AUTOLOGOUS BONE MARROW MONONUCLEAR CELL TRANSPLANTATION AND CORONARY BYPASS SURGERY FOR TREATMENT OF ISCHEMIC HEART FAILURE

Miia Lehtinen

ACADEMIC DISSERTATION

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ISBN 978-951-51-0988-0 (paperback)
ISBN 978-951-51-0989-7 (PDF)

Helsinki 2015, Unigrafia
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Abstract

**Background:** Worldwide, the leading cause of morbidity and mortality is heart failure. It is most often caused by coronary artery disease (CAD) and myocardial infarction (MI), which causes death of myocardial tissue. Although coronary interventions such as coronary bypass graft surgery (CABG) can restore blood flow to ischemic areas, and established pharmacotherapy for heart failure exists, no treatment available in the clinics can regenerate the dead cardiomyocytes. For surgical treatment, patients with heart failure represent a challenge, as they are prone to surgical complications, and suitable preoperative imaging modalities to assess possible benefit from surgery are few.

**Aims:** Cell therapies have recently emerged as a possible alternative for treating heart failure. We wanted to explore the capacity of autologous bone marrow mononuclear cells to regenerate myocardial tissue as an adjunct to CABG. The aim was to assess the therapy’s safety and detect the cells’ possible effects on cardiac function and viability. In addition, we investigated whether it would be possible to predict benefit from CABG in these heart-failure patients with 3-vessel CAD with the aid of combined nuclear imaging data. For this, we used 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) to measure cardiac viability, and 99mTc-tetrofosmin single-photon emission computed tomography (99mTc-SPECT) to measure cardiac perfusion.

**Methods:** Between 2006 and 2010, we enrolled 104 patients scheduled for CABG who suffered from CAD and ischemic heart failure. Preoperatively, pharmacotherapy was optimized, after which 39 patients still had left ventricular ejection fraction (LVEF) ≤45%. These patients received injections of bone marrow mononuclear cells (BMMCs) (N=20) or vehicle (N=19) intraoperatively into the myocardial infarction border area in a randomized and double-blind manner. During surgery and at the intensive care unit (ICU), the patients’ hemodynamics, arterial blood gases, systemic venous oxygen level, blood glucose, acid-base balance, lactate, hemoglobin, body temperature, and diuresis as well as medications needed were monitored and recorded every four hours throughout the first postoperative 24 hours. BMMC effects on the heart were evaluated by use of pre- and 1-year postoperative cardiac magnetic resonance imaging (MRI), FDG-PET, and 99mTc-SPECT and by measuring pro-B-type amino-terminal natriuretic peptide (proBNP) levels. As we later decided to extend the follow-up, these same variables, except for nuclear imaging data, as well as current quality of life were measured at a late follow-up visit in 2013. For this, we could contact 36 of the 39 patients recruited for the original study, of which 30 participated in the extended follow-up.

Preoperatively, we also analyzed FDG-PET and 99mTc-SPECT data by using three quantitative techniques with a software tool to measure defects with hypoperfused but viable and non-viable myocardium in 15 control patients. One method used solely PET, two others combined PET and SPECT at different thresholds. As a reference, we used change in LV function and volume by MRI.

**Results:** During the first-year follow-up, improvement was similar in both groups in LVEF, the predefined primary end-point measure (P=0.59), and similar improvement also occurred
in local wall thickening (WT) ($P$=0.68) in the injected segments. Neither changes in viability by PET and SPECT and levels of proBNP differed between these groups. Myocardial scar size by MRI in injected segments rose by a median of 5.1% in the control group (interquartile range, IQR -3.3 to 10.8) but fell by 13.1% in the BMMC group (IQR -21.4 to -6.5) ($P$=0.0002). During surgery and ICU stay, hemodynamics, arterial blood gases, systemic venous oxygen level, blood glucose, acid-base balance, lactate, hemoglobin, body temperature, and diuresis and levels of medications administered were similar between the study groups.

For the extended follow-up, the median period was 60.7 months (IQR 45.1 to 72.6). No statistically significant difference was observable in change in proBNP values or in quality of life between groups. LVEF in both groups remained similarly improved ($P$=0.65), as also did WT ($P$=0.43). For controls, scar size in injected segments increased with a median of 2% (IQR -7 to 19); for BMMC patients it remained reduced with a median change of -17% (IQR -30 to -6) ($P$=0.01).

When assessing the benefit-predictive capacity of the two techniques combining FDG-PET and $^{99m}$Tc-SPECT with different thresholds and one technique using FDG-PET data only, no correlation appeared with preoperative PET- or PET-SPECT-derived viable or non-viable tissue, when compared with global functional outcome (change in LVEF) or local change in WT.

**Conclusions:** In patients with 3-vessel disease and heart failure, the three techniques using SPECT perfusion and PET viability imaging data failed to predict the functional benefit received from CABG. Thus, these imaging modalities may provide no additional advantage to preoperative patient selection, which should be considered when planning treatment for this patient group in the clinics.

In the treatment of chronic ischemic heart failure, during surgery and perioperatively in the ICU, both intramyocardial BMMC and placebo injections appear safe. Although failing to affect cardiac function, combining intramyocardial BMMC therapy with CABG can sustainably reduce scar size.
List of original publications

This thesis is based on the following original publications, reprinted here with permission of the publishers.

*These authors contributed equally to this work


In the text, the publications are referred to by their Roman numerals.
Abbreviations

\(^{99}\text{mTc}\) 99m-Technetium

ACE-I Angiotensin-converting enzyme inhibitor

AHA American Heart Association

AMI Acute myocardial infarction

ARB Angiotensin I receptor blocker

BMC Bone marrow-derived cell

BMMMC Bone marrow mononuclear cell

BNP B-type natriuretic peptide

CABG Coronary artery bypass graft surgery

CAD Coronary artery disease

CI Cardiac Index

CK-MBm Creatine kinase -myocardial band fraction mass

CPB Cardio-pulmonary bypass

CPC Cardiac progenitor cell

CT Computed tomography

CVP Central venous pressure

ECG Electrocardiogram

EDV End-diastolic volume

EF Ejection fraction

ESV End-systolic volume

EuroSCORE European System for Cardiac Operative Risk Evaluation

FDG \(^{18}\text{F}-\text{fluorodeoxyglucose}\)

FFR Fractional flow reserve

Gd Gadolinium

HFrEF Heart failure with reduced ejection fraction

HFpEF Heart failure with preserved ejection fraction
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl-coenzyme A</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HRQoL</td>
<td>Health-related quality of life</td>
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<tr>
<td>ICD</td>
<td>Implantable cardioverter defibrillator</td>
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<tr>
<td>ICU</td>
<td>Intensive care unit</td>
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<tr>
<td>IQR</td>
<td>Inter-quartile range</td>
</tr>
<tr>
<td>LAD</td>
<td>Left anterior descending artery</td>
</tr>
<tr>
<td>LDD-MRI</td>
<td>Low-dose dobutamine magnetic resonance imaging</td>
</tr>
<tr>
<td>LGE-MRI</td>
<td>Late gadolinium enhancement magnetic resonance imaging</td>
</tr>
<tr>
<td>LV</td>
<td>Left-ventricular /ventricle</td>
</tr>
<tr>
<td>LVAD</td>
<td>Left-ventricular assist device</td>
</tr>
<tr>
<td>LVEDV</td>
<td>Left-ventricular end-diastolic volume</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left-ventricular ejection fraction</td>
</tr>
<tr>
<td>LVESV</td>
<td>Left-ventricular end-systolic volume</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>MPAP</td>
<td>Mean pulmonary arterial pressure</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>paO₂</td>
<td>Partial pressure of arterial oxygen</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>PM</td>
<td>Pacemaker</td>
</tr>
<tr>
<td>proBNP</td>
<td>Pro-B-type amino-terminal natriuretic peptide</td>
</tr>
<tr>
<td>RAA</td>
<td>Renin-angiotensin-aldosterone</td>
</tr>
<tr>
<td>RAMLA</td>
<td>Row Action Maximum Likelihood</td>
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</tbody>
</table>
RCT  Randomized controlled trial
SD   Standard deviation
SPECT Single-photon emission computed tomography
SvO$_2$ Venous oxygen level
SVR  Surgical ventricular reconstruction
TnT  Cardiac troponin T
WT   Wall thickening
1 Introduction

Heart failure is notorious for putting a strain on millions of patients’ quality of life and a burden on national economies (Stewart et al. 2010; Teng et al. 2010). Approximately 15 million people in Europe (McMurray et al. 2012) suffer from this condition.

The most common underlying pathology behind heart failure is coronary artery disease (CAD), a disease compromising blood flow in coronary arteries supplying the heart, thus causing ischemia. Diseased arteries present with narrowed lumens with atherosclerotic plaques. These plaques are susceptible to shear stress; they rupture easily, leading to myocardial infarction and tissue death.

The disease causes restrictive symptoms such as chest pain and fatigue, severely impairing quality of life. The further the coronary artery disease develops towards ischemic heart failure, the scarcer and less efficient become treatment options. Today, applicable treatment options include pharmacotherapy, coronary artery bypass surgery (CABG), and, at the end-stage of heart failure, heart transplantation. Despite these, prognosis remains sinister: mean survival time after first hospitalization for heart failure is approximately 2 years (Jhund et al. 2009).

