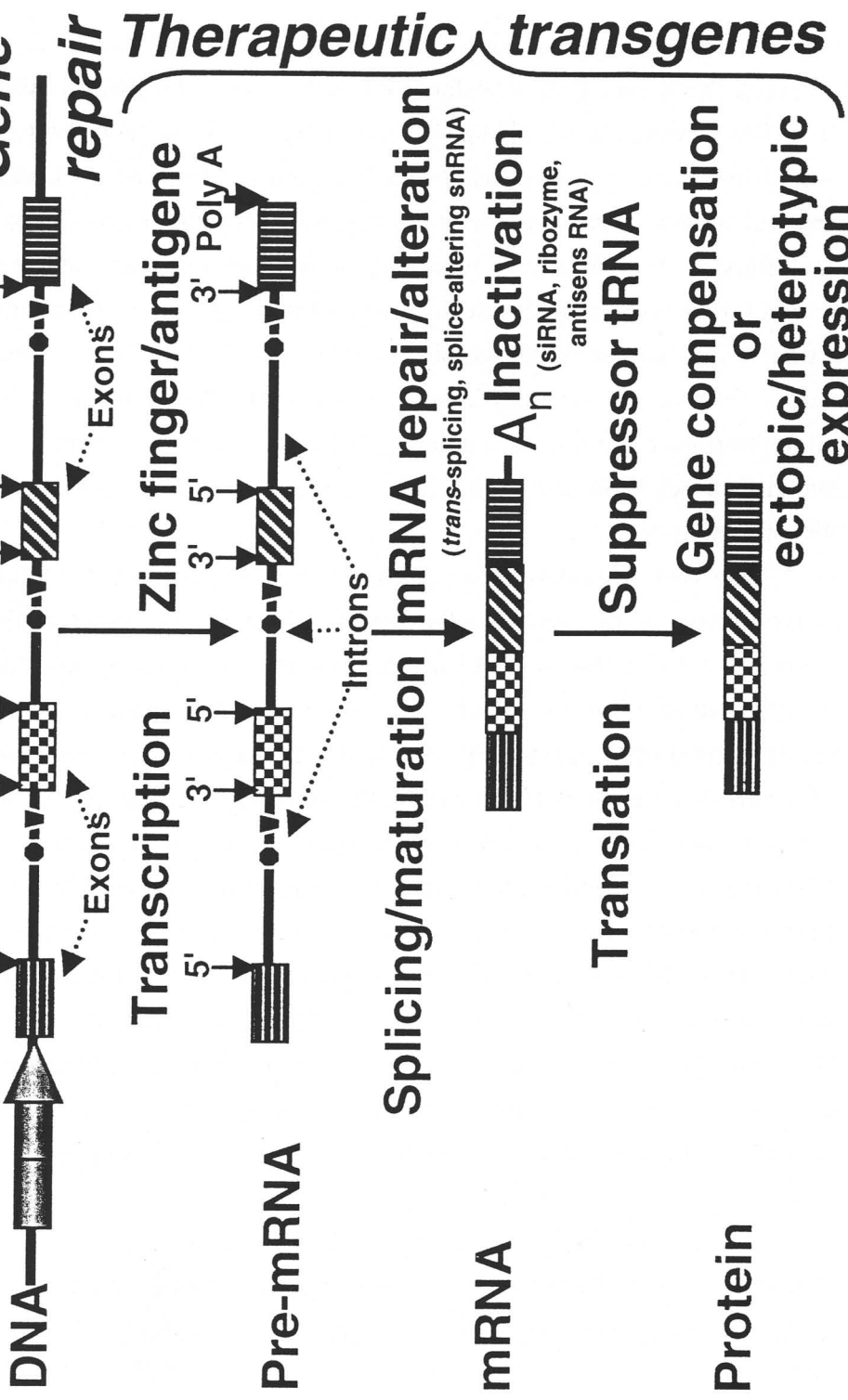
Adult stem cells have been recently identified in tissues and organs such as brain (reviews: McKay, 1997; Gage, 2000; Momma *et al,*, 2000; Alvarez-Buylla and Garcia-Verdugo, 2002) and myocardium (Kajstura *et al,*, 1998; review: Anversa and Nadal-Ginard, 2002) in which cell turn-over was reputedly absent, suggesting that stem cell targeting is most likely an universal requirement for long-term therapeutic gene expression. In this respect, hot off the press report of dopaminergic neurogenesis in the *substantia nigra* of adult rodent brain (Zhao *et al,*, 2003a) opens exciting promises for stem cell gene therapy of Parkinson's disease.

d) Random-insertional carcinogenesis as a booster for site-specific integration and gene repair technologies

Current clinical trials do not however use site-specific integrating vectors and might therefore be hampered either by random-insertional mutagenesis hazards (*e.g.* retroviral and current adeno-associated viral vectors) or by vanishing transmission of non-integrated therapeutic genes (*e.g.* adenoviral and herpes simplex viral vectors, or naked DNA) during stem/progenitor cell proliferation (see Bertolotti, 1998 and 2000a). In this respect, it is noteworthy that the aforementioned very first unequivocal success for gene therapy (Cavazzana-Calvo *et al,*, 2000) has been recently associated to the first cases (2 out of 9 successfully treated patients) of retroviral vector-mediated random insertional pathology (Marshall, 2002a and 2003; Check, 2002 and 2003; Hacein-Bey-Abina *et al,*, 2003). Importantly enough, consistent with a major long-term safety concern (see

Anderson, 1998), the pathologic symptoms appeared almost three years after successful therapeutic gene transfer (Marshall, 2002b; Hacein-Bey-Abina *et al,*, 2003; Check, 2003). Although a theoretically low-probability risk, random insertional mutagenesis is therefore a true potential drawback of retroviral vectors, in particular with repopulating stem cell targets. Stem cells stand thus as a two-faceted tool with which the corollary of efficient retroviral transduction and selective engraftment is high probability of random-insertional mutagenesis with potential carcinogenesis. In addition, random integration into host chromosomal DNA does not provide for optimal transgene expression/regulation, a major concern when unlike with gamma-C or ADA we deal with a tightly regulated function (see Bertolotti, 1998 and 2000a).

The two aforedescribed cases of random-insertional pathology resume interest in the genesis of efficient chimeric site-specific retroviral integrases based on the fusion of native enzyme to sequence-specific DNA-binding domains (review: Bushman, 2002). On the other hand, non-retroviral site-specific integrating vectors are under intensive investigations and are now emerging both for viral and non-viral gene therapy protocols (see Bertolotti, 2003). In addition, directed homologous recombination (gene targeting: Capecchi, 1989) that would provide for the ultimate gene therapy technology both for site-specific (*i.e.* targeted) integration into host chromosomal DNA and gene repair/modification (see Bertolotti, 1998, 1999, 2000b and 2000d) is currently the subject of breakthrough improvements (Bibikova *et al,*, 2003; Porteus and Baltimore, 2003; Miller *et al,*, 2003; Porteus *et al,*, 2003) that could raise its efficiency to a level compatible with gene therapy protocols. As illustrated in Figure 2, gene repair stands as the ideal therapy for inherited disease while therapeutic gene transfer has a wide field of applications in which optimized therapy relies either on site-specific integration for long-term expression or unintegration for transient expression (see Bertolotti, 1998, 2000a and 2000c).


