HEP (2007) 180:117–165

Comparison of Intracardiac Cell Transplantation: Autologous Skeletal Myoblasts Versus Bone Marrow Cells

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Abstract An increasing number of patients living with cardiovascular disease (CVD) and still unacceptably high mortality created an urgent need to effectively treat and prevent disease-related events. Within the past 5 years, skeletal myoblasts (SKMBs) and bone marrow (or blood)-derived mononuclear cells (BMNCs) have demonstrated preclinical efficacy in reducing ischemia and salvaging already injured myocardium, and in preventing left ventricular (LV) remodeling, respectively. These findings have been translated into clinical trials, so far totaling over 200 patients for SKMBs and over 800 patients for BMNCs. These safety/feasibility and early phase II studies showed promising but somewhat conflicting symptomatic and functional improvements, and some safety concerns have arisen. However, the patient population, cell type, dose, time and mode of delivery, and outcome measures differed, making comparisons problematic. In addition, the mechanisms through which cells engraft and deliver their beneficial effects remain to be fully elucidated. It is now time to critically evaluate progress made and challenges encountered in order to select not only the most suitable cells for cardiac repair but also to define appropriate patient populations and outcome measures. Reiterations between bench and bedside will increase the likelihood of cell therapy success, reduce the time to development of combined of drug- and cell-based disease management algorithms, and offer these therapies to patients to achieve a greater reduction of symptoms and allow for a sustained improvement of quality of life.

Keywords Acute myocardial infarction · Bone marrow · Cell therapy · Heart failure · Stem cells

1 Introduction

Cardiovascular disease (CVD) has become a major health issue throughout the world, exceeding infection and cancer as the leading cause of death in the Western world and in many developing countries (LeGrand 2000; Thom et al. 2006). Although CVD mortality has decreased because of advances in therapies for atherosclerosis, hypercholesterolemia, hypertension, diabetes, and post-acute myocardial infarction (post-AMI) left ventricular (LV) remodeling (Pearson et al. 2002; Smith et al. 2006), CVD still accounts for 1 in every 2.7 deaths in the United States, translating into approximately 2.5 million deaths each year (Thom et al. 2006). In addition, the prevalence of the risk factors for CVD, such as hypertension, obesity, and type 2 diabetes, has been on the rise in recent years (Appel et al. 2006; Haffner 2002; Pearson et al. 2002; Wyatt et al. 2006). Current data show that the incidence of clinical CVD in the 30- to 50-year-old age group is increasing (Juonala et al. 2006; Yan et al. 2006). Moreover, as a result of improved prevention, recognition, and treatment of AMI, the percentage of patients surviving AMI has grown, but unfortunately so has the prevalence of post-AMI heart failure (HF)—with at least a third of patients manifesting HF symptomatology in the first year following AMI (Miller and Missov 2001). Currently, the causes are attributed to both the fairly limited efficacy of pharmacological agents at reducing LV remodeling and hospitalizations for HF exacerbations (Bertrand 2004; Cohn 2002; Doggrell 2005; Hernandez et al. 2005; Jong et al. 2003; Jost et al. 2005; Reiffel 2005; Thattassery

and Gheorghiade 2004; Torp-Pedersen et al. 2005), as well as to underutilization of these drugs, which precludes translating the successes observed in trials into clinical settings (Lenzen et al. 2005; Levy et al. 2002). In addition to the increasing number of patients, survival of HF patients has also increased following wider recommendations for clinical use of implantable cardioverter-defibrillators (ICDs) (Moss et al. 2002). The number of patients with an unmet medical need is likely to continue to increase, as the number of people over 65 years of age in the United States doubles in the next 25 years because of aging of the "baby-boomers," with nearly 15% of this population projected to develop HF due to aging, CVD, and type 2 diabetes (Thom et al. 2006).

The urgency of this growing problem has created an unmet need for a more advanced understanding of the entire continuum of CVD so that we can design therapies to treat the entire spectrum of disease. New therapies are needed to prevent LV remodeling after acute injury and to stop the progressive loss of cardiac function in a chronically failing myocardium. Finally, therapies should be designed to halt the CVD process, beginning with improvement of vascular health.

All these needs have fueled research directed at cell-based therapies. The main cell types that have been evaluated clinically are skeletal myoblasts (SKMBs) and bone-marrow derived mononuclear cells (BMNCs), or subsets thereof. In this chapter, we provide a brief overview of the therapeutic effects of each of these types of cells, and also of several important challenges associated with the development of cell-based therapies.

2
The Goals of Cell-Based Therapies

As with every new therapy, be it drug-, device-, or cell-based, potential applications drive the conception and progression of the idea. Cell therapy was envisioned for use after AMI to prevent LV remodeling and onset of HF. Ultimately, cell therapy should be applicable in a broader CVD context: from the beginning stages of atherosclerosis to advanced HF. Such versatility could be afforded by creating "clinical products" in which cell type, purity, dose, route, and criteria for optimal administration (timing of injection relative to injury and degree of injury, frequency of therapeutic application), are based on the continuum of disease. Potential adverse effects will also have to be well-characterized. Taking a multi-faceted approach should generate products with a significant capability of repairing underlying cardiac injury and thus promoting functional recovery to a degree better than current drug therapies can offer; that result alone would represent a paradigm shift in the treatment of CVD.

A realistic goal of cell therapy is restoring at least some degree of function and perfusion to the injured and remodeled myocardium. Full myocardial regeneration is currently not yet achievable, although progress has been made

in developing cell-based patches and sheets that could be applied to injured areas of the LV—again, to promote repair (Hata et al. 2006; Liu et al. 2004; Miyagawa et al. 2005). The two primary cell types that have shown capabilities for repair in the heart to date are SKMBs and BMNCs, mostly studied in HF and AMI, respectively.

3
Autologous Skeletal Myoblasts: Future Tools for Repair of Failing Myocardium?

SKMBs, derived from muscle "satellite cells," can expand and form neofibers after muscle injury, thereby regenerating skeletal muscle (Mauro 1961). Because of those properties and because SKMBs express contractile proteins very similar to those in the heart, SKMBs were the first candidate for cardiac repair. The idea muscle-based repair emerged in 1987 and was translated into dynamic cardiomyoplasty, when previously paced latissimus dorsi muscle was surgically wrapped around the failing heart in an attempt to provide some contractile support to the LV (Chachques et al. 1987). Although dynamic cardiomyoplasty did not deliver the results hoped for, cellular cardiomyoplasty (transplantation of SKMBs into the heart) did (Chiu et al. 1995; Murry et al. 1996; Scorsin et al. 1997; Zibaitis et al. 1994). Transplanted cells survived and formed striated muscle grafts within the damaged cardiac tissue, which at the time was considered a success.

3.1
Overview of Preclinical Data

In 1998, we demonstrated for the first time that engraftment of SKMBs into injured myocardium improved LV function and attenuated remodeling (Taylor et al. 1998). In that study, SKMBs improved the contractility of scarred segments of the heart without strict differentiation into cardiomyocytes. Rather, SKMBs yielded myogenin-positive SKMB-like cells (situated in the center of the scar) and myogenin-negative more primitive cardiac muscle-like cells (found around the scar periphery) (Atkins et al. 1999c). The transplanted SKMBs adapted to the surrounding myocardium by forming myofibers that were electrically isolated from host cardiomyocytes and yet improved LV performance (Atkins et al. 1999a).

However, the mechanism(s) of that improvement presented a puzzle that remains unresolved. Numerous pathways have been suggested, from modulation of LV wall stress to active contraction of the injected cells (Ott and Taylor 2006). It is more likely that the improvement of LV function comes from a combination of both a direct effect of the transplanted cells on LV geometry and performance, and a "paracrine" effect of exogenous cells on LV

remodeling and angiogenesis (Van Den Bos and Taylor 2003). Because these mechanisms are still not fully understood, there is a considerable variance of opinion with regards to the long-term effect of SKMB transplantation: do autologous myoblasts improve contractility or only prevent further deterioration of the injured myocardial segments? Whether SKMBs are better or worse than other cells to do the former or the latter may not matter, as both SKMBs and BMNCs could be beneficial in patients with HF. It could, however, have implications for the timing of cell therapy. Preclinical data substantiate the claims that autologous SKMBs improve both diastolic and systolic myocardial performance after both acute and chronic injury (Agbulut et al. 2004; Fuchs et al. 2001; Hiasa et al. 2004; Horackova et al. 2004; Hutcheson et al. 2000; Ohno et al. 2003; Ott et al. 2004, 2006; Taylor et al. 1998; Thompson et al. 2003).

3.2 Initial Clinical Experience

The advantages of autologous SKMBs in CVD/HF extend beyond benefits observed in animal models. Their autologous nature (Koh et al. 1993) overcomes the two major limitations of cardiac transplantation in CVD: a shortage of donor tissue and the complexities of immunosuppression. Their capacity for myogenesis increases the likelihood of improved contractility. Finally, their relatively high resistance to ischemia may be crucial for survival in infarcted regions, where ischemia dominates (Reffelmann et al. 2003).

The first observational clinical study using cell therapy to treat CVD was initiated by Menasche and colleagues (2003) in 2000. In this trial, an average of 871×10^6 cells (at least 85% SKMBs) was injected into a nonrevascularizable LV segment as an adjunct to coronary artery bypass grafting (CABG). Significant improvements in LV ejection fraction (EF) and regional wall thickening in the treated segments were observed, suggesting anti-remodeling effects of SKMBs. More recently, Dib et al. (2005) administered SKMBs concurrently with CABG, or as an adjunct to an LV assist device (LVAD) implanted as a bridge to transplantation. Following the combined (with cells) procedure, myocardial perfusion improved and LVEF increased. Several explanted hearts were examined post-LVAD at the time of cardiac transplantation, and engrafted SKMBs were seen in 4 of the 5 specimens within the infarcted regions. In another clinical study, a lower dose of SKMBs (mean of 196×10^6) was injected—as a sole therapy—into infarcted myocardium [via a catheter system capable of percutaneous transluminal non-fluoroscopic LV electromechanical mapping—e.g., NOGA system (Biosense Webster, Inc., California, USA)]) yielding improved regional wall motion and a mildly increased LVEF over 3–6 months (Smits et al. 2003). Data continue to emerge (Chachques et al. 2004; Gavira et al. 2006; Herreros et al. 2003; Ince et al. 2004; Siminiak et al. 2004, 2005; Table 1) showing that SKMBs can be delivered in the context of HF (reduced LVEF, ongoing ischemia, neurohormonal activation, potential hemodynamic instability, risk

of arrhythmias, etc.) and not only survive within the infarcted regions of myocardium, but most importantly attenuate LV remodeling (Pagani et al. 2003). The degree of functional improvement may not only depend on the baseline LVEF, but also on the route of delivery. Overall, patients who received SKMBs as an adjunct to CABG demonstrated a mean increase in LVEF ranging from 6% to 18%, while those who received the cells without the concomitant surgical procedure showed 6% to 24% improvement. However, upon a close examination of the data, it is apparent that in those patients whose baseline LVEF is quite low (mean of 24%), the presence or absence of CABG may not matter clinically because of the predominance of irreversibly damaged myocytes, scar, or both. With higher baseline LVEFs (around 35%), CABG could increase blood flow and augment tissue perfusion to improve engraftment so that a larger proportion of cells would contribute to repair. A comparison of patients' treatment regimens and the extent of revascularization of the cell-treated area should offer insights into of whether or not the differences in outcome simply represent variations in the use of SKMBs or geographical and institutional differences in the treatment of HF.