A major problem is that patients with ischemic heart failure are often old and have many comorbidities (Ferguson et al. 2002; Braunwald 2013). Thus, patients possibly benefiting from revascularization surgery require proper selection. But although surgical treatment options could be beneficial (Passami et al. 1985; Velazquez et al. 2011), severely ill patients may not even survive surgery.

Since 2001, the focus of attention has been on an interesting notion: bone marrow-derived cells could potentially regenerate dead myocardium and improve cardiac function (Orlic et al. 2001). After promising results in animal studies, clinical trials have also been numerous (Donndorf et al. 2011; Delewi et al. 2012), by varying approaches: with intramyocardial or intracoronary injection, given shortly after myocardial infarction or in a chronic stage of CAD. After a decade of vigorous investigation, results, however, remain mixed.

Trials have suffered from heterogeneity in study methods and quality (Francis et al. 2013; Leri et al. 2013), especially concerning intramyocardial cell therapy, which is often combined with CABG. We set up a prospective, controlled, double-blinded trial evaluating the safety and efficacy of bone-marrow cell injections as an adjunct to CABG in treatment of ischemic heart failure. We also investigated the predictive role of preoperative nuclear medicine imaging for outcome of revascularization in ischemic heart failure.
2 Review of the literature

2.1 Heart failure

2.1.1 Epidemiology and etiology
Heart failure is one of the major causes for morbidity and mortality in the world. Its prevalence increases with age (Ho et al. 1993): it affects 1 to 2% of adults and even over 10% of people aged more than 70 years (Mosterd et al. 2007).

Heart failure is a common condition resulting from various diseases, including hypertension, valvular diseases, hypertrophic cardiomyopathy, and congenital heart diseases, but the most common culprit is coronary artery disease (CAD).

2.1.2 Coronary artery disease
CAD causes lipid accumulation in the heart vessels, the coronary arteries. The part of the vessel most susceptible to this lipid accumulation is the layer right next to the arterial lumen, the intima. It loses its normal cellular structure and function and becomes vulnerable to the shear stress of blood flow. When the amount of intimal lipid further increases, an extracellular lipid pool develops, covered by a fibrous collagen-rich cap (Pasterkamp et al. 2000). This atherosclerotic plaque is unstable and may ultimately rupture from the vessel wall, causing local thrombosis and obstruction leading to ischemia and myocardial damage (Pasterkamp et al. 2000).

When ischemia has struck the heart, damage to the myocardium remains reversible for less than 30 minutes; after that, damaged myocardium is progressively destroyed (Jennings et al. 1960). Within 6 hours, programmed cell death, apoptosis, and necrosis of the insulted myocardial area is complete, extending from the subendocardium to the subepicardium (Reffelman et al. 2002; Reimer et al. 1977; Buja et al. 2005).

The dead heart muscle tissue is then progressively replaced by a fibrotic scar (Frangoannis 2006). First, within 24 hours, cardiomyocytes become swollen, release their intracellular proteins to the extracellular space, and become necrotic, after which, inflammatory response proceeds and polymorphic leukocytes infiltrate the infarction area. Within one week after infarction, in an attempt to repair the damaged tissue, lymphocytes and macrophages emerge, and phagocytosis of the dead cells begins, starting from the infarction periphery towards its center. These cells stimulate fibroblasts, which start their proliferation and collagen production. Extracellular matrix also transforms. First, a fibrin-based matrix forms, promoting cell proliferation and migration at the site of infarction. This preliminary matrix is then replaced by a more organized network of fibronectin and hyaluronan. To provide nutrients and oxygen to the site of healing with active metabolism, neovessels form. During the following month, as maturation of the myocardial scar proceeds, with increasing amounts of cross-linked collagen, the tensile strength of the scar is enhanced, but the elasticity of the heart is compromised, leading to tissue stiffness.

However, ischemia’s effects are not restricted to the necrotic area killed by ischemia. During ischemia, a large area next to the dying myocardial tissue undergoes pathological changes due to oxygen shortage, but this area remains potentially viable even though it also often presents
with impaired contractile function. If reperfused, these viable cells may recover from the detrimental changes induced by ischemia and regain normal function, or progress to cell death (Lim et al. 1999).

In the area of viable but dysfunctional myocardium, some cells are stunned (Braunwald and Kloner 1982). Stunned myocardium usually recovers spontaneously after reperfusion, but myocardial dysfunction may be present for several days despite normalized coronary blood flow (Bolli et al. 1988). The mechanism of stunning involves induction of oxygen radicals, modification of calcium homeostasis, and a contracted protein structure (Kloner 2001a and 2001b).

Myocardium may also go into hibernation if hypoperfusion persists chronically (Braunwald and Rutherford 1986). After revascularization, the hibernating myocardium is capable of regaining normal contractile function. The chronic ischemic burden of hibernating myocardium is reflected in microscopic changes: whereas stunned myocardium shows minimal microscopic change, hibernating myocardium has a shape indicative of degenerating cardiomyocytes, with large perinuclear glycogen and mitochondria pools and myofilaments restricted to the cell periphery (Kloner 2001a and 2001b).

In the past, acute heart infarction was immediately lethal. Today, mortality has decreased thanks to established medical treatment. Even acutely administered treatment cannot, however, heal myocardium affected by infarction, leaving the heart permanently damaged. Gradually proceeding atherosclerotic arterial obstruction compromises cardiac blood flow and further impairs myocardial function. Thus, morbidity remains high (Chen et al. 2011), leading to a dramatic increase in number of patients suffering from heart failure.

2.1.3 Systolic and diastolic heart failure

Regardless of the underlying pathology, the failing heart activates specific pathological processes. In systolic type heart failure, deteriorating contractile capacity fails to pump enough blood into the circulation. This leads to a reduced ejection fraction (EF), the proportion of ventricular blood pumped to the aorta during a contraction. Today, the systolic type of heart failure is more often referred to as heart failure with reduced ejection (HFrEF). Its leading cause is CAD and myocardial infarction. The infarction leads to loss of the sufficient systolic function by causing cell death and loss of contractile activity in the affected zone (Dorn et al. 2009; Fraccarollo et al. 2012). With failing contractility, the hemodynamic burden increases and mechanical forces stretch the abnormally stressed tissue (Dorn et al. 2009), contributing to systolic failure.

In the diastolic type (heart failure with preserved ejection fraction, HFpEF), ventricular filling is impaired, but not contractility, as in HFrEF; this leads to a decreased amount of blood passing from the heart to the circulation, although the ejection fraction is not affected. This subtype of heart failure has long been poorly recognized, but now the estimate is perhaps even 50% of heart failure patients have a preserved ejection fraction (Burchfield et al. 2013). Initially, HFpEF was considered to result from pathological characters of the left ventricle (LV), leading to diastolic stiffness, prolonged isovolumic LV relaxation, and slow LV filling (Soufer et al. 1985). It now seems, however, that pathological LV is maybe not the actual culprit but is suffering from dysfunctional filling caused by volume overload, insufficiency of
perfusion, excessive volume resulting from extrinsic factors, or inadequate filling times (Burchfield et al. 2013). Patients with vascular stiffening and vascular dysfunction may also be more predisposed to HFpEF (Owan et al. 2006, Melenovsky et al. 2007).

Often, heart failure is a combination of these two. To compensate for these changes, the sympathetic nervous system becomes activated. The heart rate rises, and with the increased workload, myocardial circulation suffers. The renin-angiotensin-aldosterone (RAA) system is also stimulated, which leads to vasoconstriction, reduced renal blood flow leading to fluid retention, and elevated blood pressure, all further burdening the heart. Angiotensin II affects the heart mainly through its receptor type 1, the activation of which causes vasoconstriction, and induces hypertrophy and fibrosis in cardiac muscle. It also causes an increase in secretion of aldosterone, further inducing cardiac fibrosis, to fight against the overwhelming workload of the failing heart (Fyhrquist and Sajinmaa 2008).

As the increasing workload stretches the atria and ventricles, B-type natriuretic peptide (BNP) levels undergo stimulation to increase. It tries to cause vasodilatation, increased excretion of natrium and water, and reduced activity of both the RAA system and sympathetic nervous system. However, its efforts fail to counteract the accelerating deleterious processes leading to heart failure (Kupari and Lommi 2004).

2.1.4 Cardiac remodeling
Continuous ventricular wall stress and neurohumoral alterations induce morphological changes, a process called cardiac remodeling. Although first an adaptive process, it soon causes the heart to decompensate (Mann et al. 1999). Progressive remodeling is associated with a poor prognosis (Cohn et al. 2000).

The remodeling process of ischemic heart failure has been under extensive study, as it represents a logical course of pathological molecular events leading to visible changes in heart morphology. In the ischemic heart, the magnitude of cardiac remodeling depends directly on the extent of myocardial damage, infarction-caused (Fraccorollo et al. 2012). In the healing process after myocardial infarction, as the inflammatory response subsides and cardiac fibroblasts proliferate, the resulting tight, fibrotic scar, with significant tensile strength, serves to prevent rupture. Even though this process is essential for the post-insult heart to continue functioning, the remodeling process continues progressively in response to increases in wall stress, causing compensatory molecular, histological and morphological heart changes (Gajarsa et al. 2011).