Therapeutic transgenes
Gene repair
DNA
Exons
Transcription
Pre-mRNA
Introns
Splicing/maturation
mRNA repair/alteration
(trans-splicing, splice-altering snRNA)
mRNA
Translation
Protein
Zinc finger/antigene
Poly A
Inactivation
(siRNA, ribozyme, antisens RNA)
Suppressor tRNA
Gene compensation or ectopic/heterotypic expression

2. STEM CELLS AS TARGETS OF TRANSIENT GENE THERAPY

a) Therapeutic angio/vasculogenesis as a transient gene therapy breakthrough and a key clinical step for regenerative gene therapy

Stem cells are also the targets of transient gene therapy protocols where they are mobilized and recruted in a regenerative process such as the formation of new blood vessels (Fig. 1). By its transient nature relying on the safe use of non-integrating DNA, early therapeutic angio/vasculogenic gene therapy for critical limb ischemia (1994-1998) has been instrumental in the establishment of gene therapy as an effective medical practice (see Bertolotti, 2002a and 2002b). The clinical outcomes of these pioneering Phase I/Phase II trials conducted by Isner and co-workers (Isner *et al,*, 1996; Baumgartner *et al,*, 1998; Isner *et al,*, 1998) have been promising enough to prompt the recruitment of new patients (Baumgartner *et al,*, 2000; see Baumgartner, this Volume) and to promote the extension of the approach to myocardial ischemic diseases both by Isner and co-workers (Losordo *et al,*, 1998 and 2002; Symes *et al.*, 1999; Vale *et al,*, 2000 and 2001; see Symes *et al,*, this Volume) and other groups (Rosengart *et al,*, 1999; Grines *et al,*, 2002; see Penny and Hammond, this Volume).

b) Bone marrow-derived endothelial progenitor cells as a key target of therapeutic angio/vasculogenesis

Therapeutic angiogenesis, coined by Isner and co-workers (Takeshita *et al,*, 1994), consists in the therapeutic induction of neovascularization into an ischemic tissue upon transient topical production of an angiogenic growth factor such as vascular endothelial growth factor (VEGF)-A (Isner *et al,*, 1996) or fibroblast growth factor (FGF)-4 (Grines *et al,*, 2002).

Figure 2. Gene therapy: therapeutic gene transfer or gene repair. Current gene therapy protocols rely on therapeutic gene transfer both for inherited disease (gene compensation) or acquired/degenerative disorders (artificial expression patterns such as synthesis of foreign proteins, ectopic production of tissue-specific proteins or transcription of designed non-coding RNA; Bertolotti, 1998 and 2000a). Gene repair stands as the ideal therapy for inherited disease; it is under intensive investigation (Bertolotti, 2000b and text).

Although endothelial cells (ECs) from preexisting vessels were originally thought to be the main targets of therapeutic angiogenesis (Takeshita *et al,*, 1994), the critical role of bone marrow (BM)-derived endothelial progenitor cells (EPCs or angioblasts) in this revascularization process was subsequently shown (Asahara *et al,*, 1997 and 1999b; Kalka *et al,*, 2000a and 2000c). Indeed, early VEGF gene therapy trials drove Isner and co-workers to the identification of circulating EPCs in peripheral blood (Asahara *et al,*, 1997) and to the demonstration that neovascularization in adult ischemic tissue is not restricted to angiogenesis (sprouting of ECs from preexisting vessels; Folkman, 1971) but also involves vasculogenesis (Asahara *et al,*, 1997) where mobilization of bone marrow EPCs (Takahashi *et al,*, 1999; Asahara *et al,*, 1999a) is increased by VEGF gene therapy (Asahara *et al,*, 1999b; Kalka *et al,*, 2000a and 2000c), culminating in active contribution of EPCs to the formation of new blood vessels (incorporation into foci of neovascularization and differentiation into mature ECs: Asahara *et al,*, 1997 and 1999b; Kalka *et al,*, 2000a and 2000c).

Importantly enough, a key feature of circulating EPCs is their propensity to home into ischemic tissues (Asahara *et al,*, 1997 and 1999b; Kalka *et al,*, 2000a and 2000c). Such a therapeutic homing of EPCs has been instrumental in the development of 1) stem cell-mediated gene delivery to ischemic/neovascularizating tissues (Asahara *et al,*, 1997; Iwaguro *et al,*, 2002) and, most importantly 2) autologous vasculogenic stem cell therapy and tantalizing regenerative cardiovascular stem cell/gene therapy (see section 3 and 4 below).

c) Transient topical gene therapy as a basic tool for regenerative medicine

In addition to angio/vasculogenesis, transient topical gene therapy is currently used in cancer gene therapy (*e.g.* Jacobs *et al,*, 2002; see Jacobs *et al,*, this Volume) and in some DNA vaccine protocols (www.DNAvaccine .com). However, it stands as a potential breakthrough for regenerative

medicine in which the mobilization, proliferation, therapeutic homing and lineage-commitment of stem/progenitor cells are the key therapeutic features. Indeed, transient topical gene therapy has been used in early experimental bone regeneration (Bonadio *et al,*, 1999) and is anticipated to be essential in the improvement of many healing processes. Importantly enough, it is expected to have a major impacts in the treatment of many major diseases such as diabetes. In this respect, the induction of pancreatic islet genesis in the liver of diabetic mice has been recently achieved by adenoviral co-delivery of two minigenes encoding an endocrine-pancreas growth factor (betacellulin) and a basic pancreatic transcription factor (Neurod 1), respectively (Kojima *et al,*, 2003). In the potential clinical counterpart of this experimental trial, topical liver infusion and transient minigene expression should substitute for tail vein injection and long-lasting gutless adenoviral vector. Yet, putative stem cell targets have first to be identified or potential hepatocyte reprogramming documented. On the other hand, although liver might eventually be a good choice for auto-immune diabetes, diseased pancreas stands as a potential better target for transient topical gene therapy since subcutaneous injection of purified recombinant betacellulin has been shown to promote partial endocrine pancreas recovery in diabetic mice (Yamamoto *et al,*, 2000).

3. AUTOLOGOUS STEM CELL THERAPY AS A BREAKTHROUGH IN CARDIOVASCULAR REGENERATIVE MEDICINE

a) Therapeutic homing of EPCs as the key to experimental vasculogenic stem cell therapy

The propensity of circulating EPCs to home into ischemic regions and incorporate into foci of neovascularization (vasculogenesis) paved the way for autologous vasculogenic stem cell therapy (Asahara *et al,*, 1997). Purified EPCs (or angioblasts) have thus been used in a series of experimental angiogenic/vasculogenic trials both by Isner's group (Kalka *et*

al,, 2000b; Kawamoto *et al,*, 2001) and others (Kocher *et al,*, 2001) where they were either expanded *ex vivo* or *in vivo* prior to intravenous transplantation (see Asahara *et al,*, 2002 and Itescu *et al,*, 2002).

Such an experimental stem cell therapy has been successful both for peripheral arterial diseases (Kalka *et al,*, 2000b) and myocardial ischemia (Kawamoto *et al,*, 2001 and 2003; Kocher *et al,*, 2001). However, as clearly shown by Kocher *et al,* (2001), the progeny of transplanted EPCs is restricted to vessel wall cells: transplanted EPCs are instrumental to the salvage of intrinsic myocytes but do not contribute to the cardiomyocyte population.

b) Bone marrow multipotent stem cells for myocardium regeneration

The stem cell plasticity breakthrough (Ferrari *et al,*, 1998; Bittner *et al,*, 1999; Gussoni *et al,*, 1999; Bjornson *et al,*, 1999; Galli *et al,*, 2000; Jackson *et al,*, 1999; Petersen *et al,*, 1999; Theisse *et al,*, 2000a and 2000b; Mezey *et al,*, 2000; Brazelton *et al,*, 2000; Clarke *et al,*, 2000; Lagasse *et al,*, 2000) prompted another approach where transplantation involves multipotent stem cells from adult bone marrow (BM) and is aimed at infarcted myocardium regeneration (Orlic *et al,*, 2001a). Indeed, sorting and transplantion of BM-multipotent stem cells resulted in the regeneration of significant amount of contracting myocardium where transplanted cells differentiated into both vascular cells (ECs and vascular smooth muscular cells) and cardiomyocytes (Orlic *et al,*, 2001a). Interestingly enough, these multipotent stem cells can be mobilized from the bone marrow to the peripheral blood by co-administration of stem cell factor (SCF) and granulocyte-colony-stimulating factor (G-CSF) (Bodine *et al,*, 1994), thereby increasing their circulating number to a potential therapeutic level (Orlic *et al,*, 2001b).