3.3 Important Issues of SKMB Transplantation to Be Resolved

3.3.1 Expansion of Cells

The first hurdle associated with any autologous cells is the need for cell expansion in the clinical setting. This process necessitates a sufficient time between injury, harvest of cells, and therapeutic application. In many patients (as well as healthy donors), this window of time ranges from several days to several weeks, which does not seem to pose a problem in the context of chronic injury (typical for HF). However, in the case of AMI, a treatment without delay may be significantly more beneficial than a postponed intervention with regards to its effects on the acutely ischemic area. Therefore, alternative cells could be employed, such as BMNCs, or—if myoblasts are truly superior—allogeneic cells from a healthy appropriately matched donor may offer a solution. Randomized studies comparing SKMBs and BMNCs in this context would bring substantial clarity to this issue.

3.3.2 Risk of Arrhythmias

The reports of electrical adverse events in patients after autologous SKMB transplantation have generated broad skepticism within the clinical community about the safety of this potential treatment option. Despite the appropriateness of these concerns, these events should be placed in the pathophysiological context of systolic HF, where arrhythmias are inherent to the disease process

Table 1 Published cell therapy trials with SKMBs to date. Included are studies with five or more patients enrolled and for which results were published prior to 2 November 2006, where methodological and outcome details were available. For details, please refer to original publications

Investigator (Country)	No. of patients	Dx	Average SKMB dose[a] $\times 10^6$	Delivery route	Length of follow-up (months)	Baseline LVEF, %	Follow-up LVEF, %	Functional outcomes; change in symptomatology (NYHA class)
Dib et al. 2005 (USA)	30	Post-AMI HF	2.2–300	Transepi+CABG or LVAD	24	28	35 (y 1) 36 (y 2)	Myocardial viability (by PET) increased. NYHA class significantly improved (baseline: mean of 2.1; 1-y follow-up: mean of 1.4) but increased at 2-y follow-up (no differences with baseline).
Chachques et al. 2004 (France)	20	Post-AMI	300	Transepiw/o CABG	14±5	28	52	Wall motion score improved, glucose uptake (by PET) increased NYHA class significantly improved (baseline: mean of 2.5; follow-up: mean of 1.2).
Herreros et al. 2003; Gavira et al. 2006 (Spain)	12 (+14 historical controls)	Ischemic HF+prior AMI	221	Transepi+CABG	3 and 12	36; controls: 36	54 (3 m); 55 (12 m); Controls: 39	Regional wall motion (by E) and viability (glucose uptake by PET) improved 7/12 patients improved by 1 NYHA class.
Menasche et al. 2003 (France)	10	Post-AMI HF	871	Transepi w/o CABG	10.9	24	32	14/22 segments demonstrated increased systolic thickening NYHA functional class significantly improved (baseline: 2.7±0.2; follow-up: 1.6±0.1).

Table 1 (continued)

Investigator (Country)	No. of patients	Dx	Average SKMB dose[a] $\times 10^6$	Delivery route	Length of follow-up (months)	Baseline LVEF, %	Follow-up LVEF, %	Functional outcomes; change in symptomatology (NYHA class)
Siminiak et al. 2004 (Poland)	10	>3 m post-AMI	0.4–50 (range)	Transepi+CABG	4 and 12	35 (25–40)	42 (29–47)	Of 9 previously dyskinetic segments, 5 became akinetic, and of 10 akinetic segments, 4 became hypokinetic at 4 m, this effect was maintained at 12 m Reduction in symptomatology not assessed.
Siminiak et al. 2005 (Poland)	9	Post-AMI HF	17–106 (range)	Transcoronary	10 wks	41 (30–49)	44 (33–49)	Symptoms improved in all patients by at least 1 NYHA class Symptoms improved in all patients by at least 1 NYHA class.
Ince et al. 2004 (Germany)	6 (+6 controls)	Ischemic HF	210	Transendo	12	24; controls: 24	32; controls: 21	Walking distance and NYHA class significantly improved.
Smits et al. 2003 (Netherlands)	5	Post-AMI (anterior) HF	196	Transendo EMM-guided	3 and 6	36	41	Regional contractility (by MRI) significantly increased.

Abbreviations: AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; Dx, diagnosis; E, echocardiography; EMM, electromagnetic mapping; HF, heart failure; ICM, ischemic cardiomyopathy; LV, left ventricle; LVEF, left ventricular ejection fraction; m, month(s); MRI, magnetic resonance imaging; NYHA class, New York Heart Association functional class; PET, positron emission tomography; SKMB, skeletal myoblast; Transendo, transendocardial; Transepi, transepicardial; wks, weeks; y, year(s)

[a]"(Range)" denotes dose-escalation or variation was used

(Moss 2003). In fact, many of the patients in recent trials (Table 1) met the Multicenter Automatic Defibrillator Implantation Trial (MADIT)-II and now Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT) criteria (Mark et al. 2006; Moss et al. 2002), which were presented after those cell therapy trials had begun. These criteria suggest that patients with systolic HF of ischemic etiology (post-AMI) with LVEF less than or equal to 30%–35% may significantly benefit from ICDs in terms of survival, as these devices are highly successful in terminating lethal arrhythmias. Whether SKMBs augmented arrhythmogenesis in these patients is difficult to discern. Dib and colleagues (2005) have not reported a dramatically increased incidence of electrical instability after SKMB administration. In fact, there were only 2 patients with ventricular tachycardia (VT) post-cell transplantation with no clear-cut connection to cellular cardiomyoplasty. Specifically, 1 patient displayed a VT of ischemic etiology (attributable to stenoses of the bypass grafts), and another patient displayed VT, bigeminy, and junctional rhythm—all of which disappeared after discontinuation of digoxin and initiation and up-titration of carvedilol. Supplementation with low-dose amiodarone has shown beneficial effects: a reduction of VT from 40% to 10% (Siminiak et al. 2004, 2005). It is also possible that some of the patients that exhibited arrhythmogenesis could have had a suboptimally treated HF.

Alternatively, arrhythmias as much may have been avoided in more recent studies as a result of the shift in clinical practice owing to low-dose amiodarone and the inclusion of patients who met the MADIT-II and SCD-HeFT criteria and had already received ICDs. However, this strategy may be problematic in terms of quantifying efficacy of cell-based treatments. In currently published studies (Table 1), patients with a mean baseline LVEF of 30% or lower exhibited symptomatic benefits following the SKMB procedure, but the anti-remodeling effects were not as pronounced as in patients whose LVEF were in the 30% to 40% range. The case might very well be that the patients who are eligible for an ICD under the MADIT-II or SCD-HeFT criteria may have a degree of injury too large to be repaired by SKMBs any other cell type, or the ischemic process (or both) and the chronic downregulation of blood flow could create conditions that are relatively harsh for transplanted cells to survive, even taking into consideration that SKMBs are considered relatively resistant to ischemia.

The presence of arrhythmias highlights another potential limitation to cell-based therapy: the unclear (so far) ability of any transplanted cells to electrically integrate with native tissue (Abraham et al. 2005). Because SKMBs are the best-studied and most myogenic cell type, more experience exists with these cells than others. Scorsin et al. (2000) did not observe any electrical integration of myoblasts preclinically. Similarly, Suzuki and co-authors (2001) reported that in the absence of connexin-43 overexpression, SKMBs did not couple very well with surrounding myocardium but the treated area synchronously contracted with surrounding tissue and contributed to overall cardiac performance. Our group has reported similar findings (Thompson et al. 2003).

So is coupling important? Are connexin-43 and N-cadherin (Reinecke et al. 2000, 2002) the only requirements for electrical coupling of cells? Or does the coupling involve additional specific molecular factors? To what extent is electrical integration of the graft with the surrounding myocardium related to the function of the myocardial segment? Some answers to these questions are emerging, but more research on mechanistic insights into this process is much needed. In this regard, Marban's group had made progress on antiarrhythmic engineering of SKMBs by genetically modifying SKMBs to express gap junctions (and connexin 43) (Abraham et al. 2005). Interestingly, cocultures of human SKMBs with rat cardiomyocytes produced spiral reentry waves—similar to VT/ventricular fibrillation in humans—that were terminated by nitrendipine, L-type Ca^{2+} channel blocker, but not by lidocaine (standard treatment for reentry). The genetically modified SKMBs had a much higher proportion of cells that did not exhibit arrhythmias in culture. Therefore, there is much to be learned with regards to mechanisms of arrhythmias after cell transplantation.

3.3.3 Location of Transplantation: Does It Matter?

The location of transplanted cells (center of scar or its periphery), the homogeneity of the scar and its contractile properties relative to the border zone, the environment of the scar and the border zone, and the number of cells engrafted play major roles in electrical outcome. However, we have yet to dissect the variables involved and to assign primary and secondary order of importance. For example, there are data showing increased incidence of arrhythmias in animals who receive SKMBs into the border zone versus the center of the infarct (Atkins et al. 1999a, b), and there are preliminary observations showing the exact opposite (J. McCue, C. Swingen, T. Feldberg, C. Caron, S. Prabhu, R. Motillal, D.A. Taylor, unpublished data).

Notwithstanding the heterogeneity of the data, the importance of location in terms of functional outcome has recently been highlighted by several studies where delivery of cells into revascularized versus nonrevascularized scar augmented outcome. More recently, Ott et al. demonstrated that the close proximity of small injections of transplanted SKMBs (microdepots) led to a more uniform contractile improvement compared with larger volume injections with greater distances from each other (macrodepots), likely due to lesser environmental stress per cell and possibly paracrine influences (Ott et al. 2005a). Recently, we have developed several approaches to increase the ability to direct cell delivery to specific locations. First, we developed a thoracoscopic approach (Thompson et al. 2004), and more recently a robotic approach (Ott et al. 2006). Specifically, SKMBs have been transplanted into the embolization-induced HF myocardium using the da Vinci robotic system (Ott et al. 2006). A high degree of precision has been reached in the placement of the cells into apical, anterior, and lateral

segments. LVEF, wall thickening, regional wall motion, and LV end-diastolic volume have improved following the procedure as demonstrated by magnetic resonance imaging (MRI). This approach may bring better outcomes due to a higher capacity to control the placement of the cells in a hypo/akinetic versus dyskinetic myocardium. Clearly, the location of SKMB transplantation into the myocardium does matter. As more preclinical and clinical studies emerge, we will be moving closer to a more complete understanding of why cell location matters from a mechanistic standpoint.