As part of the remodeling process, intracellular adaption of the cardiomyocytes is evident. In addition to apoptotic and necrotic processes of the dying heart muscle, as a response to the stress, autophagy occurs, the role of which is debatable: it may be adaptive, serving to promote cell survival, or maladaptive, contributing to the process of cell death (Burchfield 2013). Another warning of stress and pressure overload is cardiomyocyte hypertrophy. Similarly with physiological (exercise) hypertrophic growth, in this pathological growth, increased expression occurs of genes responsible for cardiomyocyte structure, ion transport, and proteolysis (Sheehy 2009). However, in the disease process, cardiomyocyte growth is so overwhelming that adaptive capillary growth fails to meet its oxygen demand, leading to further hypoxia (Shiojima et al. 2005).
The pathology of heart failure is also linked to changes in the immune system. The immune system plays a significant role in remodeling, activating many inflammatory pathways, including the complement system, T cells, and the formation of autoantibodies (Aukrust et al. 2001; Diwan et al. 2003; Caforio et al. 2007). If activation persists, these inflammatory processes may cause long-term heart injury.

Probably the most distinctive feature of ventricular remodeling is the accumulation in the heart of fibrosis. This excessive fibrotic extracellular matrix provokes contractile dysfunction and functions as an arrhythmogenic area (Spinale et al. 2007), leading to increased morbidity and mortality (Assomull et al. 2006; Yan et al. 2006). Induced by pathological stress, cardiac fibroblasts proliferate and differentiate into contracting myofibroblasts which secrete collagen I, collagen III, and fibronectin into the extracellular matrix (Spinale et al. 2007). In addition to fibrosis aggravating the risk for arrhythmias, rhythm disturbances can also derive from pathological electrophysiological changes in the heart, causing disordered electrical currents arising from prolongation of ventricular action potentials (Burchfield 2013).

2.1.5 Symptoms and clinical course of heart failure
The symptoms of heart failure are dyspnea, swelling, fatigue, and chest pain (angina pectoris). Dyspnea occurs when fluid accumulates in the lungs; fluid accumulation in the lower extremities, usually in the ankles, causes swelling. Breathing is difficult, especially when supine because of larger intrathoracic volume and pressure. A deteriorated ability to withstand exercise causes weakness and fatigue. Increased cardiac stress demands more oxygen, leading to myocardial ischemia and chest pain. Symptom severity can be classified according to the New York Heart Association (NYHA) classes (Table 1). Symptom severity often fails to correlate with ventricular function, however. Although symptom severity and survival are clearly related to each other, patients with mild symptoms may still have worse prognosis, with a relatively high risk of hospitalization and death (McMurray et al. 2012). Sudden worsening of symptoms may occur due, for example, to infections or nutritional changes, and cause hospitalization periods. Heart failure is one of the most common reasons for recurrent hospitalizations in those of older age (Jencks et al. 2009) and, in western countries, these hospitalizations are a major economic burden.

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
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<tbody>
<tr>
<td>NYHA 1</td>
<td>no limitation of physical activity</td>
</tr>
<tr>
<td>NYHA 2</td>
<td>slight limitation of physical activity</td>
</tr>
<tr>
<td>NYHA 3</td>
<td>marked limitation of physical activity</td>
</tr>
<tr>
<td>NYHA 4</td>
<td>unable to perform any physical activity without discomfort</td>
</tr>
</tbody>
</table>

Table 1. New York Heart Association classes.

A key characteristic of heart failure is inevitable disease progression. Within five years after diagnosis, as many as half the patients die (Stewart et al. 2010; Chen et al. 2011).
2.1.6 Medical treatment for ischemic heart failure

Since heart failure is a condition caused by multiple diseases, the main goal is to treat these diseases. In the case of CAD, a major focus is on reducing the levels of cholesterol, the lipid accumulating in arteries. An established group of medicines to counteract this process is the statins. They execute their effect by inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme is engaged in production of cholesterol in the liver. In addition, CAD patients benefit from medicines improving blood circulation. These include acetasalisylic acid and clopidogrel; for CAD, statins and these two have proven important for improving these patients’ prognosis (Task Force Members 2013).

Medication necessary for all heart-failure patients aims at 1) alleviating symptoms; 2) preventing worsening of heart failure; 3) improving prognosis. Because different drug groups often affect different combinations of these aims, multiple drugs must be given in combination to achieve the desired outcome.

In easing fluid retention symptoms, dyspnea and swellings, an effective medication is the diuretics. They remove salt and water from the kidneys by preventing reabsorption of sodium chloride. One of them, spironolactone, has even shown an effect on prognosis in HFrEF (Pitt et al. 1999). It is speculated (Kupari and Lommi 2004) that other diuretics might even cause activation of a vicious circle for heart failure, as they induce activation of the RAA system. Thus, a combination of drugs counteracting RAA system effects is recommended.

Beta-blockers act through blocking adrenergic beta-receptors and thus reducing heart rate giving the heart time to relax and fill with blood more properly between contractions. They also deactivate the induced renin-angiotensin system and lower blood pressure. This can also be accomplished by drugs inhibiting an enzyme that converts angiotensin I to angiotensin II (angiotensin-converting enzyme inhibitors, ACE-I) or by blocking angiotensin-receptor type I (angiotensin-receptor blockers, ARB). All of these three groups of pharmaceuticals have improved systolic heart-failure patients’ prognosis (The SOLVD Investigators 1991; Pfeffer et al. 1992; Packer et al. 1996; CIBIS-II Investigators and Committees 1999; MERIT-HF Study group 1999; Cohn et al. 2001; Dargie et al. 2001; Packer et al. 2001; Granger et al. 2003).

Digoxin alleviates heart-failure symptoms and reduces hospitalizations when combined with ACE-I and diuretics (Digitalis Investigation Group 1997). It increases cardiac inotropy by blocking sodium pumps on cell membranes, reduces sodium re-uptake in kidneys, and dampens sympathetic activation.

Calcium-channel blockers and nitrates can be useful, both of them causing vasodilation helping coronary blood flow and alleviating symptoms of chest pain and dyspnea. Despite their alleviating effects on symptoms, they have no effect on a heart-failure patient’s prognosis (Kupari and Lommi 2004).
2.1.7 Interventional treatment for ischemic heart failure

*Surgical techniques*

Since one of the major pathologies behind heart failure is CAD, CABG is a logical surgical treatment alternative. Its aim is to redirect blood back to the areas with obstructed arteries and thus, with impaired blood flow. In addition to non-viable necrotic tissue killed by ischemia, these areas contain heart muscle tissue that has reserved its viability and, after return of blood flow, can perform normal contractile myocyte function. As mentioned in section 2.1.2, these areas are called “stunned” if the ischemia time has been short and reversible, and “hibernating” if ischemic conditions have been evolving and continuing for a long time. These areas are an excellent target for revascularization procedures (Kloner et al. 2001b).

CABG has established its position especially in treatment of patients with triple-vessel disease or stenosis of the left main coronary (Authors/Task Force Members 2012). In addition, patients with less diffuse disease but low EF (<35%), who would otherwise be suitable for surgery and survive at least a year with good function, may benefit from CABG (Velazques et al. 2011). Treating patients without angina is controversial. Few trials addressed to enlighten this problem have emerged. Evidence suggests that patients without myocardial viability or severely dilated left ventricle show no benefit from revascularization (Authors/Task Force Members 2012).

Some patients amenable to CABG may further benefit from surgical ventricular reconstruction (SVR) (Jones et al. 2009). This aims at removing scar tissue from the left ventricle to restore the normal morphology. According to guidelines, this procedure should, however, be applied only for patients with heart-failure symptoms more predominant than their angina, with large LV dimensions, and with transmural scar. In addition, SVR should be performed only in centers with long surgical experience (Authors/Task Force Members 2012). Regarding secondary valvular problems, effective medical therapy is often the treatment of choice, except perhaps for secondary, ischemic mitral regurgitation, which is usually a transient dynamic process induced by exercise and might be suitable for repair or replacement, for example in combination with revascularization surgery (Authors/Task Force Members 2012).

When heart failure progresses to the end-stage state, surgical options become fewer. Today, the gold standard remains heart transplantation (Mehra et al. 2006). To become eligible, patients have to meet strict selection criteria, because donor organs are few and the operation risky. After transplantation, the patient has to receive life-long immunosuppression with consistent risk for severe infections. However, for properly selected patients, it improves prognosis, quality of life, and physical capacity (Authors/Task Force Members 2012).

The number of donor organs cannot meet the increasing number of patients with heart failure. Thus, for the treatment of end-stage heart failure, mechanical support devices for the left ventricle assist device (LVAD) raise hope (Kirlin et al. 2012). They are now increasingly used, instead of as just a bridge therapy to transplantation, rather as a destination therapy for patients ineligible for transplantation. When compared to medical therapy, these devices improve survival rates (Jokinen et al. 2011, Rose et al. 2001). However, despite some potential of LVAD to aid in recovery from heart failure caused by other etiologies (Birks et
al. 2011), in patients with ischemic cardiomyopathy and history of myocardial infarction, the myocardial destruction is likely to be so extreme that without regeneration of the dead cardiac tissue, the heart has no change of true recovery. As foreign material, they also carry risk for infections and bleeding, and it is recommended to implant them only after careful consideration and in transplantation centers only (Authors/Task Force Members 2012).