Although stem cell fusion with relevant host cells (*e.g.* liver repopulation experiments: Vassilopoulos *et al,*, 2003 and Wang *et al,*, 2003)

cannot be excluded, such a regenerative achievement is consistent with the plasticity of adult BM multipotent stem cells that have been shown 1) to exhibit multi-organ multi-lineage engrafment capability at the single-cell level (Krause *et al,*, 2001) and 2) to include rare true pluripotent mesenchymal stem cells (multipotent adult progenitor cells or MAPCs: Jiang *et al,*, 2002). However, whether the aforedescribed three cell types (Orlic *et al,*, 2001a) arose from true multipotent stem cells (Orlic *et al,*, 2001a and 2001b; Anversa and Nadal-Ginard, 2002) or from a mixed population of BM-stem cells in which angioblasts/pre-angioblasts could provide for vascular cells, and mesenchymal stem cells for cardiomyocytes (see Itescu *et al,*, 2002) remains to be unambiguously shown (Bertolotti, 2002b). Indeed, BM-mesenchymal stem cells (Prockop, 1997; Pittenger *et al,*, 1999) can differentiate into cardiomyocytes under appropriate conditions (Makino *et al,*, 1999; Toma *et al,*, 2002; Mangi and Dzau, 2002); however, their cardiomyocytic repopulation ability awaits careful evaluation.

c) Pioneering autologous stem cell therapy clinical trials for cardiovascular ischemia

The breakthrough data of Orlic *et al,* with purified multipotent BM stem cells (2001a) and pre-clinical trials performed with unfractionated BM cells (BMCs) or whole BM mononuclear cells (BM-MNCs) either on experimental ischemic limb (Shintani *et al,*, 2001) or infarcted myocardium (Tomita *et al,*, 1999; Kamihata *et al,*, 2001; Fuchs *et al,*, 2001) models prompted the initiation of pioneering autologous stem cell therapy clinical trials both for critical limb ischemia (Tateishi-Yuyama *et al,*, 2002; see Takahashi *et al,* and Matsubara, this Volume) and infarcted myocardium/ chronic ischemic heart disease (Strauer *et al,*, 2002; Assmus *et al,*, 2002; Tse *et al,*, 2003; Perin *et al,*, 2003; Fuchs *et al,*, 2003; see Brehm *et al,*, Assmus *et al,*, Matsubara, and Takahashi *et al,*, this Volume). In addition, autologous BM cell transplantation has been associated to coronary artery

bypass graft surgery (CABG) in two other pioneering trials involving only a few patients (Hamano *et al,*, 2001; Stamm *et al,*, 2003).

Importantly enough, unfractionated autologous BMCs/BM-MNCs have been used in the aforementioned clinical trials except in one case where transplantation was restricted to AC133+ BM cells (Stamm *et al,*, 2003), *i.e.* a sub-population which includes the aforementioned rare pluripotent mesenchymal stem cells (Jiang *et al,*, 2002) with strong pre-angioblast potentialities (Reyes *et al,*, 2002). The rationale for using whole BMCs/BM-MNCs is that current *in vivo/ex vivo* expansion technology does not usually provide enough stem cells for regenerative autologous transplantation (Iwaguro *et al,*, 2002; Anversa and Nadal-Ginard, 2002; Asahara *et al,*, 2002) and that different cell sub-populations are potentially amenable to therapeutic cardiomyoplasty, *i.e.* CD34+ angioblasts/pre-angioblasts (Takahashi *et al,*, 1999; Asahara *et al,*, 1999a; Tateishi-Yuyama *et al,*, 2002), CD34- mesenchymal stem cells (Makino *et al,*, 1999; Toma *et al,*, 2002; Reyes *et al,*, 2002; Jiang *et al,*, 2002) and, possibly, other multipotent stem cells (Orlic *et al,*, 2001a). In addition, whole BMCs/BM-MNCs provide significant amounts of a cocktail of angio/vasculogenic growth factors (VEGF, FGF, angiopoietin 1, ...) which is synergistic to the action of vasculogenic/regenerative stem cells (Kamihata *et al,*, 2001; Fuchs *et al,*, 2001; Tateishi-Yuyama *et al,*, 2002), and may thus partially compensate for the scarcity of these active stem cells. Interestingly enough, cell sorting experiments show that mRNA encoding relevant growth factor receptors are restricted to the EPC/angioblast fraction while mRNA encoding the corresponding growth factors are restricted to the other BM-MNC fraction (Tateishi-Yuyama *et al,*, 2002; see Matsubara, this Volume).

The clinical outcome of the aforedescribed angio/vasculogenic trials shows that the approach is safe and promising, and deserves thus large-scale, placebo-controlled, double blind and randomized trials (see Takahashi *et al,*, Matsubara, Brehm *et al,* and Assmus *et al,*, this Volume).

d) Peripheral blood as an attractive source for autologous stem cells

Comparative transplantation analysis of BM-MNCs and *ex vivo* expanded peripheral blood (PB)-EPCs suggests that peripheral blood might be a more desirable source of autologous stem cells than bone marrow, at least for angio/vasculogenic cell therapy (Assmus *et al,*, 2002). Indeed, plain PB-MNCs appear to essentially provide angio/vasculogenic growth factors (Iba *et al,*, 2002; Kamihata *et al,*, 2002) while *ex vivo* expanded PB-EPCs (Assmus *et al,*, 2002) or *in vivo* mobilized PB-CD34+ cells (Inaba *et al,*, 2002) appear to efficiently act like in the seminal experimental trials of Isner and co-workers (Kalka *et al,*, 2000b; Kawamoto *et al,*, 2001 and 2003) or of Kocher *et al,* (2001). Importantly enough, *in vivo* mobilization of CD34+ cells is currently used for transplantation of HSCs from peripheral blood and appears to be associated to the incorporation of differentiated tranplanted cell derivatives into various organs of grafted patients (Korbling *et al,*, 2002). Efficient mobilization of BM stem cells with current clinical protocols is therefore not restricted to EPCs: it is also effective for repopulating HSCs and other multipotent stem cells. On the other hand, a recent report by Huberman and co-workers (Zhao *et al,*, 2003b) might underly a potential breakthrough for efficient production of autologous pluripotent stem cells from a subset of human PB monocytes.

4. STEM CELL GENE THERAPY: COMBINING *EX VIVO* PROTOCOLS AND TRANSIENT *IN VIVO* TOPICAL GENE THERAPY

a) Amplification of the proliferative/regenerative potential of stem cells by transient or regulated *ex vivo* gene therapy

An efficient way to overcome autologous stem cell scarcity has been pioneered by Isner and co-workers (Iwaguro *et al,*, 2002; Asahara *et al,*, 2002) where stem cell therapy and transient *ex vivo* gene therapy protocols are combined. Indeed, in this experimental trial, *ex vivo* expansion and

subsequent adenoviral transduction with a VEGF minigene strongly increased the *in vitro* proliferative index and the *in vivo* revascularizative action of adult EPCs. VEGF transduction was such a powerful booster that it reduced the effective therapeutic dose of EPCs to one thirtieth of its original value (Iwaguro *et al,*, 2002; Asahara *et al,*, 2002). The relative contribution of VEGF-induced angiogenesis and EPC-mediated vasculogenesis remains to be evaluated. Yet, this stem cell gene therapy approach stands as a potential breakthrough for therapeutic angiogenesis/ vasculogenesis and, possibly, for myocardium regeneration.