3.3.4 Role of the Environment

The environment of the infarcted myocardium consists of the border zone with viable or partially viable cells and a scarred center. These two areas have distinctly different oxygen concentrations, which are primarily dependant on the blood flow. The border zone and the scar also possess different diffusion characteristics. Recent experiments from our group (B.H. Davis, T. Schroeder, M.D. Dewhirst, K. Olbrich, D.A. Taylor, unpublished data) performed in C_2C_{12} myoblasts placed in an artificial three-dimensional infarction construct have shown that the survival of transplanted cells decreased toward the center of the scar, where the milieu becomes more ischemic (Fig. 1). Availability of oxygen remains one of the main limiting factors in cell survival, even with SKMBs that are believed to be relatively ischemia-resistant. An attempt to improve cell survival by increasing available glucose did not rescue the cells from hypoxia-induced apoptosis. However, improving biochemical alterations (such as amino acids) may have a better impact on cell viability rather than blood flow alone. In this regard, glutamine deprivation reduced oxygen consumption rate (OCR) in the myoblasts within the infarct (Fig. 1). The myoblasts with reduced OCR survived better in an ischemic milieu because of increased oxygen penetration depth. Together, these observations suggest that submersion of cells into the glutamine-free media could improve survival. Alternatively, glutamine antagonists could be used to pretreat the cells prior to transplantation. Unfortunately, the use of glutamine antagonists in patients to precondition the ischemic myocardial tissue is not a viable option due to a high rate of central nervous system toxicity (lethargy, confusion, and decreased mental status) (Hidalgo et al. 1998), an adverse reactions profile that would not be suitable for CVD patients undergoing cell therapy. However, treating cells in vitro could overcome these limitations. Esterified L-cysteine-*S*-*N*-methylcarbamate, which showed reduction of glutamine concentration in several tumors, could also be potentially examined as an adjunct glutamine reducer in SKMB media (Jayaram et al. 1990).

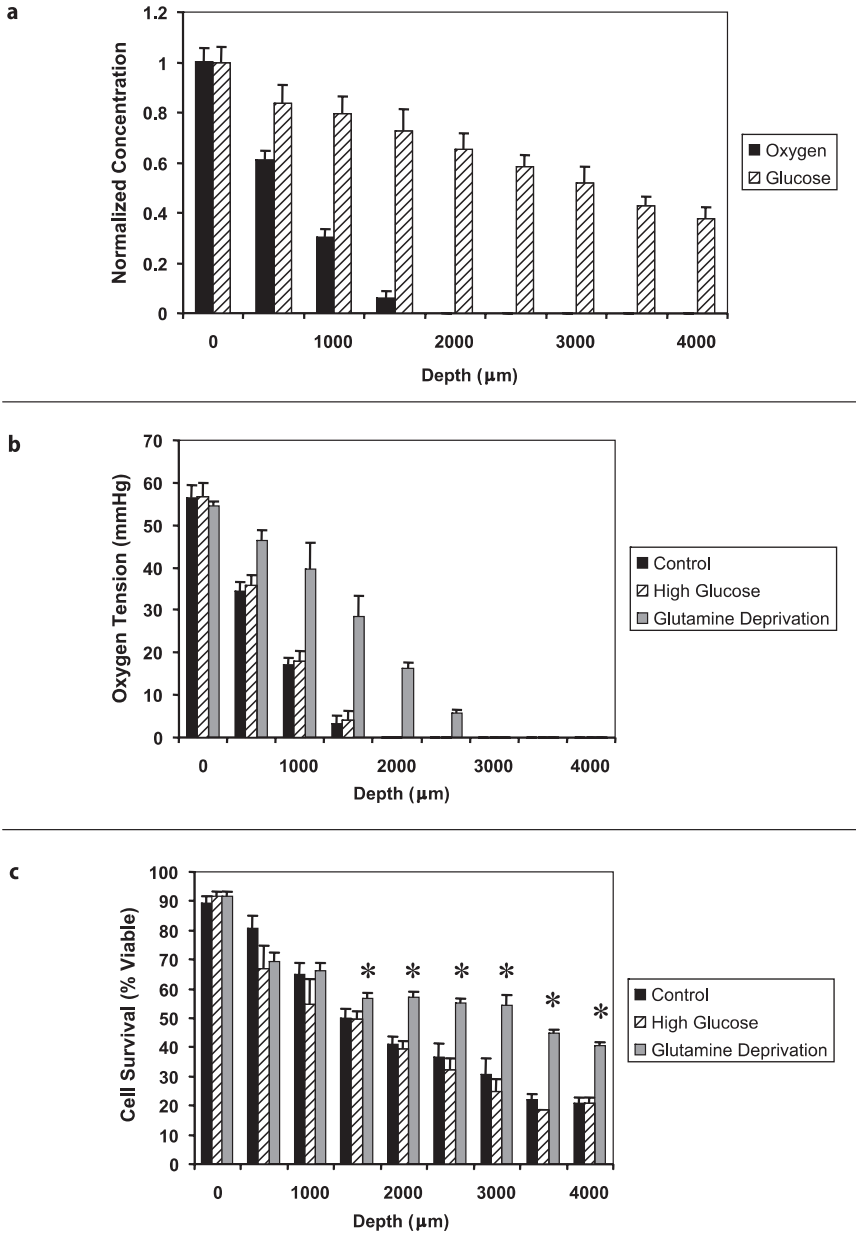
3.3.5 Inflammation and SKMBs

Inflammation is likely a cue for endogenous recruitment and repair. Cytokines and inflammatory mediators may also be important in the survival of cells. More than half of HF patients have atherosclerosis (Thom et al. 2006), which has now been established as an inflammatory disease (Hansson 2005). As cytokines mediate inflammatory responses and are largely responsible for the progression of atherosclerotic plaque (Hansson 2005), they could also negatively affect SKMB survival, most likely either via upregulation of NF-κB or downregulation of Akt (Tan et al. 2006). A considerable amount of work needs to be done to understand the interactions of SKMBs (or any transplanted cell type) with proinflammatory factors and the importance of those interactions in relationship to outcome. So far, it is unclear if cytokines that increase smooth muscle cell proliferation in atherosclerosis, such as interleukin (IL)-10 and IL-18 (Raines and Ferri 2005), would impact cells' fate, and whether monocyte chemoattractant protein (MCP)-1, IL-1β, fractalkine, interferon (IFN)-γ and/or stromal-derived factor (SDF)-1α participate in cell survival.

3.3.6 Autologous Versus Allogeneic Cells

As the development of therapeutic SKMB transplantation continues, the use of allogeneic (instead of autologous) cells may advance into clinical studies. The main reason for the use of allogeneic cells is the availability of an off-shelf product with known potency and defined characteristics. These factors are likely to be important both for use in an acute injury setting and in patients

Fig. 1a–c **a** Penetration depth of oxygen (*solid black bars*) and glucose (*ascending bars*) in a tissue-engineered construct seeded with primary SKMBs. Oxygen (55 mmHg) and glucose (5 mM) concentrations at the surface of the gel were designed to approximate values seen proximal to capillaries in mature myocardial tissue. As seen in this panel, oxygen concentration drops to zero in a fraction of the distance of glucose, indicating that oxygen (and not glucose) may be the limiting factor for survival of transplanted SKMBs. **b** Oxygen penetration depth in tissue-engineered constructs seeded with primary SKMBs maintained in control conditions (*solid black bars*) (5 mM glucose and 4 mM glutamine at the construct surface), high glucose conditions (*ascending bars*) (20 mM glucose and 4 mM glutamine at the construct surface), and glutamine deprivation conditions (*gray bars*) (5 mM glucose and 0.05 mM glutamine at the construct surface). As shown in this panel, constructs under glutamine deprivation allowed oxygen to penetrate much deeper into the constructs. **c** Cell survival in constructs described in panel b as measured by nitro blue tetrazolium chloride (NBT) staining. Cell survival was statistically equal (p=0.589) under control (*solid bars*) and high glucose conditions (*ascending bars*). Cell survival was significantly increased in constructs under maintained glutamine deprivation by 1,500 μm depth (*, p=0.009). Improved viability was secondary to improved oxygen penetration


a
Normalized Concentration
1.2
1
0.8
0.6
0.4
0.2
0
0
1000
2000
3000
4000
Depth (μm)
Oxygen
Glucose
b
Oxygen Tension (mmHg)
70
60
50
40
30
20
10
0
0
1000
2000
3000
4000
Depth (μm)
Control
High Glucose
Glutamine Deprivation
c
Cell Survival (% Viable)
100
90
80
70
60
50
40
30
20
10
0
0
1000
2000
3000
4000
Depth (μm)
Control
High Glucose
Glutamine Deprivation

with advanced HF, where SKMBs have been subjected to a prolonged period of stress: globally reduced cardiac output, alterations in levels of oxygen, amino acids and other metabolites, neurohormonal activation, etc. These cells are likely to be suboptimal and may potentially offer less functional benefits. In this regard, important progress has been made by Skuk et al. who have carried out allogeneic SKMB transplantation in nonhuman primates (Skuk et al. 2002). Obviously, the use of allogeneic cells will require carefully optimized immunosuppression. The bad news is that HF patients are already receiving extensive pharmacological regimens, and therefore, additional medications could impose the risks of drug interactions and synergistic adverse reactions. The good news is that immunomodulatory strategies are being actively explored, and so far the combination of tacrolimus and mycophenolate mofetil has been able to achieve efficient immunosuppression with reduced side effects following allogeneic SKMB transplantation. We can also learn from allogeneic myoblast transplantation in Duchenne muscular dystrophy (Camirand et al. 2004), where insights into SKMB survival and transplant tolerance will determine the potential of the use of allogeneic SKMBs in patients with CVD.

3.4 Latest Information

The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, a phase II randomized clinical trial to examine the efficacy and safety of CABG plus SKMBs versus CABG alone in approximately 300 patients in North America and Europe, has been recently reported at the Scientific Sessions of the American Heart Association in November 2006 (Menasche 2006). The trial was halted in the first part of 2006 because the design of the trial was no longer considered state-of-the-art (as the number of CABG cases declined), and recruitment fell much below projected targets. France, Belgium, the United Kingdom, Germany, Italy, and other European countries actively participated in this trial.

The inclusion criteria were LVEF between 15% and 35%, and a history of AMI prior to screening with residual akinesia, affecting at least three contiguous LV segments, that was unresponsive to administration of dobutamine. In addition, patients had to have a clinical indication for CABG. This trial utilized two doses of SKMBs: a mean of 400×10^6 and 800×10^6 cells. The cells were harvested and expanded under guidelines Good Manufacturing Practice in Paris, France, and Cambridge, MA, USA, achieving viabilities of 95% and a purity of 89%. Thirty patients received high dose of SKMBs, 33 received the low-dose, and 34 a placebo. CABG was performed in noncell-transplanted LV segments in all patients who received cell therapy, and the mean cross-clamp time ranged from 59 to 64 min without significant differences between the three groups.

Kaplan-Meier analysis of survival free of major adverse cardiac events (MACE) showed no differences between high-dose and placebo and low-dose versus placebo at 30 days and 6 months following the procedure (p=0.12 and

0.87 for the high-dose group and p=0.43 and 0.09 for the low-dose group, respectively). MACE curves separated early in the course of follow-up (at 1 month), although the trial was not powered to detect these differences statistically. Nevertheless, patients in the high-dose SKMB group appeared to have survived equivalently or slightly better compared to placebo, indicating no increase in MACE attributable to SKMB transplantation. Time to first ventricular arrhythmia was not different among the three groups at both 30 days and 6 months (p=0.30 and 0.12 for the high-dose group, and p=0.20 and 0.23 for the low-dose group). These data appear to significantly decrease if not dissolve concerns about potential augmentation of arrhythmogenesis with SKMBs.