Surgical risks

Heart failure is a major risk factor affecting survival after all kinds of surgery. It associates with both postoperative morbidity and mortality (Rosenberg et al. 2014a and 2014b). Among heart-failure patients, even CABG, the most common and least invasive of the aforementioned surgical treatments, carries an increased risk of mortality ranging from 3 to 11% (Vitali et al. 2003). When deciding whether a heart-failure patient should undergo an operation, risks must thus be carefully considered. The risk is especially high for patients with EF less than 20 to 30% (Algarni et al. 2011) and with three-vessel disease (Rao et al. 1996). Their hearts are susceptible to the manipulation encountered during cardiac surgery. Heavy blood loss causes hypovolemia and anemia which threaten the failing heart’s capacity to keep up with oxygen demand. A cardiopulmonary bypass machine has foreign surfaces that can cause coagulopathies and peripheral vasodilation, the etiology of which remains unknown. Aortic clamping causes myocardial edema and ischemia, which in combination with hypothermia and direct mechanical manipulation of the heart can lead to arrhythmias. Possible arrhythmias include atrial fibrillation, affecting 25 to 30% of CABG patients and ventricular fibrillation, affecting 1%; mortality to the latter is high, 20-25%. Transient atrioventricular block is also common: among CABG patients, it develops in 25% (Rosenberg et al. 2014a and 2014b).

To reduce surgery-related mortality, special attention should focus on patient selection. For this purpose, scoring systems are in use to assess the risk to each patient possibly amenable to surgery. In Europe, the European System for Cardiac Operative Risk Evaluation (EuroSCORE) and its updated version, EuroSCORE II, have gained popularity. By using various patient-related characteristics, for example age, comorbidities, ejection fraction, it can well predict individual death risk (Roques et al 2000).

To prevent adverse events during surgery, hypothermia and chemical protection are useful. Hypothermia has a positive effect by reducing cardiac oxygen consumption. Chemical cardioplegia solutions boost this decrease. Careful patient monitoring is vital and requires invasive techniques. Arterial cannulation and a pulmonary artery catheter (Swan-Ganz catheter) are introduced, the former monitoring systemic arterial pressure, and the latter the pressures in the right atrium, right ventricle, and pulmonary arteries and the filling pressure of the left atrium (pulmonary wedge pressure). Cases of acute exacerbation of heart failure require inotropic drugs. To treat postoperative myocardial dysfunction, guidelines (Mebazaa et al. 2010) recommend dobutamine and epinephrine, both catecholamines (Fowler et al. 1984); phosphodiesterase III inhibitors (milrinone) (Wynauds et al. 1994); and levosimendan, a calcium sensitizer (Raja et al. 2006). Cases of low blood pressure require norepinephrine (Mebazaa et al. 2010).
Percutaneous interventions

If an ischemic heart failure patient is not amenable to CABG, proceeding to percutaneous coronary intervention (PCI) to relieve angina pectoris symptoms may be one choice for the attending physicians. Often, however, coronary artery disease in ischemic heart failure patients is diffuse, giving a clear indication for surgery, but if anatomy is suitable, imaging indicates viable myocardium, and surgery is not applicable, PCI can be the choice (Windecker et al, 2014).

Because dysfunction of an enlarged ischemic ventricle impairs systolic function, improving performance through resynchronization of the ventricular function may be advantageous (Nelson et al. 2000). For ischemic heart failure patients, cardiac-resynchronization therapy with an implanted pacemaker has proven beneficial (Moss et al. 2009; Tang et al. 2010; Cleland et al. 2005; Bristow et al. 2004; Chen et al. 2014). Guidelines recommend this therapy for patients expected to survive with good functional status for more than a year, if they are in sinus rhythm, their LVEF is ≤30%, they have a prolonged QRS duration (≥150 ms), and a left bundle branch block seen in electrocardiogram (ECG), irrespective of symptom severity. Half of the deaths in heart failure patients occur suddenly and unexpectedly, many of which are related to ventricular arrhythmias (McMurray et al. 2012). Because antiarrhythmic drugs are not sufficient to reduce risk of death for heart-failure patients (Zipes et al. 2006), introducing an implantable cardioverter-defibrillator (ICD) can be beneficial, reducing the risk of death as primary prevention (Moss et al. 1996) or secondary prevention (The Antiarrhythmics versus Implantable Defibrillators (AVID) Investigators 1997).

2.1.8 Cardiac imaging of heart failure

When diagnosing, choosing treatment for, and assessing prognosis of a heart-failure patient, imaging techniques play a critical role. These techniques obtain valuable information on heart morphology, function, and circulation.

Important widely used morphological parameters include LV volumetric measurements. Luminal volumes of LV in end-diastole (EDV) and in end-systole (ESV) both show enlargement especially in systolic dysfunction (McMurray et al. 2012). Use of these two volumes yields an estimation of the heart’s EF and stroke volume (SV), that is the volume pumped to aorta during one systole. EF is regarded as the best parameter assessing global cardiac function and is a major prognostic marker: with lower EF values survival is less (Pocock et al. 2006).

For CAD patients, imaging the obstruction in coronary arteries is important when considering the need for revascularization procedures (Task Force Members et al. 2013). Conventionally, this can be accomplished by coronary angiography. This technique shows a two-dimensional X-ray view of the coronary arteries filled with contrast agent, so that stenotic parts of the coronaries are easily detectable. Coronary angiography fails to evaluate the viability of myocardium, which is essential when weighing between treatment options. Furthermore, hibernating myocardium, the main target of revascularization, cannot remain viable indefinitely; delayed revascularization is linked with worse prognosis (Schwarz et al. 1998, Shah et al. 2013).
2.1.8.1 Echocardiography
Clinically, a suitable, feasible technique for assessing morphological and functional measurements is echocardiography, based on ultrasound. Echocardiography is usually performed with a transthoracic approach giving a two- or, less frequently, three-dimensional view. A trans-esophageal approach may enable better visualization of the heart but requires usually anesthesia. Echocardiography is applicable for diagnosing heart failure and for re-evaluation of the disease to guide therapy (American College of Cardiology Foundation Appropriate Use Criteria Task Force 2011). Although inexpensive and widely available, it has limitations, however: its reliability depends quite strongly on the performer’s experience and on patient-related factors (subcutaneous fat amount, anatomical variations). Echocardiography allows left-ventricular volumes, wall thickness, and ejection fraction to be measured. It also gives information about pulmonary arterial pressure, valve function, and the pericardium (McMurray et al. 2012).

2.1.8.2 Nuclear Imaging
Single-photon emission computer tomography (SPECT) is in wide clinical use for assessment of cardiac perfusion. It takes advantage of three alternative intravenously administered tracers, $^{201}$thallium, $^{99m}$technetium-sestamibi ($^{99m}$Tc-sestamibi), or $^{99m}$technetium-tetrofosmin ($^{99m}$Tc-tetrofosmin), which all give rise to photon emission. The uptake of these tracers by myocardial cells depends on myocardial blood flow and active transport. Differences between the three tracer alternatives are quite small, but when compared to $^{201}$thallium, $^{99m}$Tc-sestamibi and $^{99m}$Tc-tetrofosmin should yield higher energy photons resulting in more accurate image quality (Slart et al. 2006).

During the SPECT imaging procedure, a gamma camera records emitted photons at multiple projection angles around the patient within a 180- or 360-degree arc. This acquisition process continues from one R wave to the next (i.e. one cardiac cycle), and is repeated multiple times to generate satisfactory count density and to produce a plot representing cardiac perfusion. SPECT imaging can be performed both at rest and after exercise or pharmacological stress. Comparing the rest and stress images is common in clinics since it helps in detecting areas with inducible coronary blood flow impairment. These areas with low tracer activity in stress images but near to normal activity in rest images are suggested as more likely benefiting from revascularization procedures (Hachamovitz et al. 2003).

For the detection of CAD, a large trial called the ROBUST study with 2560 patients randomized to SPECT with either $^{201}$thallium, $^{99m}$Tc-tetrofosmin, or $^{99m}$Tc-sestamibi and using mainly adenosine stress reported that average sensitivity in the subgroup of patients undergoing coronary angiography was 91% and specificity 87% (Kapur et al. 2002). No significant difference was detectable between these tracers. In another trial, approximate sensitivity of SPECT for predicting global functional cardiac outcome after coronary revascularization was 84 and specificity was 68% (Schinkel et al. 2007).
Positron emission tomography

Positron emission tomography (PET) is based on radio nucleotides that give rise to positrons. Positron emission results in a positron-electron interaction which leads to their annihilation. This produces two 511-kev gamma photons traveling 180 degrees apart. Detection of these photons colliding at the same time with a circular detector device leads to acquisition of PET images. Even though it was introduced into cardiac applications more than 30 years ago (Schelbert et al. 1982; Tillisch et al. 1986), it still has not established a strong position in CAD imaging.