A similar approach has been used for repopulating adult HSCs in which the rate of expansion and the *in vivo* regenerative potential has been shown to be dramatically increased by *ex vivo* HOXB4 gene therapy (Antonchuk *et al,*, 2001 and 2002). However, in this case, instead of using non-integrating adenoviral vectors that are diluted and lost in proliferating cells, they transduced stem cells with integrative retroviral vectors. In this system, transient expression is not required because HOXB4 does not override extrinsic physiologic mechanisms that appear to control the population size of HSCs *in vivo* (Antonchuk *et al,*, 2001).

b). Transient topical gene therapy as a booster for the therapeutic homing/regenerative potential of transplanted stem cells

Another way to increase the regenerative potential of both natural and genetic-engineered transplanted stem cells is to couple *ex vivo* protocols to synergistic transient topical gene expression protocols (Bertolotti, 2001 and 2002b). Topical production of a growth/homing factor may of course increase the proliferative/differentiative index of transplanted (engineered) stem cells and differentiative progeny in the same way it acts on endogenous stem cells (*e.g.* aforedescribed angio/vasculogenic VEGF/FGF gene therapy). Yet, the main inerest of transient local overproduction of a growth/homing factors may be to be an essential booster for therapeutic homing and lineage-commitment of transplanted (engineered) stem cells (Bertolotti, 2001).

Noticeably, topical brain infusion of epidermal growth factor (EGF) has been shown to act as a chemoattractant for transplanted embryonic neuro-progenitor cells: directed migration was associated to selective proliferation (Fricker-Gates *et al,*, 2000). Importantly enough, topical injection of the stroma cell-derived factor-1 (SDF-1) has been recently used to increase the recruitement of transplanted EPCs in ischemic muscle tissues (Yamaguchi *et al,*, 2003). Such an improvement in the local accumulation of transplanted EPCs had a dramatic therapeutic effect on the neovascularizating process of experimental hind limb ischemia (Yamaguchi *et al,*, 2003). Like for VEGF, FGF and other growth factors (*e.g.* Isner, 2002; Grines *et al,*, 2002; Baumgartner, Symes *et al,*, Penny and Hammond, this Volume), transient topical SDF-1 gene therapy should be more efficient than the mere topical infusion of the purified protein. Synergistic transient topical SDF-1 gene therapy is thus expected to be a potent booster for stem cell gene therapy for cardiovascular ischemic disease. In this respect, preliminary data on rats show that topical transplantation of cardiac fibroblasts engineered to constitutively express SDF-1 increases therapeutic homing of BM-derived CD117+ stem cells in the infarcted zone (Unzek *et al,*, 2003). Another field of application might involve HSCs since SDF-1 is also a potent chemoattractant for these cells (Mohle *et al,*, 1998).

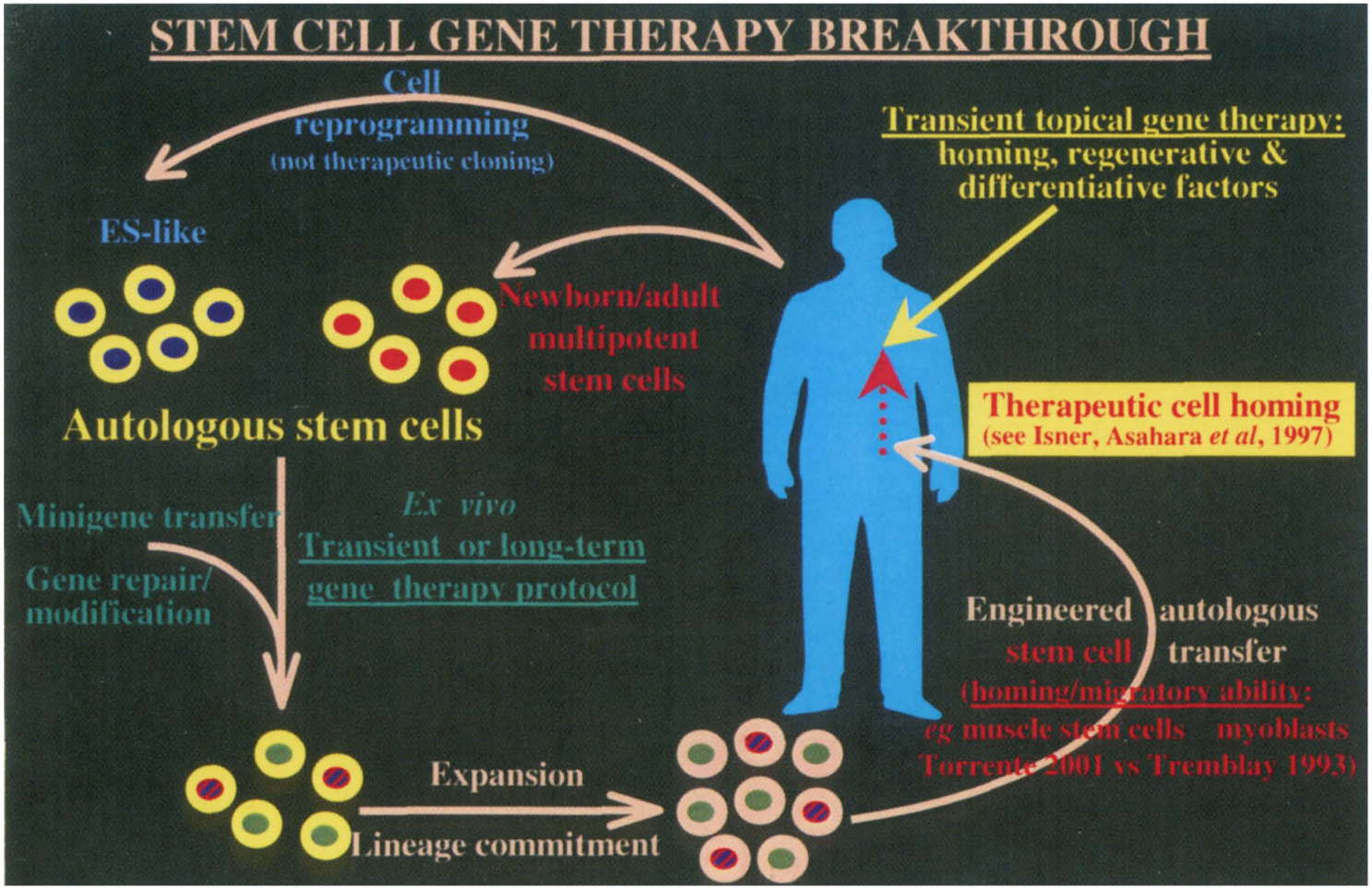
In addition to therapeutic homing, the regenerative activity of transplanted stem cells requires an efficient commitment to the target tissue/organ function. Such a fonctional integration may depend on transient topical expression of lineage-commitment and specific growth/differentiative factors, and is crucial for multipotent/pluripotent stem cells (see Bertolotti, 2001). Such a process could also benefit from transient expression of a "regenerative" inducer (Bertolotti, 2001) such as msx1 for skeletal muscle (Odelberg *et al,*, 2000) or IGF-1 for many tissues including cardiac and skeletal muscles (*e.g.* Reiss *et al,*, 1996; Barton-Davis *et al,*, 1998; Musaro *et al,*, 2001; Shiotani *et al,*, 2001; Barton *et al,*, 2002; Takahashi *et al,*, 2003).

c) Combining *ex vivo* protocols with transient topical gene therapy

Upon mobilization both by transient gene therapy and/or recombinant protein application, autologous stem cells are thus amenable to *ex vivo* protocols where they can be purified, expanded, genetic engineered either for transient amplification of their proliferative potential or/and for long-term transgene expression/gene repair and, eventually commited to a specific cell-lineage before being returned to the patient. Such *ex vivo* manipulations can then be combined to synergistic transient topical gene therapy culminating in an optimization of the homing, regenerative and differentiative capabilities of transplanted stem cells. Importantly enough, transient gene therapy may rely on exogenous drug control (*e.g.* Baron and Bujard, 2000; Pollock *et al,*, 2000; Ye *et al,*, 2002; Saez *et al,*, 2000; Fussenegger, 2001) when fine tuning and transient gene expression have to be inforced in a very tight way.