Importantly, administration of high-dose SKMBs reduced LV end-diastolic volume by the mean of 23 ml (range: −42 to 0 ml), p=0.006, and decreased LV systolic volume by the mean of 18 ml (−34–6.0 ml), p=0.008 versus placebo. These changes translated into a mean 3.0% change in LVEF (3.0–14.0), p=0.04 compared with the placebo group. Of course, the limitations of this trial are in the small number of patients, relatively short length of follow-up, and the inability to perform MRI for a more precise quantification (versus echocardiography) of the anti-remodeling effects of SKMB therapy. Another important remark is that no patient in either the low- or high-dose group exhibited overt acute HF.

Overall, the MAGIC trial showed that SKMB administration has favorable effects on the LV remodeling process, which is the culprit of HF progression. This outcome was achieved without compromising safety of patients. The results of the MAGIC trial in conjunction with earlier studies in the United States and Europe suggest, at the very least, that SKMBs deserve a more in-depth evaluation.

In summary, autologous SKMB administration for patients with HF has the potential of being a relatively efficacious treatment, if such holds true in definitive phase III trials. The results of the MAGIC trial have certainly brought back the enthusiasm for SKMB-based repair of myocardial damage and the possibility of SKMB therapy being a part of the treatment paradigm in patients with HF. The role of SKMBs in the treatment of AMI remains to be determined.

4
Bone Marrow Mononuclear Cells: Recent Studies Show Positive Effects in Ischemic Injury

BMNCs have received attention recently following presentation of the latest clinical trials showing reduction in a composite endpoint of death, recurrence of myocardial infarction and revascularization in patients post-AMI (Schachinger et al. 2006b). In the past bone marrow cells were thought to give rise only to hematopoietic cells. We now serow that BMNCs are a heterogeneous population of hematopoietic precursors, containing endothelial pro-

genitor cells (EPCs) and their subsets (e.g., AC 133^+, or VEGF $R2^+$, or $CD34^+$ progenitors), mesenchymal stem cells (MSCs), and multiple other populations including monocyte precursors, T and B cell precursors, CD14 cells, etc. (Saulnier et al. 2005; Verfaillie et al. 2003). Similar cell populations have been isolated from umbilical cord bloodw (Zhai et al. 2004). As these cell types show satisfactory results in treatment of AMI, several aspects merit a discussion.

4.1 Bone Marrow Mononuclear Cells: Brief Overview

Several BMNCs populations have been targeted for cardiac repair. These include c-kit^+ cells, EPCs, mixed BMNC fractions, and other subsets. At issue is their potential for cardiac-related angiogenesis and myogenesis. Bone marrow-derived progenitors by their nature respond to the microenvironment and develop a correspondent phenotype (Orlic et al. 2001). The differentiation of BMNCs into cardiomyocyte-like cells has been demonstrated (Makino et al. 1999; Pittenger et al. 1999). Tomita et al. have substantiated that finding showing that BMNCs transplanted into cryoinjured myocardium differentiate into myogenic cells expressing myosin heavy chain and troponin I—hallmarks of muscle cells (Tomita et al. 1999). Therapeutic agents, such as dexamethasone (Grigoriadis et al. 1988) and 5-azacytidine (Wakitani et al. 1995), accelerated the formation of myotube-like structures in vitro, and the cells then started beating spontaneously. Wang and colleagues (2000) demonstrated differentiation of BMNCs (in the environment of a normal myocardium) into cardiomyocytes that contained not only myosin heavy chain but also gap junctions. Orlic and coauthors confirmed that a BMNC population (lin^- c-kit^+ cells) could repair myocardial scar when delivered subcutaneously together with granulocyte-colony stimulating factor (Orlic et al. 2001). Other cell populations isolated from BMNCs also achieved positive results (i.e., reduction of LV remodeling and lessening the degree of cardiomyocyte apoptosis) (Kocher et al. 2001). In that regard, our group has also shown that when injected into the center and the peripheral regions of the scar, some BMNCs differentiated into striated muscle and improved LV function (Thompson et al. 2003). Whether BMNC populations do or do not produce functioning cardiomyocytes may not be clinically relevant, as Murry et al. (2004) have shown that lin^- c-kit^+ cells did not produce cardiomyocytes after transplantation into the ischemic myocardium, but instead differentiated into hematopoietic cells; however, despite the lack of differentiation into cardiomyocytes, these cells did prevent LV remodeling. These and other (Jackson et al. 2001) findings indicated that bone marrow administration may deliver beneficial results when transplanted into a patient with CVD, without ethical dilemmas associated with the use of embryonic progenitors. Moreover, the techniques of collection and expansion of bone marrow cells have been established because of the use of bone marrow transplantation in hematology and oncology as a therapeutic procedure.

4.2 Endothelial Progenitor Cells

EPCs are bone marrow-derived cells that express CD133 (AC133), CD34, and vascular endothelial growth factor (VEGF)-R2 (KDR) markers on their surface at various times in their maturation process. EPCs are considered to play an important role in maintaining vascular integrity and mediating angiogenesis (Hill et al. 2003; Kalka et al. 2000). Recent data have shown associative evidence between the quantity and function of circulating EPCs and the risk for CVD. For example, the number and the functional capacity of $CD34^+KDR^+$ EPCs was inversely related to the level of risk for CVD in 519 patients and also correlated with a composite measure of events (AMI, hospitalization, revascularization, or CVD-related death) at follow-up after adjustments for age, gender, and other risk factors and covariates (Hill et al. 2003; Werner et al. 2005). Event-free survival increased proportionately to the baseline level of EPCs. Fadini et al. showed that the same population of EPCs was an independent predictor of early atherosclerosis measured by carotid intima-media thickness in 137 subjects (Fadini et al. 2006). However, we believe that the risk for CVD could be better reflected by a combined assessment of several BMNC-derived, EPC-related populations—"reparative" (such as $CD34^+$, $AC133^+$ EPCs) and "pro-inflammatory" (such as $CD45^+$, $CD14^+$, and $CD3^+$, and the like) cells. Our group recently showed that a reduction in $CD31^+CD45^-$ vascular progenitor cells, thought to be related to EPCs, is associated with aging and disease state in the mouse *ApoE*$^{-/-}$ model of atherosclerosis (Rauscher et al. 2003). In our hands, the delivery of functionally viable cells could prevent the progression of atherosclerosis and reduce inflammation, as reflected by decreasing circulating IL-6.

Recent research has shown that the number of circulating EPCs is increased in patients following AMI, most likely representing an attempt for endogenous repair (Shintani et al. 2001). EPCs are presumed to be mobilized by the ischemic damage in the heart (and other tissues) and migrate to damaged areas to induce neovascularization. When EPCs were injected into the rats tail vein or LV cavity after a period of ischemia, more than a twofold increase in the homing of infused EPCs was observed when compared to animals undergoing sham surgery (Aicher et al. 2003). LV dimensions, fractional shortening, and regional wall motion improved only in rats that received EPCs and not in the controls (Kawamoto et al. 2001). It is likely that the improvements seen in part depended on increased myocardial perfusion and decreased inflammation following administration of EPCs. Indeed, after an intravenous infusion of EPCs into an infarcted region, a marked increase in capillary density in the infarcted area and its borders occurred (Kocher et al. 2001). That effect has been attributed to a combination of vasculogenesis and angiogenesis, and several paracrine properties have recently been attributed to these cells (Kinnaird et al. 2004), although the mechanism of these beneficial action on the

ischemic myocardium remain to be investigated. Currently, $CD34^+$ cells are being evaluated for effects in patients with refractory angina, chronic ischemia, and intermittent claudication.

4.3 Mesenchymal Stem Cells

MSCs are multipotent progenitors derived from the marrow stroma. These cells are negative for the $CD34^+$ marker that is characteristic of EPCs, but express a series of other distinguishing markers, such as CD29, CD44, CD71, CD90, CD105, CD106, CD120a, CD124, and Src homology domains (SH) (Haynesworth et al. 1992; Pittenger and Martin 2004). MSCs can differentiate into most cell types of mesodermal origin including fat, bone, cartilage, and skeletal muscle precursors (Jiang et al. 2002). There is also conflicting evidence on the capacity of MSCs to differentiate into cardiomyocyte-like and endothelial cells after intramyocardial injections (Kawada et al. 2004; Shake et al. 2002; Toma et al. 2002). The prevailing thought is that such differentiation can only happen when these cells are in contact with native cardiomyocytes and does not happen within the scar (Strauer et al. 2002), where instead cells can give rise to other mesodermal cells including fibroblasts, osteoblasts, chondrocytes, and adipocytes. If this holds true, the optimal time for therapeutic MSC administration is more likely to be from early after injury (within days), when surviving cardiomyocytes are still present in the infarcted territory, than when the scar has fully matured. Functionally, MSCs engraft in relatively high numbers, and appear to increase neovascularization and improve regional contractility and diastolic function (Schuster et al. 2004). Several other studies have suggested that MSCs can home to sites of injury following injection into the coronary or peripheral vasculature, reduce the size of the infarcted territory, and restore functional characteristics of the injured myocardium (Amado et al. 2005; Bittira et al. 2003; Strauer et al. 2002). However, it has also been reported that intracoronary administration of MSCs can cause microinfarctions and induce damage of otherwise healthy myocardium (Vulliet et al. 2004). These safety aspects need to be carefully evaluated in the ongoing clinical trials.

MSCs are the only allogeneic progenitor population in clinical trials for treatment of CVD. Recently, MSCs have been defined as "immunoprivileged" (Amado et al. 2005; Jiang et al. 2005) because they do not express MHC-class II and B-7 molecules, which prevents their engaging in the usual T cell responses to produce soluble mediators of rejection (Zimmet and Hare 2005). Although a certain degree of skepticism about that fact remains, such a property definitely increases the attractiveness of these cells for future clinical use. Observing functional improvement of the myocardium with an off-the-shelf cell that lacks negative immunological effects could accelerate the development of a commercial cell therapy product. However, whether MSCs are going to hold up to their promise in clinical studies remains to be seen. A safety/feasibility

study is underway, and the early data appear promising, but whether MSCs will equal or outperform any other cell type remains to be seen. A recent preclinical study from our group suggests that MSCs and SKMBs both improve function after ischemia-induced cardiac injury to a similar degree (Thompson et al. 2003).

4.4 Cardiac Progenitor Cells

Recently, several undifferentiated cell populations have been isolated from neonatal and acutely infarcted, failing hearts by their expression of c-kit, multidrug resistant gene (MRD)-1, isl-1, or sca-1 stem cell markers and by concomitant lack of expression of hematopoietic markers (Anversa and Nadal-Ginard 2002; Oh et al. 2003; Urbanek et al. 2005). Interestingly, the number of some these cells was increased after AMI, but was very low in failing hearts, suggesting that these cells take part in ongoing minor repair, which becomes insufficient in HF (Beltrami et al. 2001). Mouquet et al. have recently identified a similar side population within the bone marrow (Mouquet et al. 2005). More recently, we have isolated an upstream progenitor population in neonatal and adult hearts, which appears to give rise to these downstream (more mature) cardiac progenitor populations. These stage-specific embryonic antigen-1-positive uncommitted cardiac progenitor cells (UPCs) could be expanded in vitro and differentiated down myocyte, smooth muscle, and endothelial cell pathways (Ott et al. 2007). To date, methods for harvest, expansion, and in vitro growth of all cardiac progenitor cell (CPC) populations are limited. Smith and colleagues demonstrated that CPCs could be isolated from biopsy specimens obtained from humans and grown under in vitro conditions (Smith et al. 2005). We have shown that we can expand UPCs to large numbers in vitro over several weeks, providing numbers sufficient for cardiac repair (Ott et al. 2007).