PET can serve for both perfusion and viability imaging. Tracers for perfusion studies include $^{13}$N-ammonium and $^{82}$rubidium. They have both been extensively described in clinical trials showing good sensitivity and specificity for CAD (Machac et al. 2005). Both tracers have a short half-life: for $^{13}$N-ammonium it is 10 min, and for $^{82}$rubidium, only 75 s. Because this demands on-site tracer generation, it restricts their clinical use.

PET for viability testing has gained more popularity on a global level since it was introduced by Tillisch et al (Tillisch et al. 1986). A traditional metabolic tracer for PET imaging is $^{18}$F-fluorodeoxyglucose (FDG). Its use is based on a shift in metabolism caused by ischemia. In ischemic conditions, the heart’s usual preferred energy source, fatty acids, is replaced by glucose. FDG behaves similar in heart metabolism to the way glucose does, and is thus a convenient tracer to visualize glucose metabolism. Its accumulation in the heart can be optimized by simultaneous injection of insulin (also known as the euglycemic hyperinsulinemic glucose clamp) and acipimox, a nicotinic-acid derivative, which efficiently inhibits the heart’s preferential use of free fatty acids for energy and shifts the cardiac metabolism even more towards glucose utilization (Knuuti et al. 1994).

PET is considered an accurate method of assessing myocardial viability (Tillisch et al. 1986; Tamaki et al. 1989; DiCarli et al. 1994). In the clinical routine, it is usually evaluated semi-quantitatively based on the relative regional uptake of FDG scaled to the maximal uptake of the heart in question. An experienced physician is required for data analyses (Abraham et al. 2010). Pooled from various studies, its specificity for predicting global functional cardiac outcome after coronary revascularization is calculated to be 83%, with a specificity of 64% (Schinkel et al 2007).

Combining FDG-PET and $^{99m}$Tc-SPECT

To increase the accuracy of cardiac viability studies, the latter two study methods have also been combined (Zhang et al. 2001; Yamakawa et al. 2004). Normal or elevated FDG tracer activity in regions of low perfusion tracer uptake (i.e. perfusion-metabolism mismatch) is regarded as presenting areas of hibernating but viable myocardium in potential need of revascularization. If both perfusion and FDG accumulation are low (flow-metabolism match) the area is interpreted to be scar. In fact, it has been shown that patients with an extensive rest perfusion-metabolism mismatch may have an increasing risk of cardiac death when treated without revascularization with pharmacotherapy only (Desideri et al. 2005).
2.1.8.3 Magnetic Resonance Imaging

*Magnetic Resonance Imaging Fundamentals*

Magnetic resonance imaging (MRI) is based on the uneven nuclear numbers of protons and neutrons in an atom, particular in hydrogen atoms, which are abundant in the human body. They have a magnetic spin, which can be manipulated with a magnetic field aligning them parallel and anti-parallel to the direction of the primary field producing a net vector. This vector can be modified with a temporary radiofrequency pulse and different pulse sequences varying in strength and duration. These pulses are echoed back (i.e. resonated) from the patient and the echo data is collected to produce an image. The most common sequences used in cardiac MRI are spin and gradient echo (Pettigrew et al. 1999).

*Cardiac volume and function*

The heart is a challenging organ to image because it is constantly moving. Respiratory movement affects the heart’s location; thus, most of the MRI study protocols use breath-holding to avoid artifacts. Heart contractions change cardiac morphology during each cardiac cycle. Electrocardiography (ECG) -gating synchronizes imaging with the cardiac cycle. In retrospective ECG-gating, ECG and MRI are acquired at the same time but independently. Then, a computer calculates retrospectively cardiac phases from the images with the use of ECG data. The cardiac cycle can be imaged efficiently (Feinstein 1997). Prospective ECG-gating, in contrast, binds ECG and MRI tightly throughout the procedure: image acquisition starts immediately after one R peak in ECG, which represents the initiation of systole, and stops just before the next R peak.

Left ventricular volume and function are measured from cine images. Common measurements are LVEDV and LVESV, LVEF, and myocardial mass. In patients with regional differences in heart function caused by, for example, myocardial infarction, local function measurements are important. According to American Heart Association (AHA) guidelines (Cerqueira et el. 2002), LV should be divided into 17 segments (*Figure 1*). First, three equidistant short-axis planes are selected. Then, the ventricular wall in the first two planes is divided into six segments and the third one into four segments. The 17th segment is assessed from longitudinal images and represents the most apical part of the ventricle.

*Myocardial damage*

When detecting myocardial scar caused by ischemia, late gadolinium enhancement MRI (LGE-MRI) has shown major potentiality. It takes advantage of a gadolinium contrast agent to distinguish between viable and non-viable tissue. Gadolinium chelate accumulates in areas with wide interstitial space. Healthy viable myocardial tissue has minimal interstitial space, whereas non-viable tissue containing necrotic and apoptotic cells with ruptured cell membranes has substantial amounts of interstitial space. Hence, gadolinium preferentially gathers in scar tissue for a longer time (Kim et al. 1996 and 2000).

For LGE-MRI, gadolinium chelate is administered intravenously. After a delay of approximately 5 to 20 minutes, T1-weighed gradient echo-images are acquired. The inversion time needs to be correctly adjusted to null the signal intensity of normal myocardium for
accurate demarcation of the infarcted area (Edelman 2004). As a result, in the appropriately acquired image, viable tissue appears dark, and non-viable bright.

Figure 1. Segmentation model for left ventricle as recommended by American Heart Association. Reprinted with Permission, Circulation. 220;105:539-542, ©2002 American Heart Association, Inc. http://circ.ahajournals.org/content/105/4/539.full

2.2 Heart regeneration
The heart has traditionally been regarded as a post-mitotic organ that gains its permanent histology and morphology during embryogenesis, when mesoderm contributes to the formation of most cardiac cells; a few cells are derived from the cardiac neural crest and proepicardium. Early in embryogenesis, mesodermal cells that are destined to become part of the heart segregate into two anatomically distinct groups, termed the first and second heart fields (Chien et al. 2008). The left ventricular cardiomyocytes are derived from the first heart field, for which a unique phenotype marker and pool of specific progenitor cells are as yet unestablished (Ptaszek et al. 2012). The second heart field is marked by expression of the LIM-homeobox transcription factor Isl1, and its progenitor cells show great multipotency: they can give rise to cardiomyocytes in the right and left atria, the right ventricle, the outflow tract, the proximal coronary arteries, and most of the conduction system in vivo (Laugwitz et
al. 2005). *In vitro*, human fetal-derived cells expressing ISL1 can be differentiated into the three cell lineages present in the heart: cardiomyocyte, smooth muscle and endothelial cell lineages (Bu et al. 2009).

Although already challenged in the 1980’s, only a decade ago, more and more studies contradicting the assumption of the adult heart’s inability to regenerate started to emerge and gain trust. It soon became evident that also the human heart had mitotic cells that could divide (Quaini et al. 1994; Beltrami et al. 2001; Bergman et al. 2009). The regenerative heart cell subpopulation is referred to as cardiac progenitor cells (CPC). They have been detected in various areas of the heart, including in the atrial appendages and in the outflow tract. Although identified by many different laboratories (Beltrami et al. 2003; Messina et al. 2004), the exact CPC marker profile is still under debate. Proposed, yet controversial identifying markers are c-kit and Sca-1 (both stem cell-related surface antigens) (Garbern and Lee 2013); progenitor cells expressing the aforementioned Isl1 seem to be absent from the adult heart (Weinberger et al. 2012). Moreover, an epicardial progenitor population expressing an embryonic epicardial factor, Wilm’s tumour 1 (Wt1), has been identified in the adult heart. These cells reside primarily on the proepicardial surface during embryogenesis, persist in the epicardium of the adult mammalian heart, and can proliferate in response to myocardial injury and secrete trophic growth factors into the underlying myocardium (Smart et al. 2011; Zhou et al. 2011).

The natural regenerative capacity of CPCs in the adult human heart is, however, quite limited: only 1 to 4% of myocardial cells divide after infarction (Beltrami et al. 2001).

2.3 Cell therapy for heart failure

Despite inert cardiac regenerative capacity the heart cannot repair itself with its progenitor cells. Yet, repair of the heart, for example after infarction, is necessary to regain its lost function. Thus, in the beginning of the 21st century, finding alternative cell types (*Figure 2*) to establish regenerative cell therapy methods became a popular research area.

2.3.1 Bone marrow cells

The bone marrow is a diffuse organ comprising numerous sub-units in the approximately 206 bones of an adult human. In adults, the bone marrow weighs approximately 2600 g, and contains supporting stroma and 1400 g of active blood cell-forming parenchyma (Fliedner et al. 2002). The bone marrow contains cells at different stages of regenerative potential, all belonging to the mononuclear cell pool. The most potent of them, hematopoietic stem and progenitor cells, comprise only 1 to 4% of the bone marrow cells, and are assumed to be labeled with the CD34 surface marker, since these cells can restore the whole hematopoietic system after myeloablation (Andrews et al. 1992; Berencon et al. 1988). Bone marrow also contains non-hematopoietic stem cells, mesenchymal stem cells, capable of differentiating into osteoblasts, chondrocytes, adipocytes, and even into cardiomyocytes (Makino et al. 1999). The number of these cells in the bone marrow is even more limited, ranging from 0.001 to 0.01% of nucleated cells.
Attention has been paid to bone marrow-derived cells and their rather curious wide regenerative potential in also other contexts. As noticed in autopsy studies of organ-transplantation patients, cell chimerism existed in many tissues containing both recipient- and donor-derived cells. It soon became evident that bone marrow cells were the origin of this chimerism and showed a capability to differentiate into epithelial and neural cells and hepatocytes (Mezey et al. 2000; Korbling et al. 2002; Mattsson et al. 2004). Recently, bone marrow-derived cells have also been useful in tissue engineering of, for example, trachea transplants (Macchiarini et al. 2008; Jungebluth et al. 2011).