With their self-renewing and homing/regenerative capabilities, autologous stem cells are thus amenable to sophisticated cell gene therapy protocols that have been schematized in Figure 2. Such a stem cell gene therapy in which stem cell therapy is combined with long-term and transient gene therapy protocols stands thus as a powerful mean to tackle both inherited diseases and degenerative/acquired disorders (Bertolotti, 2001 and 2002b). Indeed, transient magnification of the regenerative potential of stem cells, in which therapeutic cell homing is a key feature (see above), should culminate in an efficient targeted repopulation dynamics of therapeutic stem cells and differentiated derivatives in relevant tissues and organs, thereby providing both for appropriate gene therapeutics and life-long maintenance/ replacement of therapeutic cells.

Figure 3. Stem cell gene therapy: combining *ex vivo* protocols with transient topical gene therapy (Poster Exhibition of June 6, 2002, ASGT 5th Annual Meeting, Boston: Bertolotti, 2002c). A schematic overview of the stem cell gene therapic breakthrough in which emerging autologous multipotent/pluripotent stem cells are displayed together with potential future autologous embryonic stem (ES)-like cells that might result from intensive cell reprogramming investigations (see Bertolotti, 2001).


STEM CELL GENE THERAPY BREAKTHROUGH
Cell reprogramming
(not therapeutic cloning)
ES-like
Newborn/adult multipotent stem cells
Autologous stem cells
Transient topical gene therapy: homing, regenerative & differentiative factors
Therapeutic cell homing
(see Isner, Asahara *et al*, 1997)
Minigene transfer
Gene repair/ modification
Ex vivo
Transient or long-term gene therapy protocol
Engineered autologous stem cell transfer
(homing/migratory ability:
eg muscle stem cells myoblasts
Torrente 2001 vs Tremblay 1993)
Expansion
Lineage commitment

Serendipitously, the development of the aforedescribed stem cell gene therapy technology will most likely provide the means to handle most adult stem cells *in vivo*, thereby boostering 1) transient gene expression protocols for regenerative medicine and 2) targeted-vector technology for efficient *in vivo* genetic-engineering of patient's resident stem cell populations the expansion/re-homing/lineage-commitment of which may ultimately rely on synergistic transient gene expression therapy.

5. CONCLUSION: THERAPEUTIC STEM CELL HOMING AND STEM CELL GENE THERAPY

With bone marrow transplantation, therapeutic stem cell homing is a standard medical practice whereby repopulating HSCs efficiently engraft in conditioned host bone marrow. Transplantation of HLA-identical or haploidentical T cell-depleted allogeneic bone marrow under conditioning-free conditions is the basic treatment for X-SCID and most other SCID patients (Buckley *et al,*, 1999; Antoine *et al,*, 2003). Such a well-established medical practice has been essential to the achievement of the aforedescribed first unequivocal success for gene therapy (Cavazzano-Calvo *et al,*, 2000). In this trial, engraftment of *ex vivo* gene-corrected autologous X-SCID HSCs was achieved without bone marrow conditioning while a light busulfan conditioning has been used in the subsequent success of improved ADA-SCID gene therapy protocols (Aiuti *et al,*, 2003). Therapeutic homing of transduced HSCs and the selective growth advantage of their lymphoid progenitor derivatives over SCID cognates have been instrumental in these paradigm trials.

As discussed earlier in the chapter, therapeutic stem cell homing is also essential to the vasculogenic activity of circulating EPCs. The same holds true for other bone marrow stem/progenitor cells that, like for circulating EPCs, have first to be mobilized from their bone marrow niche and then re-home, directly or through progenitor derivatives, into injured

tissues where they contribute to various specialized cell replacement (*e.g.* Jackson *et al,*, 2001; Orlic *et al,*, 2001b). Whether multipotent/pluripotent bone marrow stem cells are directly involved in these multi-lineage commited processes remains to be clearly established. However, therapeutic homing of purified BM multipotent stem cells (lineage-negative c-kit-positive sub-population) into infarcted myocardial tissue upon topical peri-infarct transplantation (Orlic *et al,*, 2001a) is consistent with such an hypothesis. Indeed, multipotent stem cells have migration and re-homing capabilities that appear to be missing to late pre-differentiated cells as illustrated by the transplantation inefficiency of myoblasts (Tremblay *et al,*, 1993; Mendell *et al,*, 1995; El Fahime *et al,*, 2000) compared to multipotent muscle-derived stem cells (Torrente *et al,*, 2001; Gussoni *et al,*, 1999).

Importantly enough, pluripotent mesenchymal stem cells (aforedescribed MAPCs, Jiang *et al,*, 2002) appear to have a wide therapeutic homing capability although their propensity to home into injured/diseased tissues has not yet been formally evaluated (Jiang *et al,*, 2002). They might therefore eventually end as the core cells of a polyvalent therapeutic platform for autologous stem cell gene therapy. They might however be challenged 1) by other potential sources of pluripotent/ multipotent adult stem cells such as peripheral blood (Zhao *et al,*, 2003b) or 2) by more specialized stem cells such as mesodermal lineage-commited mesoangioblasts (Minasi *et al,*, 2002) that sense inflammatory cytokines and are extensively recruited by regenerating muscle fibers in dystrophic mice (Sampaolesi *et al,*, 2003).

6. REFERENCES

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.

Alvarez-Buylla, A. and Garcia-Verdugo, J.M. (2002). Neurogenesis in adult subventricular

zone. *J Neurosci.*, **22**: 629-634.

Anderson, F.W. (1998). Human gene therapy. *Nature*, **392** suppl.: 25-30.

Antoine, C., Muller, S., Cant, A., Cavazzana-Calvo, M., Veys, P., Vossen, J., Fasth, A., Heilmann, C., Wulffraat, N., Seger, R., Blanche, S., Friedrich, W., Abinun, M., Davies, G., Bredius, R., Schulz, A., Landais, P., Fischer, A., European Group for Blood and Marrow Transplantation and European Society for Immunodeficiency (2003). Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. *Lancet*, **361**: 553-560.

Antonchuk, J., Sauvageau, G. and Humphries, R.K. (2001). HOXB4 overexpression mediates very rapid stem cell regeneration and competitive hematopoietic repopulation. *Exp Hematol.*, **29**: 1125-1134.

Antonchuk, J., Sauvageau, G. and Humphries, R.K. (2002). HOXB4-induced expansion of adult hematopoietic stem cells *ex vivo*. *Cell*, **109**: 39-45.

Anversa, P. and Nadal-Ginard, B. (2002). Myocyte renewal and ventricular remodelling. *Nature*, **415**: 240-243.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G. and Isner, J.M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science*, **275:** 964-967.

Asahara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., Kearney, M., Magner, M. and Isner, J.M. (1999a). Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res.*, **85:** 221-228.

Asahara, T., Takahashi, T., Masuda, H., Kalka, C., Chen, D., Iwaguro, H., Inai, Y., Silver, M. and Isner, J.M. (1999b). VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.*, **18:** 3964-3972.

Asahara, T., Kawamoto, A., Kalka, C. and Masuda, H. (2002). Therapeutic potential of bone marrow-derived endothelial progenitor cells for cardiovascular ischemic diseases. *Gene Therapy & Regulation*, **1**: 361-374.

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.

Baron, U. and Bujard, H. (2000). Tet repressor-based system for regulated gene expression in eukaryotic cells: principles and advances. *Methods Enzymol.*, **327**: 401-421.

Barton, E.R., Morris, L., Musaro, A., Rosenthal, N. and Sweeney, H.L. (2002). Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. *J Cell Biol.*, **157**: 137-148.

Barton-Davis, E.R., Shoturma, D.I., Musaro, A., Rosenthal, N. and Sweeney, H.L. (1998). Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proc. Natl. Acad. Sci. USA*, **95**: 15603-15607.

Baumgartner, I., Pieczek, A., Manor, O., Blair, R., Kearney, M., Walsh, K. and Isner, J.M. (1998). Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation*, **97**: 1114-1123.