Functional repair is the ultimate aim of cell therapy and should at least theoretically be best initiated with cardiac-derived cells. We have recently shown that expanded UPCs were capable of functional repair when injected into infarcted rat heart at 2 weeks following the ligation of the left anterior descending artery (Ott et al. 2005b). LVEF improved in the treated animals (baseline: 34.8±4.2%, week 5:56.5±6.5%, p=0.001), and, as expected, decreased in control animals (baseline: 36.5±3.7%, week 5:28.2±3.8%, p<0.001). Overall, LV remodeling was attenuated in UPC-treated animals compared to controls. At follow-up, maximal +dP/dt was higher in UPC-treated animals and the relaxation time was shorter compared with controls. As predicted by the hemodynamic improvement and positive anti-remodeling effects, the infarct size was reduced with UPCs. Engraftment of UPCs within scars was histologically verified (Ott et al. 2005b). Similarly to the UPC population, c-kit$^+$ cells are involved in repair after being injected into an ischemic myocardium (Beltrami et al. 2003). Endogenous Sca-1$^+$ CPCs possess the ability to differentiate into functional cardiomyocytes (Oh et al. 2003). And when isl-1$^+$ cells were

co-cultured with neonatal cardiomyocytes, those cells were able to electrically integrate with the myocardial cells in vitro by forming gap junctions (Laugwitz et al. 2005).

Overall, the biology of CPCs and their capacity for repair are creating interest, and the results of preclinical use are intriguing. It is possible that future of cardiac repair may involve endogenous mobilization or recruitment of these cells.

4.5 Clinical Studies

Trials performed to date (Table 2) have focused on the use of BMNCs, EPCs, MSCs, and cardiac-derived progenitor cells (CPCs) for a broad range of CVD—from advanced coronary atherosclerosis to end-stage HF. As shown in Table 2, the outcomes of clinical studies are quite divergent—from no effect on patients' symptoms and/or objective measures of LV performance to a reduction of CVD-related events at follow-up. Whether this discrepancy represents a difference in disease context (advanced atherosclerotic CVD versus acute ischemic insult versus chronic downregulation of blood flow and contractility), patient population, cell type, and dose or some other factors remains to be resolved.

Overall, the data on the use of BMNCs in advanced atherosclerotic disease, AMI and ST-elevation myocardial infarction (STEMI) to date are encouraging. Although a small number of patients with advanced atherosclerosis (and no AMI or HF) have been studied (Tse et al. 2003, 2006), cell therapy substantially reduced anginal episodes per week to an extent that appears greater than the reduction seen with ranolazine, a new antianginal agent (Chaitman 2006). The improvement in symptomatology with BMNCs correlated with increased myocardial perfusion. Unfortunately, BMNCs in the context of reperfused and/or stented AMI were not as beneficial. Although a trend toward a reduction in the size of the infarcted area was observed, no functional improvement was gained (Lunde et al. 2006). This apparent lack of a positive effect might have occurred due to an efficient reperfusion and prompt restoration of coronary flow that precluded the potential for BMNC-based repair (Kuethe et al. 2004). When reperfusion and stenting were not uniformly utilized, BMNCs and other cell types ($AC133^+$ EPCs and MSCs) improved myocardial viability (or reduced infarct size), wall motion, coronary flow, and LVEF. However, some disappointments taint the otherwise bright picture: (1) compared to BMNCs, CPCs did not perform very well, and (2) several patients who received $AC133^+$ EPCs showed either restenosis or *de novo* lesions. Whether the cells were the primary suspects or innocent bystanders is quite difficult to discern from the small number of patients studied (Bartunek et al. 2005). It is possible that the success of BMNCs lies in the administration of unfractionated cells, which then allows for the cell–cell and cell–tissue signaling interactions in vivo (the extent of which are not entirely known at this time) that are otherwise absent when

Table 2 Cell therapy trials with BMNCs, MSCs, EPCs, and CPCs. Included are studies with 5 or more patients enrolled for which results were published prior to or on 1 November 2006, where methodological and outcome details were available. Controls comprise historical and active randomized participants and those patients who received placebo. Dose-escalation/variation was used in studies where range is provided. For details, please refer to original publications

Investigator (country)	No. of patients	Cell type	Dx	Mean cell dose, $\times 10^6$	Delivery route	Follow-up time, m	Functional outcomes
Tse et al. 2003, 2006 (China)	12	BMNCs	Advanced CAD	12–16	Transendo EMM-guided	3, 6 and 44±10	At 3 m, regional wall motion, thickening improved, hypoperfused areas lessened (by MRI); no significant changes in LVEF at 3 or 6 m (baseline LVEF 60%). Angina reduced from 26.5 to 16.4 episodes/w. At long-term follow-up, 2 patients died, 1 patient received CABG.
Hamano et al. 2001 (Japan)	5	BMNCs	Advanced CAD	300–2,200	Transepi +CABG	12	Perfusion improved in 3 out of 5 patients (by S).
ASTAMI: Lunde et al. 2006 (Norway)	47 (+50 controls)	BMNCs	AMI treated with PCI	54–130	IC	6	No differences in LVEF (by MRI), or perfusion (by SPECT); trend toward infarct size reduction (by MRI).
Kuethe et al. 2004 (Germany)	5	BMNCs	AMI (reperfused, stented)	39	IC, 6.3±0.4 d post PCI	3 and 12	LVEF and regional wall motion did not change (by E). Coronary flow (by IC Doppler) and contractility indexes (by DE) remained similar at follow-up.

Table 2 (continued)

Investigator (country)	No. of patients	Cell type	Dx	Mean cell dose, $\times 10^6$	Delivery route	Follow-up time, m	Functional outcomes
TOPCARE-AMI: Assmus et al. 2002; Schachinger et al. 2004, 2006a; Britten et al. 2003 (Germany)	30 29	CPCs BMNCs	AMI	CPCs: 13 BMNCs: 238	IC, 4.9 d after AMI	4 and 12	At 4 m, wall motion in the infarction zone improved (all by V and DE); myocardial viability increased (by PET); CPCs and BMNCs behaved similarly. Migratory capacity predicted LV remodeling in multivariate analysis. LVEF improved from 51.6% to 60%; ESV reduced At 1 y, 1 patient in each cell group died due to cardiogenic shock, no other MACE or malignant arrhythmias; MRI at 1 y showed maintenance of LVEF and reduction of infarct size, no reactive LV hypertrophy. Coronary flow normalized in infarct-related arteries.
Chen et al. 2004 (China)	34 (+35 controls)	MSCs	AMI	8,000–10,000	IC, 8±4 h after AMI	6	LVEF and regional wall motion increased (by V); perfusion defects decreased (by PET); real-time EMM showed improvements in mechanical capabilities, electrical properties and functional indexes of LV.
Strauer et al. 2002 (Germany)	10 (+10 controls)	BMNCs	AMI	28	IC, 5–9 d post AMI	3	Infarcted region decreased to 12±7% from 30±13% (by V); LV contractility and EDV improved, perfusion increased (by DE, RV, RC).
Bartunek et al. 2005 (Belgium)	19 (+16 controls)	AC133	AMI	12.6	IC, 11.6±1.4 d post AMI	4	LVEF increased from 45% to 52.1% similar to controls (by E), perfusion improved (by SPECT). 7 patients in cell therapy group developed restenosis (vs 4 in control group), 2 patients in cell therapy group had de novo lesions.

Table 2 (continued)

Investigator (country)	No. of patients	Cell type	Dx	Mean cell dose, $\times 10^6$	Delivery route	Follow-up time, m	Functional outcomes
Stamm et al. 2003 (Germany)	6	AC 133 (purified)	AMI	1.02–1.57	Transepi +CABG	3	LVEF, EDV, EDD improved (by E); perfusion (area at-risk) improved in 5 out of 6 patients.
Fernandez-Aviles et al. 2004 (Spain)	20	BMNCs	Extensive AM	78	IC, 13.5±5.5 d post-AMI	6	LVEF improved by mean of 6%, contractile reserve increased; ESV decreased in (by MRI, DE).
Galinanes et al. 2004 (UK)	14	BMNCs	Extensive AMI	Aspirated BMNCs	+CABG	1.5 and 10	Improvement in segmental and global LV function only in segments that received cells+CABG (by DE). Effect persisted through follow-up.
REPAIR-AMI: Schachinger et al. 2006b (Germany)	101 (+103 controls)	BMNCs	STEMI	236	IC, 3–6 days after AMI	4 and 12	LVEF increased by a mean of 5.5% (by V), patients with LVEF<49% benefited most At 1 y, BMNC-treated patients exhibited reduction in a combined MACE endpoint (death, AMI recurrence, revascularization).
Janssens et al. 2006 (Belgium)	33 34	BMNCs Controls	STEMI	172	IC with 3 ischemia-reperfusion cycles, 24 h post Dx	4	Contractility enhanced at follow-up in segments with severe and complete hyperenhancement (markedly reduced viability) the cell-treated group, a trend toward reduction in LV mass (by MRI); patients with larger infarctions exhibited restoration of myocardial viability (by PET).

Table 2 (continued)

Investigator (country)	No. of patients	Cell type	Dx	Mean cell dose, $\times 10^6$	Delivery route	Follow-up time, m	Functional outcomes
BOOST: Meyer et al. 2006; Schaefer et al. 2006; Wollert et al. 2004 (Germany)	30 (+30 controls)	BMNCs	STEMI	2,460	IC, 4.8±1.3 days post-PCI	12	LVEF increased by 6.7% mostly due to improved regional wall motion in the peripheral area (by MRI); LVEDV and infarct size decreased. Diastolic function improved (by E). LV functional benefits did not persist at 1 y.
Katritsis et al. 2005 (Greece)	11 (+11 controls)	MSCs +EPCs	STEMI (6 pts <1 m; 6 pts >1 m)	1–2 (MSCs: 66±11%; EPCs: 28±11%	IC, with ischemia-reperfusion cycle	4	Myocardial contractility improved in 5/11 patients but not in controls (by DE); perfusion improved in 6/11 patients (by SPECT).
Fuchs et al. 2006 (Israel)	27	BMNCs	Refractory angina +ischemia	28	Transendo EMM-guided	3 and 12	Exercise duration increased from 418±136 s to 489±142 s; ischemia lessened (by SPECT).CCS angina class improved from 3.2±0.5 to 2.0±0.9 At 1 year, 5 patients had revascularization procedures, functional and symptomatic improvements were maintained in other patients.
Fuchs et al. 2003 (USA)	10	BMNCs	Refractory angina	78	Transendo EMM-guided	3	Perfusion improved (by SPECT); CCS angina score decreased from 3.1±0.3 to 2.0±0.9.
TOPCARE-CHD: Assmus et al. 2006 (Germany)	34 35 (+23 controls)	CPCs BMNCs	Prior AMI (>3 m)	CPCs: 22 BMNCs: 205	IC, with cross-over to the other cell type	3	LVEF increased in patients that crossed over to BMNCs, absolute increase=2.9% (by MRI). No changes in LVEF with CPCs. NYHA class improved in BMNC group—reduction to of 2.0±0.7 from 2.2±0.6, no improvement with CPCs.