In 2001, a study reporting ground-breaking data suggested, that bone marrow cells (BMCs) could serve as a potential cell group also for replacing dead myocardial tissue (Orlic et al. 2001). According to this article, BMCs could form new cardiomyocytes and vasculature when injected intramyocardially into areas next to an infarction.

Later, these cells were shown to integrate into the myocardial structure, enhance angiogenesis and secrete growth factors (Fuchs et al. 2001; Kamihata et al. 2001; Orlic et al. 2001; Mäkelä et al. 2007; Burchfield et al. 2008; Korf-Klingebiel et al. 2015), even though effects on true myocyte regeneration have been challenged (Balsam et al. 2004; Murry et al. 2004).

Clinical trials with autologous BMCs were started the same year (Assmus et al. 2002) with promising results. Since then, dozens of clinical trials throughout the world have emerged, either using unfractionated BMCs or special subgroups, such as mesenchymal stem cells (Hare et al. 2012). Results have, however, been mixed (Donndorf et al. 2011; Delewi et al. 2012).

2.3.2 Timing

Since CAD may be diagnosed behind both acute and chronic symptoms, bone marrow cell therapy has also been introduced at various phases of the disease. Trials delivering cell therapy for patients with acute myocardial infarction aim at preventing myocardial damage from occurring. Unfortunately, a large number of patients never visit a hospital in this acute phase of disease progression, but visit a hospital after years of dyspnea and chest pain with permanently compromised heart function. At this point, the aim of cell therapy is to convert the on-going detrimental cardiac remodeling process in order to upgrade cardiac performance. It has also been speculated that it could actually be even more beneficial to wait until the acute infarction-induced harmful cytokine storm has abated, and the cellular environment in the heart has gained integrity in its structure.

2.3.3 Cell delivery routes

Several routes for cell therapy administration (Figure 2) have been studied to find the safest, most reliable, and efficient means for maximal cell retention. The most popular methods are catheter-based intracoronary and transendocardial injections and direct intramyocardial epicardial injections.

The intracoronary technique has been quite popular, especially in clinical trials, due to its non-invasive nature. When compared to the other two delivery routes, however, with this technique, a more worrisome number of cells are flushed away from the heart to other tissues, like the lungs (Hou et al. 2005; Mäkelä et al. 2009). In clinical trials, it is usually applied soon
after acute myocardial infarction (AMI) in combination with early revascularization with PCI. A recent meta-analysis evaluated 24 randomized clinical trials (RCTs). These trials allocated 1624 patients to intracoronary cell therapy or standard therapy for AMI. The meta-analysis concluded that intracoronary BMC treatment leads to a modest improvement in LVEF: the mean difference in improvement in LVEF within 6 months was 2.23% [95% confidence interval (CI), 1.00 to 3.47; \( P < 0.001 \)], favoring patients receiving intracoronary cell therapy. At 12 months of follow-up, this difference was sustained, with 3.91% more LVEF improvement (95% CI, 2.56 to 5.27; \( P < 0.001 \)). Sustained reduction in LVESV was also detectable, but with no significant effect on LVEDV or infarct size (Delewi et al. 2012).

The intramyocardial transendocardial technique is a more novel catheter-based method used also for patients with chronic myocardial ischemia (Beeres et al. 2006 and 2007). Being not so invasive, transendocardial cell delivery might also be applicable for severely ill patients who could not survive an open-heart surgery. With the aid of evolving imaging techniques, this technique will, one hopes, also gain more accuracy and reliability. Thus far, it has shown encouraging results. In a trial by van Ramshorst et al. (2009), LVEF improvement was significantly greater in bone marrow cell–treated patients (change, 3%; 95% CI, 0.5% to 4.7% vs −1%; 95% CI, −2.1 to 1.1; \( P = 0.03 \)). No significant differences were detectable in LVEDV and LVESV; scar size was not analyzed.

In contrast to these two techniques, intramyocardial epicardial injections are applied with the aid of direct visualization of the heart and injection sites, and in addition, cell retention is apparently most efficient (Hou et al. 2005; Mäkelä et al. 2009), leading to its being regarded as the most reliable means of cell delivery (Dib 2010 and 2011). This technique is usually applied in combination with cardiac surgery. Clinical trials using this approach have emerged, although few have been RCTs, and even fewer have included placebo treatment. A meta-analysis assessing 6 trials (4 RCTs, 2 cohort studies) showed a 5.40% difference in mean LVEF improvement (95% CI, 1.36–9.44; \( P = 0.009 \)) favoring BMC treatment. A trend toward a reduction in LVEDV by BMC treatment was apparent. LVESV and scar size were not analyzed in the meta-analysis (Donndorf et al. 2011).

### 2.3.4 Other cell types

Another cell type used in the earliest cell therapy clinical trials is skeletal myoblasts. The first clinical trial combining injections of these cells during CABG started in 2000 and suggested a measurable positive systolic effect. Later, also a multicenter study emerged (Menasché et al. 2008). One alarming event was a trend towards increased ventricular arrhythmias in the myoblast-treated group, although no difference was detectable in comparison with the control group that reached the level of significance. Despite promising findings in the early small cohort studies, myoblast therapy in this multicenter trial failed to improve regional or global LV function beyond that seen in control patients.

After mixed results with trials with quite well-differentiated cell types (unfractionated BMCs, skeletal myoblasts), interest in using more potent, “second generation” cell types (Takashima et al. 2013) has been increasing. Fat tissue has recently become one source of cells in cell therapy for myocardial infarction, as it also contains a small population of mesenchymal-like stem cells, ones possibly capable of producing beneficial regenerative paracrine factors as
well as even differentiating into cardiomyocytes (Gimble et al. 2007). In the PRECISE trial (Randomized Clinical Trial of Adipose-derived Stem Cells in Treatment of Non Revascularizable Ischemic Myocardium), autologous adipose-derived regenerative cells were injected transendocardially to treat patients with ischemic cardiomyopathy. The authors reported an improvement in global wall-motion score index in the cell treatment group by MRI, plus better preservation of maximal oxygen consumption; echocardiography analyses, however, revealed no effect on volume or function (Perin et al. 2014).

From 2011 to 2012, primary reports from two independent clinical trials using CPCs for cell therapy appeared, launching the third generation of cell therapy (Takashima et al. 2013). In the SCIPIO trial (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy), CPCs were isolated from atrial appendages and infused via coronary arteries (Bolli et al. 2011), whereas in the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction), CPCs were isolated from endomyocardial biopsies and also infused via coronary arteries (Makkar et al. 2012). Both trials have reported safety data, with no special adverse events detected. In the SCIPIO trial, the authors reported an increase in both LVEF and regional function, and a decrease in myocardial scar size in 9 of the 20 treated patients, who underwent MRI. However, no comparisons were made with the control group for these parameters, because no control patients underwent MRI (Chugh et al. 2012). In the CADUCEUS trial, comparison of 1-year follow-up data from 17 CPC-treated patients and 8 controls revealed a reduction in scar size and an improvement in regional function of infarcted myocardium in the treatment group (Malliaras et al. 2014).

Targeting cells with even more regenerative potential, a research group located in France recently announced the launch of a phase I clinical trial for treatment of myocardial infarction taking advantage of no less than human embryonic stem cells (Menasché et al. 2014). After positive results with these cells in preclinical studies in rodents (Tomescot et al. 2007) and non-human primates (Blin et al. 2010)—both animal models permanently immunodeprived as the allograft requires—the French regulatory agency has approved the group’s project plan for a six-patient feasibility and safety trial studying the effect of a stage-specific embryonic antigen (SSEA)-1-positive cell population strongly expressing the early cardiac transcription factor Isl-1. The trial is currently at its screening phase.
Figure 2. Commonly used injection routes for cardiac cell therapy: intramyocardial epicardial injection; catheter-based transendocardial injection; and catheter-based intracoronary infusion. Different cell types used for cell therapy studies are: A. skeletal myoblasts; B. embryonic stem cells; C. adipose-derived stem cells; D. bone marrow cells; E. cardiac progenitor cells. Illustration by Vanessa Valero.
3 Aims of the study

The main purpose of this study was to assess a cell therapy method combined with coronary artery bypass graft surgery (CABG) in the treatment of ischemic heart failure.