Baumgartner, I., Rauh, G., Pieczek, A., Wuensch, D., Magner, M., Kearney, M., Schainfeld, R. and Isner, J.M. (2000). Lower-extremity edema associated with gene transfer of naked DNA encoding vascular endothelial growth factor. *Ann. Intern. Med.*, **132**: 880-884.

Bertolotti, R. (1998). Gene therapy 1998: transient or stable minigene expression and gene repair/inactivation. *Biogenic Amines*, **14**: 389-406.

Bertolotti, R. (1999). Recombinase-DNA nucleoprotein filaments as vectors for gene repair/ inactivation and targeted integration of minigenes. *Biogenic Amines*, **15**: 169-195.

Bertolotti, R. (2000a). Transient or long-term transgene expression and gene repair/ inactivation. *Gene Therapy & Regulation*, **1**: 1-8.

Bertolotti, R. (2000b). RNA and gene repair/alteration: from inherited diseases to acquired disorders and tantalizing applications for non-disease conditions. *Gene Therapy & Regulation*, **1**: 115-122.

Bertolotti, R. (2000c). Gene therapy: transient or long-term minigene expression and gene repair/inactivation. In: *Progress in Gene Therapy — Basic and Clinical Frontiers*, R. Bertolotti, H. Parvez and T. Nagatsu (Eds), VSP Int. Science Press, Utrecht, N.L., pp. 1-34.

Bertolotti, R. (2000d). Gene therapy: dsDNA-cored presynaptic filaments as vectors for gene repair and targeted integration of transgenes. In: *Progress in Gene Therapy — Basic and Clinical Frontiers*, R. Bertolotti, H. Parvez and T. Nagatsu (Eds), VSP Int. Science Press, Utrecht, N.L., pp. 513-549.

Bertolotti, R. (2001). Adult and embryonic-like stem cells: toward a major gene therapy breakthrough relying on autologous multipotent stem cells. *Gene Therapy & Regulation*, **1**: 207-212.

Bertolotti, R. (2002a). In memoriam: Jeffrey M. ISNER, stem cell/gene therapy pioneer. *Gene Therapy & Regulation*, **1**: 285.

Bertolotti, R. (2002b). From therapeutic angiogenesis to myocardium regeneration. *Gene Therapy & Regulation*, **1**: 287-295.

Bertolotti, R. (2002c). Gene repair/modification and targeted integration of minigenes: toward improved ex vivo/in vivo stem cell gene therapy. *Mol. Ther.*, **5**: S133 (Abstract).

Bertolotti, R. (2003). Site-specific integration. *Gene Therapy & Regulation*, **2**: 1-5.

Bibikova, M., Beumer, K., Trautman, J.K. and Carroll, D. (2003). Enhancing gene targeting with designed zinc finger nucleases. *Science*, **300**: 764.

Bittner, R.E., Schofer, C., Weipoltshammer, K., Ivanova, S., Streubel, B., Hauser, E., Freilinger, M., Hoger, H., Elbe-Burger, A. and Wachtler, F. (1999). Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. *Anat. Embryol. (Berl.)*, **199**: 391-396.

Bjornson, C.R., Rietze, R.L., Reynolds, B.A., Magli, M.C. and Vescovi, A.L. (1999).

Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science*, **283**: 534-537.

Blaese, R.M., Culver, K., Miller, A.D., Carter, C., Fleisher, T., Clerici, M., Shearer, G., Chang, L., Chiang, Y., Tolstoshev, P., Greenblatt, J., Rosenberg, S., Klein, H., Berger, M., Mullen, C., Ramsey, W., Muul, L., Morgan, R. and Anderson, F.W. (1995). T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. *Science*, **270**: 475-480.

Bodine, D.M., Seidel, N.E., Gale, M.S., Nienhuis, A.W. and Orlic, D. (1994). Efficient retrovirus transduction of mouse pluripotent hematopoietic stem cells mobilized into the peripheral blood by treatment with granulocyte colony-stimulating factor and stem cell factor. *Blood*, **84**: 1482-1491.

Bonadio J, Smiley E, Patil P, Goldstein S. (1999). Localized, direct plasmid gene delivery *in vivo*: prolonged therapy results in reproducible tissue regeneration. *Nat Med.*, **5**: 753-739.

Bordignon C, Notarangelo LD, Nobili N, Ferrari G, Casorati G, Panina P, Mazzolari E, Maggioni D, Rossi C, Servida P, Ugazio, A.G. and Mavilio, F. (1995). Gene therapy in peripheral blood lymphocytes and bone marrow for ADA- immunodeficient patients. *Science*, **270**: 470-475.

Brazelton, T.R., Rossi, F.M., Keshet, G.I. and Blau, H.M. (2000). From marrow to brain: expression of neuronal phenotypes in adult mice. *Science*, **290**: 1775-1779.

Buckley, R.H., Schiff, S.E., Schiff, R.I., Markert, L., Williams, L.W., Roberts, J.L., Myers, L.A. and Ward, F.E. (1999). Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med.*, **340**: 508-516.

Bushman, F. (2002). Targeting retroviral integration? *Mol. Ther.*, **6**: 570-571.

Capecchi, M. (1989). The new mouse genetics: altering the genome by gene targeting. *Trends Genet.*, **5**: 70-76.

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.

Check, E. (2002). A tragic setback. *Nature*, **420**: 116-118.

Check, E. (2003). Second cancer case halts gene-therapy trials. *Nature*, **421**: 305.

Clarke, D.L., Johansson, C.B., Wilbertz, J., Veress, B., Nilsson, E., Karlstrom, H., Lendahl, U. and Frisen, J. (2000). Generalized potential of adult neural stem cells. *Science*, **288**: 1660-1663.

Culver, K.W., Anderson, W.F. and Blaese, R.M. (1991). Lymphocyte gene therapy. *Hum Gene Ther.*, **2**: 107-109.

El Fahime, E., Torrente, Y., Caron, N.J., Bresolin, M.D. and Tremblay, J.P. (2000). *In vivo* migration of transplanted myoblasts requires matrix metalloproteinase activity. *Exp. Cell Res.*, **258**: 279-287.

Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G. and Mavilio, F. (1998). Muscle regeneration by bone marrow-derived myogenic

progenitors. *Science*, **279**: 1528-1530.

Fischer, A., Hacein-Bey, S. and Cavazzana-Calvo, M. (2002). Gene therapy of severe combined immunodeficiencies. *Nat Rev Immunol.*, **2:** 615-621.

Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.*, **285:** 1182-1186.

Fricker-Gates, R.A., Winkler, C., Kirik, D., Rosenblad, C., Carpenter, M.K. and Bjorklund, A. (2000). EGF infusion stimulates the proliferation and migration of embryonic progenitor cells transplanted in the adult rat striatum. *Exp. Neurol.*, **165**: 237-247.

Fuchs, S., Baffour, R., Zhou, Y.F., Shou, M., Pierre, A., Tio, F.O., Weissman, N.J., Leon, M.B., Epstein, S.E. and Kornowski, R. (2001). Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol.*, **37**: 1726-1732.

Fuchs, S., Satler, L.F., Kornowski, R., Okubagzi, P., Weisz, G., Baffour, R., Waksman, R., Weissman, N.J., Cerqueira, M., Leon, M.B. and Epstein, S.E. (2003). Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. *J Am Coll Cardiol.*, **41**: 1721-1724.

Fussenegger, M. (2001). Dual-regulated gene expression in mammalian cells, a novel approach to gene therapy. *Gene Therapy & Regulation*, **1**: 233-264.

Gage, F.H. (2000). Mammalian neural stem cells. *Science*, **287**: 1433-1438.

Galli, R., Borello, U., Gritti, A., Minasi, M.G., Bjornson, C., Coletta, M., Mora, M., De Angelis, M.G., Fiocco, R., Cossu, G. and Vescovi, A.L. (2000). Skeletal myogenic potential of human and mouse neural stem cells. *Nat. Neurosci.*, **3**: 986-991.