Table 2 (continued)

Investigator (country)	No. of patients	Cell type	Dx	Mean cell dose, $\times 10^6$	Delivery route	Follow-up time, m	Functional outcomes
IACT: Strauer et al. 2005 (Germany)	18	BMNCs	Prior AM	15–22 per ea infusion, 4–6 total	IC, 5 m–8.5 y	3	LVEF increased by 15% (by V), infarct size fell by 30% (by SPECT), myocardial viability of infarcted zone increased by 15% (by PET).
Perin et al. 2003; Dohmann et al. 2005 (study conducted in Brazil)	14 (+7 controls)	BMNCs	Severe CAD+HF	25.5	Transendo EMM-guided	2, 4, and 6	Mean LVEF increased from 30% to 35.5% (by E); perfusion improved (by SPECT); NYHA class decreased from 2.2±0.9 to 1.1±0.4; CCS angina reduced from 2.6±0.8 to 1.3±0.6 class.
Blatt et al. 2005 (Israel)	6	BMNCs	ICM	50 ml of aspirated BMNCs	IC, after induction of ischemia by balloon inflation for 3 min	4	LVEF improved from mean of 25% to 28% (by E); wall motion (by DE) increased but only in segments with baseline hibernation. NYHA class fell to mean of 2.3 from 3.5; one patient developed postprocedure hypotension and troponin increase.
Perin et al. 2004 (study conducted in Brazil)	11 (+9 controls)	BMNCs	End-stage ICM	15 injections, 0.2 ml/ea (50 ml aspirated)	Transendo EMM-guided	2, 6 and 12	LVEF increased at 2 m, did not change at 6 and 12 m; perfusion improved (by SPECT), NYHA class decreased from a mean of 2.2 to 1.4, CCS fell from 2.6 to 1.2 class. Exercise capacity improved (by treadmill).

Table 2 (continued)

Investigator (country)	No. of patients	Cell type	Dx	Mean cell dose, $\times 10^6$	Delivery route	Follow-up time, m	Functional outcomes
Silva et al. 2004 (USA)	5	BMNCs	Pre-Trnsp HF	15 injections, 0.2 ml/ea (50 ml aspirated)	Transendo EMM-guided	2 and 6	Exercise capacity (by treadmill oxygen consumption) improved in 4/5 patients, disqualifying them from listing for Trnsp.

CABG, coronary artery bypass graft; CAD, coronary artery disease; CCS, Canadian Cardiovascular Society; d, days; DE, dobutamine echocardiography; Dx, diagnosis; ea, each; EDD, end-diastolic (LV) dimension; EDV, end-diastolic (LV) volume; EMM, electromechanical mapping of LV; h, hours; HF, heart failure; IC, intracoronary; ICM, ischemic cardiomyopathy; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; m, month(s); MACE, major adverse cardiac events; NA, not available; NYHA class, New York Heart Association functional class; PCI, percutaneous coronary intervention; PET, positron emission tomography; pts, patients; RC, right heart catheterization; RV, radionuclide ventriculography; S, scintigraphy; sec, seconds; SPECT, single photon emission tomography; STEMI, acute ST-elevation myocardial infarction; Transendo, transendocardial; Transepi, transepicardial; Trnsp, transplantation; Tx, therapy/treatment; V, ventriculography; w, week; y, years

an isolated cell population is administered. In other words, unfractionated BMNCs have both mature and immature EPCs along with other progenitors, and it is quite possible that a combination of these cells may be the best choice, although specific CPC populations have not been clinically tested.

The REPAIR-AMI (Schachinger et al. 2006b) study highlighted several important aspects with regards to efficacy of cell therapy. First, administration of the mean number of 236×10^6 BMNCs in patients with ST-elevation myocardial infarction resulted in a higher event-free survival at 1 year than patients who received placebo. That was the first randomized study showing that exogenous BMNCs do in fact participate in tissue repair and can withstand the rigorous test of clinically driven endpoints, at least in a phase II study. Second, it is becoming apparent that there needs to be a sufficient degree of tissue injury for the cells to show efficacy. For instance, in the REPAIR-AMI study, those patients that had a baseline LVEF at 48.5% or lower benefited the most from BMNCs, and those above this cut-off showed little or no benefit.

Unfortunately, despite the best efforts to reduce the progression of the pathophysiological process post-AMI, up to 50% of patients manifest symptomatic HF by year 7 post-AMI (Abbate et al. 2006; Miller and Missov 2001). HF notwithstanding, refractory angina is a growing problem post-AMI, with approximately 20% of patients remaining symptomatic despite best efforts to tailor pharmacological therapy and interventional approaches (Yang et al. 2004). BMNCs have exhibited a considerable improvement of symptomatic status and functional outcomes in the latter group, which persisted at 1 year follow-up. The reduction of anginal episodes was paralleled by increased exercise treadmill time and improved myocardial perfusion. In the former group (HF), successes of BMNCs varied, most likely dependent on the baseline LVEF and, quite possibly, on the delivery technique. Clearly, patients enjoyed a reduction of shortness of breath, angina, and other symptoms; however, the effect on LV contractility was not always very pronounced. Possibly, administration of cells can prevent HF from worsening, at least for a period of time. The data from Silva et al. (2004) showing delisting of patients from transplantation because of the increase in exercise capacity, albeit in a very small number of patients, supports these beneficial effects on BMNCs in HF. The TOPCARE-HF and BOOST-2 studies have been initiated to gain a more systematic insight into the response of the myocardium to BMNCs, when HF pathophysiology predominates. Given the reduced number and migratory capacity of EPCs shown in preclinical studies and the deficits in EPC quantity seen in patients with advanced CVD and HF (Werner et al. 2005), it will be interesting to see if BMNCs are capable of improving cardiac function or if the HF milieu only allows ischemia-resistant cells, such as SKMBs or MSCs, to survive. So far, unlike in the SKMB trials, symptomatic and functional improvements in HF patients treated with BMNCs occurred without the additional burden of electrical events. These large trials will provide more data to definitively answer the question of safety with BMNCs.

BMNCs, CPCs, and AC133$^+$ cells have been given intracoronarily and as an adjunct to CABG with granulocyte colony-stimulating factor (filgrastim and PEG-filgrastim) mostly in the context of safety and feasibility studies. These studies have been reviewed elsewhere (Boyle et al. 2006; Korbling et al. 2003).

4.6 Umbilical Cord Blood Cells

A relatively new source for progenitor cells is umbilical cord blood, which contains fetal-derived populations identical to those found in bone marrow (Erices et al. 2000). Umbilical cord blood cells (UCBCs) are easily obtained, albeit not in large volumes, have the potential to develop into multiple lineages, do not pose as many ethical questions as embryonic cells, and are less immunogenic than allogeneic bone marrow counterparts, which means a larger proportion of the population could receive cells from appropriately matched donors. If UCBCs are stored at birth, they could provide an autologous source of stem cells to treat myocardial damage later in life. Current studies in animal models show that UCBCs injected directly into the infarcted myocardium improve LV ejection fraction, anteroseptal wall thickening, and dP/dt (max), while decreasing infarct size (Henning et al. 2004). In addition, intravenous injection of UCBCs in mice following ligation-induced injury resulted in an approximately 20% higher capillary density in the border zones of the infarction—a finding not observed in untreated animals (Ma et al. 2005). Recent data have suggested that human UCBC-derived CD34$^+$ cells may be capable both of preventing injury progression and of partially reversing systolic and diastolic dysfunction, if administered shortly after AMI (Leor et al. 2006). Several other investigators published studies showing similar functional outcomes with varying populations of UCBCs (Hirata et al. 2005; Kim et al. 2005). However, no evidence to date suggests that cord blood cells injected into the infarcted myocardium are able to produce mature cardiomyocytes in humans, or that the functional benefits seen in animal models could be replicated in patients with AMI, HF, or both. Overall, it appears that UCBCs may appear to be an interesting cell of choice to be used in further studies of treatment of myocardial injury.

In summary, the data with regards to BMNCs across the continuum of CVD are not uniform, but nonetheless encouraging. Taken together, the results show a great deal of evidence toward the efficacy of BMNC therapy in settings of AMI, refractory angina, and HF. At this time, it is clear that at least in AMI patients, a phase III trial will take place soon. Current ongoing clinical trials are listed on the Internet (http://www.clinicaltrials.gov; http://www.the-scientist.com/supplementary/html/24104). There are reasons to be optimistic realizing there is much yet to be learned in the process of defining a cell-based clinical product.

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Skeletal Myoblasts Versus Bone-Marrow Cells: How Far to Go to Reach the Best Cell for Cardiac Repair?

Translating research findings from bench experiments to bedside efficacy to develop a new therapeutic product is a process of multiple interrelated steps. The first stage is the idea, which comes from the basic science of the pathophysiology of a disease. Next, that idea must be tested in clinically relevant animal models. If the data indicate a potential benefit, further testing takes place in consecutive clinical studies according to a regulatory framework. However, if unexpected issues arise or new pieces of the puzzle emerge (for example, delivery-related issues in the case of cell therapy), the process needs to move back to the bench, and only when those issues are resolved can the process return back to bedside. Cell therapy with either SKMBs or BMNCs and its subsets is in the iterative stage between bedside and bench at the present time. The success of the "clinical product" rests on these re-iterations, as these refinement cycles address issues that may hinder or even preclude clinical utilization. Ultimately, selection of the cell to exercise the fullest capacity for repair delivered via the route that is easiest to operate and least dangerous for the patient, thereby reaching the most suitable environment will define the best cell. At this time, however, we still have several things to accomplish before we can assign that status to a particular cell type. In this regard, there are several important prerequisites that merit discussion.

5.1
Creating a Centralized Registry for the Results of Trials and Biorepository for Blood Samples to Examine Accumulated Data and Set Direction for the Future of the Field

Decades of CVD research have highlighted the importance of centralized databases in advancing our understanding of a disease process. The field of cardiovascular medicine, in particular, would not have advanced as far as it has in the past 25–30 years if the Framingham Study or the Thrombolysis in Myocardial Infarction (TIMI) trials had not been initiated and executed in a centralized matter. Large databases provide the power to examine the data retrospectively while being able to control for numerous covariates—a step not possible to accomplish in a review or even a meta-analysis. Centralized databases also act as testing grounds for new hypotheses, often before clinical trials commence.

The field of cell therapy has arrived at the point where the next advancement should be creating and employing a large database of all results of clinical trials to serve as a filter for the hypotheses. With the aid of such a tool, ideas could be segregated before hundreds of thousands of dollars are spent only to find out, for example, that a specific unforeseen factor interfered with the outcome. Centralized data collecting efforts in acute HF, such as the Acute

Decompensated Heart Failure National Registry (Yancy and Fonarow 2004), have brought extremely valuable data with regards to the outcomes of clinical management of HF patients. We believe that a centralized registry of cell therapy trials could not only advance the field but could generate the next set of questions to ask, which, in turn, will greatly advance the science and will move the field closer to creating a cell-based "clinical product." With the powers of the Internet and data sharing, it is possible to design the registry in such a way that it is not only compliant with appropriate regulations on the conduct of research and on patient privacy but is also effective in reducing the work load of users, and, most importantly, could be accessible from multiple points, similar to a Web page.