The specific aims were:

1. Evaluating effects of intramyocardial BMMC injections combined with CABG in the treatment of ischemic heart failure with MRI, SPECT, PET, and clinical parameter-scales during a 1-year follow-up. (I)
2. Assessing perioperative patient safety after intramyocardial BMMC injections combined with CABG. (II)
3. Introducing new standards for clinical testing of novel methods in treatment of ischemic heart failure, including state-of-the-art imaging and perioperative safety monitoring. (I-II)
4. Evaluating effects of the combined BMMC injections and CABG with MRI and clinical parameters during extended follow-up. (IV)
5. Testing whether software-guided analysis of preoperative FDG-PET and $^{99m}$Tc-SPECT can predict CABG outcome. (III)
4 Methods

4.1 Agreements and notifications
The study protocol was approved by the institutional ethics committee (Dn:o HUS 456/E6/05). Before its start, the trial was registered at ClinicalTrials.gov (identifier: NCT00418418).

4.2 Patient selection
Patients of either gender were evaluated in our cardiovascular laboratory in Helsinki University Central Hospital between the years 2006 and 2010. After evaluation, they were scheduled for CABG with moderate heart failure. They were eligible if meeting the following inclusion criteria: 1) age between 18 to 75; 2) informed consent available; 3) LVEF between \(\leq 45\%\) and \(\geq 15\%\); and 4) NYHA class II to IV heart-failure symptoms. Exclusion criteria were: 1) heart failure due to LV outflow-tract obstruction; 2) history of life-threatening ventricular arrhythmias or resuscitation, conditions possibly repeating, or an implantable cardioverter-defibrillator (ICD); 3) stroke or other disabling condition within 3 months before screening; 4) severe valve disease or scheduled valve surgery; 5) other disease limiting life expectancy; 6) contraindications for coronary angiogram or MRI; 7) participation in some other clinical trial.

Before proceeding to surgery, patients selected for the trial received optimal medication for heart failure and coronary disease. At least two heart-failure drugs were included at the highest tolerated dose. Alternatives were either an ACE-I or ARB, or a beta-blocker or a combination together with diuretics or an aldosterone antagonist (spironolactone). Medication for coronary disease was a statin, and anticoagulation by aspirin or clopidogrel. Drug optimization continued for 4 to 12 weeks, after which the screening echo was repeated. LVEF \(\leq 45\%\) meant that the patient could participate in the trial after informed consent. After inclusion came CABG scheduling. Baseline studies took place during the waiting period before surgery.

4.3 Cell transplantation procedure

4.3.1 BMMC harvesting
Before starting bone marrow aspiration, anesthesia was induced with etomidate or propofol, sufentanil or fentanyl, and rocuronium, and continued with infusions of propofol and sufentanil or fentanyl to maintain entropy below 50.

After anesthesia stabilization, the patient was converted from a supine to a prone position, and had 100 ml of bone marrow aspirated from each posterior iliac crest and collected in a sterile bag containing heparin. The aliquots, once transferred to Helsinki University Meilahti Hospital Stem Cell Laboratory, underwent filtration and density-gradient centrifugation (Ficoll-Paque Premium, GE Healthcare Bio-Sciences Ab, Uppsala, Sweden) to obtain the mononuclear cell fraction, by the standard methods of our laboratory. Finally, the cell
suspension was divided into 1-ml syringes, six for each treatment-group patient. Controls received the same amount of fluid by syringe but containing only vehicle medium. Treatments were masked from the surgeons by a tape covering.

For the BMMC group, cell counts and flow-cytometry for cell-surface markers CD34, CD117, CD133, and CD19 of the bone-marrow harvest were performed, by standard methods of the laboratory and the FACS Calibur (Beckton-Dickinson, San Jose, CA, USA). Monoclonal antibody for CD133 was from Miltenyi Biotech (Auburn, CA, USA), others were from Beckton-Dickinson.

4.3.2 Randomization
Before preoperative examinations, stem-cell laboratory personnel blinded to other participants sealed the numbered randomization envelopes. After delivery of the bone marrow harvest to the stem cell laboratory, randomization of each patient occurred at the time of the operation.

4.3.3 Operation
After the harvesting procedure, the patient was converted back to the supine position and standard CABG operation began under cardiopulmonary bypass (CPB) and mild hypothermia. For starting CPB, intravenous heparin (300 IU/kg) was administered, with an additional 5000 IU if needed to achieve the proper activated clotting time of over 480 seconds. Dideco Avant or Medtronic provided the membrane oxygenator for CPB. Phenylephrine and norepinephrine for hypotension and nitroglycerin for hypertension allowed maintenance of the optimal radial artery pressure of 60 to 80 mmHg. To keep blood glucose between 4 and 8 mmol/l, insulin was administered. A nonpulsatile oxygen flow of 2.4 l/min/m², partial pressure of arterial oxygen (P\text{a}O_2) over 30 kPa, and hematocrit over 22% maintained a venous oxygen level (SvO\text{\textsubscript{2}}) over 70%.

After insertion of the aortic cross-clamp, both boluses of cold (12 °C) blood cardioplegia and left ventricular venting protected the myocardium. The minimum nasopharyngeal temperature during the CPB was 32 to 33 °C. After completion of bypass anastomoses, each patient received, under cardiac arrest, 15 to 20 0.2-ml injections containing either BMMCs or placebo in the infarction border area; these sites had been decided before surgery by use of imaging data. During each surgery, the injection procedure was carefully photographed, and segments injected were indicated in patient documents for analysis.

At the end of the operation, rewarming was initiated 20 minutes before removal of the aortic cross-clamp to achieve a bladder temperature of over 35 °C while the nasopharyngeal temperature remained less than 37 °C. After declamping, the first spontaneous cardiac rhythm was recorded and was treated with defibrillation if necessary. Epicardial pacing was also administered when necessary.

When the patient was stabilized, weaning from CPB began. Before start of the weaning process, patients had to fulfill the following criteria: 1) reperfusion time more than one-third of the aortic cross-clamp time; 2) sufficient perfusion pressure; 3) no significant acidosis (pH
more than 7.3, BE more than -3); 4) serum potassium 5 to 6 mmol/l; 5) bladder temperature more than 35 °C. Pump reduction was 20% at one-minute intervals. Successful weaning required an SvO₂ over 70%, cardiac index (CI) over 2.2 l/min/m², central venous pressure (CVP) under 12 mmHg, and pulmonary capillary wedge pressure (PCWP) under 16 mmHg 10 min after the termination of pumping in the absence of inotropic agents. If weaning was unsuccessful, 10 min of CPB ensued before a reattempt with the aid of epinephrine, milrinone, or intra-aortic balloon pump, or a combination, if necessary. To counteract the anti-clotting effect of heparin, we administered 1 mg of protamine per 100 IU of the loading dose of heparin.

4.3.3 Hemodynamic monitoring during surgery (Study II)
Throughout anesthesia, the patient’s condition was monitored with hemodynamic measurements via a fiberoptic pulmonary artery catheter. We recorded heart rate (HR), mean arterial pressure (MAP), CI, mean pulmonary arterial pressure (MPAP), CVP, and PCWP with recordings made at the induction, at the opening and widening of the sternum, at the beginning of and during CPB, and at the end of the surgery.

4.4 Intensive-care unit stay (Study II)
After surgery, patients stayed in the intensive care unit (ICU) as long as necessary. To optimize recovery, we pursued the following aims: 1) CI over 2.2 l/m²; 2) hemoglobin over 80 g/l; 3) serum potassium over 4.2 mmol/l; 4) blood glucose 4 to 6 mmol/l; 5) SvO₂ over 70%. The patient was extubated at a bladder temperature of more than 36 °C, when able to respond adequately to speech and to maintain adequate spontaneous breathing (10-20 breaths/min at positive end-expiratory pressure +6 cmH₂O, oxygen fraction 40%).

During the first postoperative 24 hours in the ICU, we collected patient data every 4 hours including the following: invasive hemodynamics, arterial blood gases, venous oxygen level, blood glucose, acid-base balance, lactate, hemoglobin, body temperature, and diuresis. On the first postoperative day, we took additional blood tests including pro-B-type amino-terminal natriuretic peptide (proBNP), troponin T (TnT), and creatine kinase-myocardial band fraction mass (CK-MBm).

4.5 Follow-up
Patients underwent MRI, PET, and SPECT one week preoperatively and then one year after surgery. As a change to our original plan, after encouraging results from that one-year follow-up, we decided to continue the follow-up and invited the patients for one more follow-up visit in spring 2013, when MRI was repeated. Clinical evaluation, and proBNP measurement took place preoperatively, one year postoperatively, and at the long-term follow-up in 2013. Assessment of current health-related quality of life (HRQoL) took place at the long-term follow-up in 2013.
4.6 Cardiac MRI (Studies I, III, IV)

MRI was with a 1.5-T Siemens Sonata scanner and phase array cardiac coil (Siemens AG, Erlangen, Germany). Images were gated with ECG and obtained during breath-holding. For morphological study and for further orientation data, transaxial haste sequences covered the entire heart. Structure and function of the left ventricle we imaged by a standardized MRI protocol (Kramer et al. 2008). Truefisp cine series were acquired at the vertical and horizontal long axis for a scout to line up short-axis images. From the mitral valve plane through the apex, a stack of short-axis images was obtained with slice thickness of 8 mm with a 2-mm gap and temporal resolution of 28 to 40 ms. To detect LV scar areas, LGE-MRI imaging was with a 2D-segmented inversion recovery gradient echo sequence 12 to 20 minutes after Dotarem® (279.3 mg/ml; dose 0.2 mmol/kg) injection. LGE images had identical views and slice/gap thickness as cine imaging. Inversion time, determined from a T1-scout sequence, ranged, varied between 240 and 300 ms.