Grines, C.L., Watkins, M.W., Helmer, G., Penny, W., Brinker, J., Marmur, J., West, A., Rade, J., Marrott, P., Hammond, H.K. and Engler, R.E. (2002). Angiogenic gene therapy trial (AGENT): intracoronary delivery of adenovirus encoding FGF4 for patients with stable angina pectoris. *Circulation*, **105**: 1291-1297.

Gussoni, E., Soneoka, Y., Strickland, C.D., Buzney, E.A., Khan, M.K., Flint, A.F., Kunkel, L.M. and Mulligan, R.C. (1999). Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature*, **401** 390-394.

Hamano, K., Nishida, M., Hirata, K., Mikamo, A., Li, T.S., Harada, M., Miura, T., Matsuzaki, M. and Esato, K. (2001). Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results, *Jpn Circ J*, **65,** 845-847.

Hacein-Bey-Abina, S., 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.

Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., Le Deist, F., Wulffraat, N., McIntyre, E., Radford, I., Villeval, J.L., Fraser, C.C., Cavazzana-Calvo, M. and

Fischer, A. (2003). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med.*, **348**: 255-256.

Iba, O., Matsubara, H., Nozawa, Y., Fujiyama, S., Amano, K., Mori, Y., Kojima, H. and Iwasaka, T. (2002). Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. *Circulation*, **106**: 2019-2025.

Inaba, S., Egashira, K. and Komori, K. (2002). Peripheral-blood or bone-marrow mononuclear cells for therapeutic angiogenesis. *Lancet*, **360**: 2083-2084.

Isner, J.M. (2002). Myocardial gene therapy. *Nature*, **415**: 234-239.

Isner, J.M., Pieczek, A., Schainfeld, R., Blair, R., Haley, L., Asahara, T., Rosenfield, K., Razvi, S., Walsh, K. and Symes, J.F. (1996). Clinical evidence of angiogenesis following arterial gene transfer of phVEGF165 in a patient with ischemic limb. *Lancet*, **348**: 370-374.

Isner, J.M., Baumgartner, I., Rauh, G., Schainfeld, R., Blair, R., Manor, O., Razvi, S. and Symes, J.F. (1998). Treatment of thrombangiitis obliterans (Buerger's disease) by intramuscular gene transfer of vascular endothelial growth factor: preliminary clinical results. *J. Vasc. Surg.*, **28**: 964-975.

Itescu, S., Kocher, A.A. and Schuster, M.D. (2002). Adult bone marrow-derived angioblasts for improvement of cardiomyocyte function after myocardial ischemia. *Gene Therapy & Regulation*, **1**: 375-386.

Iwaguro, H., Yamaguchi, J., Kalka, C., Murasawa, S., Masuda, H., Hayashi, S., Silver, M., Li, T., Isner, J.M. and Asahara, T. (2002). Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation*, **105**: 732-738.

Jackson, K.A., Mi, T. and Goodell, M.A. (1999). Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc. Natl. Acad. Sci. USA*, **96**: 14482-14486.

Jackson, K.A., Majka, S.M., Wang, H., Pocius, J., Hartley, C.J., Majesky, M.W., Entman, M.L., Michael, L.H., Hirschi, K.K. and Goodell, M.A. (2001). Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.*, **107**: 1395-1402.

Jacobs, A., Voges, J., Reszka, R., Lercher, M., Gossmann, A., Kracht, L., Kaestle, C., Wagner, R., Wienhard, K. and Heiss, W.D. (2001). Positron-emission tomography of vector-mediated gene expression in gene therapy for gliomas. *Lancet*, **358**: 727-729.

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.

Kajstura, J., Leri, A., Finato, N., Di Loreto, C., Beltrami, C.A. and Anversa, P. (1998). Myocyte proliferation in end-stage cardiac failure in humans. *Proc. Natl. Acad. Sci. USA*, **95**: 8801-8805.

Kalka, C., Masuda, H., Takahashi, T., Gordon, R., Tepper, O., Gravereaux, E., Pieczek, A., Iwaguro, H., Hayashi, S.-I., Isner, J.M. and Asahara, T. (2000a). Vascular endothelial growth factor(165) gene transfer augments circulating endothelial progenitor cells in human subjects. *Circ. Res.*, **86:** 1198-1202.

Kalka, C., Masuda, H., Takahashi, T., Kalka-Moll, W.M., Silver, M., Kearney, M., Li, T., Isner, J.M. and Asahara, T. (2000b). Transplantation of *ex vivo* expanded endothelial progenitor cells for therapeutic neovascularization. *Proc. Natl. Acad. Sci. USA*, **97:** 3422-3427.

Kalka, C., Tehrani, H., Laudenberg, B., Vale, P.R., Isner, J.M., Asahara, T. and Symes, J.F. (2000c). VEGF gene transfer mobilizes endothelial progenitor cells in patients with inoperable coronary disease. *Ann. Thorac. Surg.*, **70:** 829-834.

Kamihata, H., Matsubara, H., Nishiue, T., Fujiyama, S., Tsutsumi, Y., Ozono, R., Masaki, H., Mori, Y., Iba, O., Tateishi, E., Kosaki, A., Shintani, S., Murohara, T., Imaizumi, T. and Iwasaka, T. (2001). Implantation of autologous bone marrow cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*, **104**: 1046–1052.

Kamihata, H., Matsubara, H., Nishiue, T., Fujiyama, S., Amano, K., Iba, O., Imada, T. and Iwasaka, T. (2002). Improvement of Collateral Perfusion and Regional Function by Catheter-Based Implantation of Peripheral Blood Mononuclear Cells into Ischemic Hibernating Myocardium. *Arterioscler Thromb Vasc Biol*, **22**: 1804-1810.

Kawamoto, A., Gwon, H.-C., Iwaguro, H., Yamaguchi, J.-I., Uchida, S., Masuda, H., Silver, M., Ma, H., Kearney, M., Isner, J.M. and Asahara, T. (2001). Therapeutic potential of *ex vivo* expanded endothelial progenitor cells for myocardial ischemia. *Circulation*, **103**: 634–637.

Kawamoto, A., Tkebuchava, T., Yamaguchi, J., Nishimura, H., Yoon, Y.S., Milliken, C., Uchida, S., Masuo, O., Iwaguro, H., Ma, H., Hanley, A., Silver, M., Kearney, M., Losordo, D.W., Isner, J.M. and Asahara, T. (2003). Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*, **107**: 461-468.

Kocher, A.A., Schuster, M.D., Szabolcs, M.J., Takuma, S., Burkoff, D., Wang, J., Homma, S., Edwards, N.M. and Itescu, S. (2001). Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat. Med.*, **7:** 430-436.

Kohn, D.B., Weinberg, K.I., Nolta, J.A., Heiss, L.N., Lenarsky, C., Crooks, G.M., Hanley, M.E., Annett, G., Brooks, J.S., El-Khoureiy, A., Lawrence, K., Wells, S., Shaw, K., Moen, R.C., Bastian, J., Williams-Herman, D.E., Elder, M., Wara, D., Bowen, T., Hershfield, M.S., Mullen, C.A., Blaese, R.M. and Parkman, R. (1995). Engraftment of gene-modified cells from umbilical cord blood in neonates with adenosine deaminase deficiency. *Nature Med.*, **1**: 1017-1026.

Kojima, H., Fujimiya, M., Matsumura, K., Younan, P., Imaeda, H., Maeda, M. and Chan, L. (2003). NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med.*, **9**: 596-603.

Korbling, M., Katz, R.L., Khanna, A., Ruifrok, A.C., Rondon, G., Albitar, M., Champlin, R.E. and Estrov, Z. (2002). Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med.*, **346**: 738-746.

Krause, D.S., Theise, N.D., Collector, M.I., Henegariu, O., Hwang, S., Gardner, R.,

Neutzel, S. and Sharkis, S.J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, **105**: 369-377.

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.

Losordo, D.W, Vale, P.R., Symes, J.F., Dunnington, C.H., Esakof, D.D., Maysky, M., Ashare, A.B., Lathi, K. and Isner J.M. (1998). Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation*, **98**: 2800-2804.