The second aspect of the centralized registry is pairing it with a biorepository for blood samples. Although such an initiative requires committed funding and resources, centralizing sample collection, storage, flow cytometry, and assays makes a great deal of sense and could help greatly advance the science. The goal would be to use the progenitor cell characterization in conjunction with clinical data and examine the dynamics within multiple populations of progenitors in varying states of disease when different types of cells are given. Understanding the mechanisms of repair and regeneration is the next obstacle of the field today—and the biorepository may substantially advance that knowledge. In that regard, the ability to go back to the samples when new markers, receptors, and pathways emerge is far more cost-efficient than the currently available approaches. Combining the registry for the clinical trials data and the biorepository for the blood and tissue samples seems to be exactly what the field needs to make another decade of major progress and help shape future cell therapy products.

5.2 Increased Mechanistic Understanding Should Allow Us to Create the Best Cell-Based "Clinical Product"

Nine years after the first report suggested efficacy of SKMBs in treatment of failing myocardium, cell therapy has been applied, albeit only in clinical studies, in almost 800 patients (sum of patients in all published studies, and including the recently presented MAGIC trial). In addition, many trials are still actively recruiting patients. However even after over 800 patients have been treated, we do not possess a solid understanding of the mechanism of cell engraftment, survival, and tissue repair. While a complete definition of the mechanisms of beneficial action as well as adverse effects has not been required for the regulatory approval and clinical use of pharmacological treatments [e.g., levonorgestrel for emergency contraception (Gemzell-Danielsson 2006), immunomodulatory drugs in myelodysplasia (Galili and Raza 2006), farnesyl transferase inhibitors in cancer (Appels et al. 2005), mitoxantrone in multiple sclerosis (Neuhaus et al. 2005), adverse effects of bisphosphonates on the bone

(Pickett 2006), behavioral side effects of certain triptans in the treatment of migraine headaches (Lambert 2005), etc.], optimizing of cell-based products cannot proceed without additional mechanistic insights into cell-mediated repair. Knowing critical components of how the SKMB- or BMNC-driven repair works should enable us to target these (and other) types of cells to the right pathophysiological contexts, to achieve efficacy comparable or better than that of pharmacological therapies, especially when measured by long-term follow-up studies. These insights can only be obtained when clinical and basic science work together, so that the process can balance between clinically relevant questions and scientifically important observations.

Even though our understanding of repair at this point is limited, progress has been made and pathways as well as individual components are being identified. For example, we now understand that survival of BMNCs and their actions are mediated (at least to some extent) by Akt, (Gnecchi et al. 2006) and that VEGF, (Wang et al. 2006; Zen et al. 2006) stromal-derived factor-1 (Misao et al. 2006; Ratajczak et al. 2006) and several cytokines (Takahashi et al. 2006) play a role as well. But what about thrombopoietin, erythropoietin, hypoxia-induced factor-1-α, tissue growth factor-β, and other molecules implicated in other aspects of CVD treatment or angiogenesis (Kirito et al. 2005; Vandervelde et al. 2005)? To what extent do any of these mediators act similarly on SKMBs? There is evidence that supplying VEGF decreases the amount of SKMBs undergoing necrotic/apoptotic process following transplantation into the myocardium (Yau et al. 2005). There is also initial evidence that treatment of C_2C_{12} SKMBs with tumor necrosis factor-α (TNF-α) not only suppresses morphological and biochemical differentiation, but induces apoptosis following its initial stimulation of proliferation and survival (Stewart et al. 2004). Such influence of TNF-α makes sense, since increased concentrations of this cytokine in HF correlate with reduced systolic LV function (Kaur et al. 2006). However, we have yet to define how the molecular cascades act in synergism to promote repair. This state of fragmented knowledge about signaling is very reminiscent of the early days of understanding of the clotting cascade and the mechanisms involved into coagulation and hemostasis. Eventually, however, a more or less complete understanding of pathways emerged and intrinsic and extrinsic pathways were brought together. That effort serves well even today; therapies continue to be developed targeting specific components (such as factor X versus thrombin inhibition) and the management of such serious conditions as AMI continues to evolve as new targets are being refined and introduced into clinical practice. There is no doubt that cell therapy will take a similar path. Although, because the field of cell therapy lies on the crossroads of cardiology, vascular biology, hematology, and immunology, deciphering the pathways involved in repair will take a lot more effort than the abovementioned coagulation cascade. However, borrowing the knowledge from oncology, hematology, and immunology and applying it in the context of cardiac repair will accelerate acquiring of new knowledge and will lead to refining of the eventual therapeutic product.

5.3 The Two Important Steps in Defining "Best Cell"

At present, discrepant clinical trial outcomes exist for the similar cells in different patients and different cells in the similar patients. Comparisons are difficult because timing after injury, dosing, and route of cell administration also differ. Yet some generalities are emerging. For example, SKMBs seem to engraft into the myocardium and result in functional improvement in HF, and BMNCs show positive results in treating acutely injured myocardium. What is clear from a basic science point of view is that different environments in the myocardium at the time of injury likely generate different milieus, and therefore the cells that engraft in one environment may not survive in another one. Whether the discrepant clinical results are a result of a rush to be first clinically to apply various cells types in various contexts, or if segregation of the types of cells (at least between SKMBs and BMNCs) for the appropriate types of injury (AMI and HF) has already happened inadvertently, remains to be understood.

Because developing a successful therapy, one that is based on a biological understanding of the human body and the pathogenesis of disease, requires multiple re-iterations between bench and beside, we need to go back to bench research now to compare various cell types side by side in various types of ischemic injury *in appropriate animal models*. This may seem to be a simple process, but in reality all available cells, delivery routes, and injury models taken together would result in approximately 2,400 comparisons to be done. This clearly is a prohibitive number for a promising therapy. Therefore, the field needs to come to a consensus on the clinical relevance and conduct comparisons accordingly (i.e., concentrate on the most common types of injury, such as reperfused AMI at up to 4 h from the onset of symptoms, ischemic HF with a mild-to-moderate ischemic process). As the data become available, we can then build additional hypotheses as to what may or may not work in other types of pathology and models. Such experiments should also bring additional insights into our understanding of how and when repair happens. This suggestion may sound contradictory to reality, considering the number of preclinical studies and clinical studies that have been published in the field (i.e., 1,347 Medline hits on keyword searches for "heart" and "cell transplantation" as of 19 November 2006). However, only a few side-by-side comparisons of different cell populations have been performed. We clearly lack direct comparisons of different cell types in clearly defined clinically relevant models of disease.

Comparing different cell types in various contexts of disease will also help us definite how to improve survival of transplanted cells, which is currently one of the largest hurdles of cell therapy. Most reports suggest that 70%–90% of all transplanted cells die within the first few days of transplantation into infarct scar. Studies have shown that a subset of the transplanted cells survive and multiply, but the question is how to promote survival either by genetic expression or

by pharmacological means, or by modification of the media. Preconditioning of cells before transplantation via heat shock or transfecting cells with pro-survival factors (Akt, heat shock proteins, growth factors) (Kohin et al. 2001; Zhang et al. 2001) or glutamine deprivation (or antagonists) as we described earlier may help increase their survival rate in vivo. More work will need to be done in this area to better define the relationship between the microenvironment of the infarct scar and the adjacent myocardial segments and outcome of the transplanted cells. In addition, we have to look beyond the scar and evaluate the impact of hypertension (HTN), valvular insufficiency (VI), and nocturnal dips in oxygen saturation and relate them with the ability of cells to repair the myocardium. HTN and VI are important causal factors of LV hypertrophy and remodeling (Udelson et al. 2003) and therefore will impact the signaling and quite possibly the ability to promote engraftment of exogenous cells. This may mean that adequate antihypertensive therapy and correction of valvular abnormalities may positively impact the engraftment and survival of the delivered cells. Also, correction of valve abnormalities is now feasible with percutaneously placed devices—a positive development for HF patients, many of whom are not considered to be ideal candidates for surgical procedures. Some of these devices, such as the Coapsys platform for mitral insufficiency repair developed by Myocor, positively impact LV remodeling in early clinical trials (TRACE data, OUS clinical trial; J. Price, personal communication). It is possible that correction of VI prior to cellular cardiomyoplasty will provide a better environment for cell survival and ability to perform repair, as the effects of the cells will not be counteracted by VI-associated remodeling. Nocturnal dips in tissue oxygen saturation are the result of sleep apnea, which is present in many CVD patients mainly due to HTN and obesity. In HF, however, a large proportion of patients is suffering from sleep apnea (Ferreira et al. 2006). Therefore, tissue hypoxia may be augmented by sleep apnea, thus decreasing the chances of the transplanted cells surviving. Although this aspect has not been evaluated, it intuitively makes sense. It is possible that correction of sleep apnea may be required to optimize the potential of exogenous cells to repair the myocardium, decrease the apnea-associated diastolic dysfunction (Sidana et al. 2005), and allow the cells to augment impaired systolic properties of myocardial segments.

Insights from previous research endeavors are important. A confounding inflammatory response to needle punctures during cell administration is very reminiscent of early percutaneous or transmyocardial revascularization studies where the creating channels with laser promoted inflammation (Fleischer et al. 1996). The possibility that needle-based cell delivery is proinflammatory should be explored further; and if so, we need to define specific cytokines that might be involved. If that suggestion holds, the proinflammatory action of the delivery vehicle should be evaluated against the etiology of the disease, as atherosclerosis is now considered an inflammatory disorder (Hansson 2005) and cytokine abnormalities have been found in HF (Kotlyar et al. 2006; Toth et al. 2006).

In addition to surviving in the ischemic environment at the time of implantation, the ideal cell for myocardial repair will be able to, become, despite ischemia, a fully functioning cardiomyocyte or an endothelial cell. However, none of the progenitor cells currently used satisfies both of these criteria at the numbers sufficient for maximal repair or recovery of function. Therefore, it is important to continue working toward understanding the differentiation of progenitor cells in a cardiomyocyte phenotype at the bench level, even if it does not seem clinically relevant. The goal, then, might be to design specific pharmacological/molecular tools to induce a differentiation pathway prior to implantation so that a specific phenotype would manifest slowly enough to allow neovascularization to become functional.

Moreover, injected cells have significantly different electrical properties than cardiomyocytes. These differences have led to VT observed in some of the clinical trials as previously described. For cardiovascular cell therapy to reach its potential, it will be critical to electrically integrate transplanted cells into the surviving myocardium and to ensure the absence of negative electrical consequences. This problem may be approached by genetically altering transplanted cells (to promote electrical coupling), developing new adjunctive safety measures (such as co-administration of antiarrhythmics), delivery of cells only in patients who meet the MADIT-II or SCD-HeFT criteria and have ICDs, or modifying the transplanted cells to become true cardiomyocytes that can survive in a harsh milieu.

Lastly, and perhaps most importantly, there is an urgent need for a taskforce to define the nomenclature of progenitor cells to arrive to a consensus of which cells we are going to call "progenitor cells." Similar taxonomy efforts have been recently accomplished by Krumholz et al. (2006) for clarification of nomenclature in CVD. A writing group consisting of experts in cell biology, taxonomy, cell differentiation, cell therapy, hematology, and translational research could very rapidly accomplish this task. Efforts in this direction will advance the field—and may help avoid unfortunate outcomes. Even though a task force of the European Society of Cardiology released a guiding document on the clinical investigations of autologous adult stem cells for cardiac repair (Bartunek et al. 2006), taxonomic questions were not covered.

5.4 Evaluating the Best Delivery Route for a Cell-Based Clinical Product Will Be Beneficial for Clinicians and Patients

It is clear that choosing the best delivery route is an important prerequisite for success, closely following the choice of cell for the right environment. A major obstacle to achieving efficacy is a rather poor engraftment seen when cells are administered by intracoronary, intravenous, and intracardiac routes. This limitation is likely to have emerged due to multiple factors, of which the technical difficulties of injecting the cells exactly into the center or the periphery of the

scar or precise catheter manipulations in the coronary tree cannot be overemphasized. Therefore, training of the operators gains a pivotal importance. Recently, concerns of operator error halted the GENASIS trial (Genetic Angiogenic Stimulation Investigational Study—Corautus Genetics phase IIb clinical trial to evaluate safety and efficacy of VEGF-2 for treatment of patients with severe angina). As we go forward, creating a specialized network of centers for cell therapy, as recently proposed by the National Heart, Lung, and Blood Institute (NHLBI), would allow for training of interventional cardiologists by experts in delivery techniques. Alternatively, it may also make sense to restrict the number of centers per region that act as referral centers to utilize cell therapy—at least until the techniques come to solid maturity. We have learned that operator volume and experience was a critical determinant of success in CABG and percutaneous coronary intervention (PCI) clinical trials and also in routine clinical practice. As the field of cell therapy goes forward, we cannot ignore the importance of appropriately trained specialists.

The data thus far have suggested that intracoronary delivery, at least in the context of AMI, can provide a comparable level of engraftment of cells to surgical delivery. This outcome again highlights the need for side-by-side comparisons of various delivery methods in controlled, well-designed experiments. Understanding of the biology involved in the delivery route–engraftment interaction could translate into the development of optimal situation-specific delivery systems. No doubt this process will take some time. However, the technological progress in the past 10–15 years has been so rampant that it will not at all be surprising if the next 5–10 years brings major advancements in this regard.

5.5 Arriving at a Consensus Regarding Trial Design and Outcome Measurements

At the present time, clinical trials in cell therapy suffer from several major shortcomings primarily involving design and selection of endpoints. For example, most studies have been accompanied by an additional revascularization procedure, either by percutaneous coronary intervention or CABG, making any functional improvement due to exogenous cells nearly impossible to distinguish from the standard of care. In addition, patient characteristics at study entry need to be matched more carefully in prospective trials, which would include baseline comparisons beyond the standard regimen of demographic and basic clinical disease-defining parameters, such as assessments of biomarkers, cytokine profiles, and levels of circulating progenitors to characterize the milieu and relate the impact of exogenous cells to outcome appropriately. Furthermore, medication regimens need to be tracked more carefully throughout the course of the trial, as illustrated by the recently published trial (Lunde et al. 2006) where the patients who received BMNCs were prescribed more diuretics (40% in the cell therapy group versus 26% in the control group), which might

have negatively impacted the engraftment of the cells and the overall outcome of the study. We also need to account for the stimulatory effects of drugs, such as statins, peroxisome proliferator-activated receptor agonists, erythropoietin, estrogen, angiotensin receptor blockers, and possibly others in various disease states. Right now, it is completely unclear which, if any, of these combinations of drugs alter the number and the function of progenitor cells available for repair. Clearly, if cell therapy is to be adequately evaluated as a therapy, we will need this type of information for the design of definitive phase III trials and also going forward with clinical applications. The best chance to obtain this information prospectively is through a centralized registry, as the number of covariates to discern the drug–cell effect is going to be disproportionately large and may require a large number of patients. In addition, we lack data that evaluate time in disease progression as well as time in dynamics of transplanted cells as an additional factor in treatment. Over all, there is a lack of standardization in the current preclinical approach to cell therapy; for example, cell types, doses, preclinical models, and endpoints all differ. Attempts to standardize these parameters and to decide on a consensus will move us forward.

What we select to be an "endpoint" in cell therapy likely matters. So far, clinical trials have been geared toward measuring functional improvement of the LV by assessing global EF. As we know from the HF trials, improvement of regional contractility may not always translate into better HF numbers because of differences in loading conditions. Since the data with both SKMBs and BMNCs so far suggest that exogenous cells are capable of anti-remodeling effects, measuring those as an endpoint in prospective trials will require using a technique with a high sensitivity and specificity in measurements of regional contractile parameters. However, we have begun to appreciate observer-dependence of those measurements. Even though cardiac magnetic resonance imaging (CMR) offers the best topographic assessment of the heart, the variability is minimized by conducting clinical trials with centralized core laboratories where the personnel undergo regular inter- and intra-observer reproducibility assessments. More attention needs to be paid to peri-infarct zone and scar volume and myocardial perfusion quantification. Over the last 10 years, CMR has matured to offer quantitative assessment of myocardial perfusion (Jerosch-Herold et al. 1998; Zenovich et al. 2001). Measuring changes in blood flow was proposed as an endpoint for angiogenesis studies (Wilke et al. 2001), and it is now becoming apparent that cell therapy will need a sensitive measure of blood flow as well. CMR has been used to detect the presence of exogenous cells in the myocardium, as well as to characterize the myocardium prior to transplantation of cells to delineate the areas of myocardial damage (Zhou et al. 2006).

Concurrently, we need to critically evaluate the endpoints that are used at the present time and come to an agreement, most likely through an AHA/ACC-sponsored consensus document, similarly to available data standards for AMI, HF, and atrial fibrillation, that would outline the standard sets of data to be captured in the cell therapy trials. As that process goes along, some endpoints

with high subject variability, such as exercise treadmill time, will be critically evaluated and new, biologically relevant, and clinically translatable endpoints will be introduced. Such a process will also enormously aid acceptance of new endpoints by the Food and Drug Administration and will over time accelerate bringing cell therapies to market.

5.6 Testing Cell-Based Models in Drug Development to Accelerate Design of Therapies Targeted at Repair

Recognition of involvement of progenitor cells in tissue repair and investigation of its mechanisms may provide a foundation for a new approach in drug development—testing the effects of candidate molecules on BMNCs, EPCs, MSCs, and other cell types. At the time when the cost of bringing a new drug to market ranges from US $500 million to US $2 billion, dependent on the therapeutic application (Adams and Brantner 2006), new avenues must be thought to reduce the price tag of drug discovery research as well as clinical trials to allow more candidate molecules to reach phase I and II trials. It is clear that the drugs that impact major pathways in CVD, such as renin-angiotensin-aldosterone or HMG-co-α reductase, have already been discovered, tested, and brought to market, and have shown their clinical abilities to save patients' lives. Therefore, the next generation of therapeutics will be directed at initiation of atherosclerotic lesions and arresting their development via reduction of inflammation and targeting specific receptors. We propose that along with those avenues, new compounds should also be evaluated for their ability to aid exogenous cells engraft and survive. In HF, the increasing number of patients creates an opportunity to design new therapies to reduce symptomatology and reverse remodeling. Recently, trials of endothelin antagonists (darusentan, tezosentan) (Anand et al. 2004; O'Connor et al. 2003), TNF-α antagonists (e.g., etanercept) (Mann et al. 2004), and a Ca^{2+} sensitizer (levosimendan) (Cleland et al. 2006) have been disappointing either due to a lack of beneficial effect or because of adverse reactions that created a prospective application to a wide patient cohort problematic. SKMBs, on the other hand, have a great potential to be a part of a therapeutic armamentarium in HF, as the evidence for efficacy, at least so far, points in a positive direction. Pharmacological approaches to aid engraftment, survival, and electrical integration of SKMBs would be beneficial to the development of cell-based therapies.

In addition to testing new drugs for their effects on exogenous repair and seeking approaches to improve survival of cells, we propose that models involving cells with reparative potential could be employed in drug discovery science. If a new drug is targeting repair, it makes sense that models that utilize reparative cells are used early in the process to reduce costs of further development in case of a negative outcome. Progenitor cells could also be employed as tools for toxicogenomics and safety evaluations. After all, if a progenitor

cell dies, then the efficacy of a compound as well as its potential safety should be reexamined. Here, the use of biomarkers together with evaluations of progenitor cells may bridge bench science and human pharmacology, providing additional insights into biological mechanisms of disease and advancing the science at the same time.

Overall, the use of SKMBs, BMNCs, EPCs, MSCs, and other cell types may be extremely helpful both early in drug development and also in the human testing of new candidate molecules.

In summary, we believe we have outlined the major issues in cell therapy today. As the field develops further and products moves closer to market, addressing each prerequisite will increase the likelihood of a successful outcome. The ultimate success, however, will be the prevention of atherosclerosis and CVD, the reduction of hospitalization and major adverse cardiac events, and in prolonging a healthy life for patients who currently have limited options available to them.

6 Summary

Cell transplantation has opened a new frontier in CVD. The concept of repairing or regenerating ischemic cardiac tissue creates a real possibility, and while many questions still remain, it has an excellent chance of eventually becoming a clinical reality. Cell-based therapies have the potential of providing physicians with alternatives that extend beyond revascularization and medical management to reverse damage that, in many cases, has already been done and may not be truly controllable for a large patient population.

To further advance cell therapy for CVD, we now have to come to a field-wide consensus and standardize future studies. The diversity of cell types, application techniques, and disease stages can be a hurdle and an opportunity, and only collaboration will allow us to move forward as a field instead of expanding information that cannot be combined or compared. Recent clinical trials have shown that cell therapy with SKMBs and BMNCs is able to demonstrate clinical benefits in AMI and HF. Promising results evoked the scientific enthusiasm to warrant large-scale controlled clinical trials to determine the best and safest application of this technology, and to gain a better understanding of its mechanism(s).

As the field progresses, we have a responsibility to promise patients (and the press) only what we can deliver; that is, to tell the truth about cardiac repair. BMNCs, MSCs, SKMBs, or other types of cells hold a great promise to modify pathophysiological process in specific ways. It is crucial to understand for clinicians, patients, and the press that specificity precludes a panacea. As we go forward, some applications will succeed and some will fail. Cells may not be found guilty of failures. On the contrary, the disease contexts may come to

be the primary determinants of efficacy. We have already experienced a similar process with angiogenic growth factors in CVD, and we now realize that those trials should have more carefully targeted the disease process, as the results uniformly showed that sicker patients had larger therapeutic benefits. As investigators, we need to be realistic of the expectations we place on cell therapy, and ultimately we need to under-promise and over-deliver, based on rigorous science; otherwise, the great potential will eventually be destroyed. Cell therapy is, however, a new and very promising alternative that warrants much further exploration, inspiration, and investment of our time and resources.

Acknowledgements This work has been supported in part by NHLBI/NIH award to Dr. Doris A. Taylor (R01-HL-063346), Minnesota Partnership for Biotechnology and Medical Genomics award, and by funding from the Center for Cardiovascular Repair, University of Minnesota. The authors sincerely thank Harald C. Ott, MD, for his continuous contributions to the ongoing success of the Center for Cardiovascular Repair.

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http://www.springer.com/978-3-540-68975-1

Bone Marrow-Derived Progenitors
Kauser, K.; Zeiher, A.-M. (Eds.)
2007, IX, 289 p., Hardcover
ISBN: 978-3-540-68975-1