Losordo, D.W., Vale, P.R., Hendel, R.C., Milliken, C.E., Fortuin, F.D., Cummings, N., Schatz, R.A., Asahara, T., Isner, J.M. and Kuntz, R.E. (2002). Phase 1/2 placebo-controlled, double-blind, dose-escalating trial of myocardial vascular endothelial growth factor 2 gene transfer by catheter delivery in patients with chronic myocardial ischemia, *Circulation*, **105**: 2012-2018.

Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., Hata, J., Umezawa, A. and Ogawa, S. (1999). Cardiomyoctes can be generated from marrow stromal cells in vitro. *J Clin Invest*, **103**: 697-705.

Mangi, A.A. and Dzau, V.J. (2002). Expansion and genetic modification of bone marrow mesenchymal stem cells for *in vivo* repair of damaged myocardium. *Mol. Ther.*, **5**: S296 (Abstract).

Marshall, E. (2002a). Clinical research: Gene therapy a suspect in leukemia-like disease. *Science*, **298**: 34-35.

Marshall, E. (2002b). Gene therapy. What to do when clear success comes with an unclear risk? *Science*, **298**: 510-511.

Marshall, E. (2003). Gene therapy. Second child in French trial is found to have leukemia. *Science*, **299**: 320.

McKay, R. (1997). Stem cells in the central nervous system. *Science*, **276**: 66-71.

Mendell, J.R., Kissel, J.T., Amato, A.A., King, W., Signore, L., Prior, T.W., Sahenk, Z., Benson, S., McAndrew, P.E., Rice, R., Nagaraja, H., Stephens, R., Lantry, L., Morris, G.E. and Burghes, A.H. (1995). Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *N. Engl. J. Med.*, **333**: 832-838.

Mezey, E., Chandross, K.J., Harta, G., Maki, R.A. and McKercher, S.R. (2000). Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science*, **290**: 1779-1782.

Miller, D.G., Petek, L.M. and Russell, D.W. (2003). Human gene targeting by adeno-associated virus vectors is enhanced by DNA double-strand breaks. *Mol Cell Biol.*, **23**: 3550-3557.

Minasi, M.G., Riminucci, M., De Angelis, L., Borello, U., Berarducci, B., Innocenzi, A., Caprioli, A., Sirabella, D., Baiocchi, M., De Maria, R., Boratto, R., Jaffredo, T., Broccoli, V., Bianco, P. and Cossu, G. (2002). The meso-angioblast: a multipotent,

self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. *Development*, **129**: 2773-2783.

Mohle R, Bautz F, Rafii S, Moore MA, Brugger W, Kanz L. (1998). The chemokine receptor CXCR-4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. *Blood*, **91**: 4523-4530.

Momma, S., Johansson, C.B. and Frisen, J. (2000). Get to know your stem cells. *Curr Opin Neurobiol.*, **10**: 45-49.

Murayama, T., Tepper, O.M., Silver, M., Ma, H., Losordo, D.W., Isner, J.M., Asahara, T. and Kalka, C. (2002). Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization *in vivo*. *Exp. Hematol.*, **30**: 967-972.

Musaro, A., McCullagh, K., Paul, A., Houghton, L., Dobrowolny, G., Molinaro, M., Barton, E.R., Sweeney, H.L. and Rosenthal, N. (2001). Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet.*, **27**: 195-200.

Odelberg, S.J., Kollhoff, A. and Keating, M.T. (2000). Dedifferentiation of mammalian myotubes induced by msx1. *Cell*, **103**: 1099-1109.

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. (2001a). Bone marrow cells regenerate infarcted myocardium. *Nature*, **410**: 701-705.

Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D.M., Leri, A. and Anversa, P. (2001b). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl. Acad. Sci. USA*, **98**: 10344-10349.

Perin, E.C., Dohmann, H.F.R., Borojevic, R., Silva, S.A., Sousa, A.L.S., Mesquita, C.T., Rossi, M.I.D., Carvalho, A.C., Dutra, H.S., Dohmann, H.J.F., Silva, G.V., Belem, L., Vivacqua, R., Rangel, F.O.D., Esporcatte, R., Geng, Y.J., Vaughn, W.K., Assad, J.A.R., Mesquita, E.T. and Willerson, J.T. (2003). Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation*, **107**: 2294-2302.

Petersen, B.E., Bowen, W.C., Patrene, K.D., Mars, W.M., Sullivan, A.K., Murase, N., Boggs, S.S., Greenberger, J.S. and Goff, J.P. (1999). Bone marrow as a potential source of hepatic oval cells. *Science*, **284**: 1168-1170.

Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S. and Marshak, D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*, **284**: 143-147.

Pollock, R., Issner, R., Zoller, K., Natesan, S., Rivera, V.M. and Clackson, T. (2000). Delivery of a stringent dimerizer-regulated gene expression system in a single retroviral vector. *Proc. Natl. Acad. Sci. USA*, **97**: 13221-13226.

Porteus, M.H. and Baltimore D. (2003). Chimeric nucleases stimulate gene targeting in human cells. *Science*, **300**: 763.

Porteus, M.H., Cathomen, T., Weitzman, M.D. and Baltimore, D. (2003). Efficient gene targeting mediated by adeno-associated virus and DNA double-strand breaks. *Mol Cell Biol.*, **23**: 3558-3565.

Prockop, D.J. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*, **276**: 71-74.

Reiss, K., Cheng, W., Ferber, A., Kajstura, J., Li, P., Li, B., Olivetti, G., Homcy, C.J., Baserga, R. and Anversa, P. (1996). Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice. *Proc. Natl. Acad. Sci. USA*, **93**: 8630-8635..

Reyes, M., Dudek, A., Jahagirdar, B., Koodie, L., Marker, P.H. and Verfaillie CM. (2002). Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest.*, **109**: 337-346.

Rosengart, T.K., Lee, L.Y., Patel, S.R., Sanborn, T.A., Parikh, M., Bergman, G.W., Hachamovitch, R., Szulc, M., Kligfield, P.D., Okin, P.M., Hahn, R., Devereux, R., Post, M., Hackett, N., Foster, T., Grasso, T., Lesser, M., Isom, O. and Crystal, R.G. (1999). Angiogenesis gene therapy: phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. *Circulation*, **100**: 468-474.

Saez, E., Nelson, M.C., Eshelman, B., Banayo, E., Koder, A., Cho, G.J. and Evans, R.M. (2000). Identification of ligands and coligands for the ecdysone-regulated gene switch. *Proc. Natl. Acad. Sci. USA*, **97**: 14512-14517.

Sampaolesi, M., Torrente, Y., Innocenzi, A., Tonlorenzi, R., D'Antona, G., Pellegrino, M.A., Barresi, R., Bresolin, N., De Angelis, M.G., Campbell, K.P., Bottinelli, R. and Cossu, G. (2003). Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science*, **301**: 487-492.

Shintani, S., Murohara, T., Ikeda, H., Ueno, T., Sasaki, K., Duan, J. and Imaizumi, T. (2001). Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation*, **103**: 897-903.

Shiotani, A., Fukumura, M., Maeda, M., Hou, X., Inoue, M., Kanamori, T., Komaba, S., Washizawa, K., Fujikawa, S., Yamamoto, T., Kadono, C., Watabe, K., Fukuda, H., Saito, K., Sakai, Y., Nagai, Y., Kanzaki, J. and Hasegawa, M. (2001). Skeletal muscle regeneration after insulin-like growth factor I gene transfer by recombinant Sendai virus vector. *Gene Ther.*, **8**: 1043-1050.

Stamm, C., Westphal, B., Kleine, H.D., Petzsch, M., Kittner, C., Klinge, H., Schumichen, C., 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., Kostering, M., Hernandez, A., Sorg, R.V., Kogler, G. and Wernet, P. (2002). Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans, *Circulation*, **106**, 1913-1918.

Sussman, M. (2001). Cardiovascular biology. Hearts and bones. *Nature*, **410**: 640-641.