For LV volumnetry, we analyzed all short-axis cine images with Qmass software (Medis Medical Imaging Systems, Leiden, the Netherlands). Endocardial and epicardial contours were planimetered at end-diastole and end-systole with papillary muscles included in the ventricular blood pool. Planimetry resulted in LVEDV, LVESV, and LVEF. LV was divided into 16 segments according to AHA guidelines (Cerqueira et al. 2002), excluding the apical segment due to its susceptibility to movement artifacts. For each segment, wall thickening (WT) was assessed with Siemens Leonardo workstation and Argus software (Siemens AG), and LGE scar volume with Qmass software. For scar tissue, the threshold value was set at 5 standard deviations (SD) above normal myocardium (Bondarenko et al. 2005); for transmural scar the threshold value was set at 50% indicating the percentage of the LGE scar encompassing more than 50% of ventricular wall thickness per segment. For each patient, pre-and postoperative values for analyses of WT, scar volume, and transmural scar were considered as the averages over all injected segments. One investigator analyzed all MRI imaging data in random order.

4.7 Nuclear imaging with SPECT and PET (Studies I, III)

For assessment of viability and hibernating myocardium with PET, FDG served as the tracer. After at least 12-hour fasting, patients initially received 250 mg acipimox and acetylsalicylic acid 500 mg (to reduce flushing and the vasodilatory effect of acipimox), and one hour later again 250 mg acipimox. Another hour later, they received injections of 250 MBq FDG, and one hour after the injection the imaging procedure started with the PET computed tomography (CT) device (Gemini GXL 16, Philips, Cleveland, OH, USA). First a CT surview (30mA, 120 kVp) was obtained, and then, a non-gated FDG-PET scan of the cardiac area (35 mAs, 140 kVp), 1 hour (16-cm frame, 10 min) and 3 hours (16-cm frame, 15 min) after the injection. PET transaxial images were reconstructed by the 3-dimensional Row Action Maximum Likelihood (RAMLA) method. The images were converted to short-axis slices, each of 4-mm thickness.

Within one week after PET-CT, an ECG-gated rest \(^{99m}\text{Tc}\)-SPECT at a dose of 250 MBq of \(^{99m}\text{Tc}\)-tetrofosmin was performed for assessment of myocardial perfusion. We acquired data
at 64 angles using a 64x64 matrix and 30-second frame time with a double-headed ADAC Forte SPECT gamma camera (Philips) equipped with low-energy, high-resolution collimators. With Cedars AutoSPECT Plus software, 6.6-mm thick gated and summed (non-gated) short-axis slices were generated.

4.7.1 Evaluation of nuclear imaging data

For Study I, FDG polar plot images reconstructed and evaluated in comparison with cardiac perfusion SPECT images, allowed assessment of areas of scar (perfusion-metabolism match) and ischemic myocardium (perfusion-metabolism mismatch). Analysis was by two blinded, experienced nuclear-medicine physicians. By semi-quantitative analysis (semi-quantitative polar map displays), FDG uptake was normalized to the zones of maximal perfusion in SPECT images. Pre- and postoperative PET studies reporting changes in overall outcome for metabolism compared to perfusion changes in SPECT were evaluated and visually scored: 1) significant deterioration, 2) mild deterioration, 3) no change, 4) mild improvement, 5) significant improvement.

For quantitative analysis of myocardial viability used in Study III, we evaluated FDG-PET and summed $^{99m}$Tc-tetrofosmin-SPECT short-axis images with Emory Cardiac Toolbox software (ECT; Syntermed, Inc., Atlanta, GA, USA). We used two quantitative methods comparing FDG-PET images to $^{99m}$Tc-SPECT perfusion images normalized with the program supplier’s normal perfusion database. In addition, one method utilized FDG-PET data only.

In the PET-only study, we calculated the size of the nonviable and viable LV tissue in each of the three coronary territories and in the total myocardium using thresholds of below 50% for nonviable and over 50% for viable tissue. The percentage was calculated from the maximal FDG uptake of the LV, and the investigator adjusted the threshold.

For the second and third methods, we applied the normalization protocol provided by the ECT program. By comparing them to the normal perfusion database, areas with hypoperfusion were identified. These were excluded, and for the remaining normally perfused areas, we calculated the average count value. This count then helped to scale the average count value for normal areas of FDG study to be equal to that of normal areas of the perfusion study. After this, we obtained two overlapping areas representing the normalized perfusion distribution and the normalized metabolism distribution.

For the second method (the 10%-threshold method), a threshold of 10% identified an area scaled as hypoperfused where FDG uptake value was more than 10 percentage units of the perfusion value in the same area. This area was considered as a mismatch, that is, viable but hibernating/ischemic myocardium.

For the third method (the 50%-threshold method), a mismatch area was adjusted to be a region defined as hypoperfused and had an FDG uptake value of more than 50% of the maximal FDG uptake area. In both of these methods, hypoperfused areas with an FDG value below the respective threshold were considered as a match, that is, nonviable myocardium.
Mismatch and match defect areas we calculated for each of the three coronary territories and for the total myocardium.

4.8 Laboratory parameters, NYHA class, and health-related quality of life (Studies I, II, IV)
Clinical evaluation with NYHA class and proBNP measurement from plasma took place preoperatively, one year postoperatively, and for the long-term follow-up in the spring of 2013. For the long-term follow-up, we measured HRQoL with a questionnaire in spring 2013. We chose the SF-36 questionnaire because it has been validated for the Finnish adult population (Hagman 1996) and has performed successfully in our institution also previously (Jokinen et al. 2010). It is a standardized questionnaire with specified mean and SD values for the different dimensions of the instrument per age and gender cohorts. The SF-36 contains questions concerning eight dimensions that reflect: 1) physical functioning, measuring performance of physical activities (walking shorter or longer distances, lifting heavy objects, climbing stairs); 2) role-physical, measuring role limitations (performing work or other daily activities) resulting from physical health problems; 3) bodily pain, evaluating limitations in performing other daily activities because of pain; 4) subjective general health perceptions and future expectations of one's health; 5) vitality, assessing feelings of energy or fatigue; 6) social functioning, measuring ability to perform normal social activities; 7) role-emotional, assessing role limitations as a result of emotional impairment; and 8) mental health, measuring state of mind. The investigator then transformed the scores for each dimension into a 0- to 100-point scale for comparisons between groups. A lower score meant greater limitations to the person's activity or more distress from social and emotional problems. As a reference, we used values for a Finnish cohort with any chronic disease. For comparisons, we calculated SF-36 mean scores for our study patients and compared them with means and SD of the chosen aggregate of the standardized Finnish population.

4.9 Statistical analysis
We concluded from published cell therapy trials involving heart failure that we would achieve a power of 80% (with 2-sided alpha of 0.05) with 20 patients per group to detect an up to 8% difference in LVEF change. Based on these trials, we estimated a rise in LVEF only in the treatment group. Achieving a good marginal would have required enrolling a population of 30 patients per group. We excluded, however, a large number of patients due to the high efficacy of drug therapy optimization that improved their LVEF above our inclusion criteria. We thus were able to enroll 20 and 19 patients per group. Variables were tested by Student’s independent samples t-test for quantitative normally distributed variables, and by the Mann-Whitney-U for non-normally distributed variables. For repeated quantitative variables measured during the surgery or at the ICU, we used the analysis of variance for repeated measures model with Mauchly’s test of sphericity and, if necessary, a Huynh-Feldt correction. For correlations of non-normally distributed variables, we calculated Spearman’s coefficient. Categorical variables were analyzed with Fisher’s exact test. To test for the impact of the differences in length of the extended follow-up periods, we used these lengths as a covariate in the ANCOVA model. All P-value testing was two-sided with statistical significance set at $P<0.05$. Computation was achieved with PASW Statistics 18 (IBM Inc., Armonk, NY, USA).
5 Results

During the selection process, we initially enrolled 104 ischemic heart-failure patients (Figure 3). They were scheduled for CABG and underwent the preoperative 4- to 12-week (average, 8-week) pharmacotherapy optimization and standardization period. After this, the screening echo was repeated to select patients left with LVEF still remaining ≤45%. The number of eligible patients was then 39 (36%). Of these, a randomly selected 18 men and 1 woman comprised the controls and 19 men and 1 woman the BMMC group. Patient characteristics (Table 2) and drug therapy were similar in both groups preoperatively.

Figure 3. Study flow diagram of the allocation process.

5.1 Patient characteristics
Table 2. Pre- and perioperative patient and surgical characteristics with medians (IQR).

Since Study III was methodological, we chose to exclude the possible confounding effect of BMMC therapy. Thus, we included only the control group in Study III.

Initially, we had not planned to continue the follow-up after the first postoperative year. However, after encouraging results in Study I, we decided to organize one more follow-up visit for our patients. In the spring of 2013, we were able to contact 36 of the 39 patients. One control patient and 2 BMMC group patients had died after the first postoperative year. Since
informed consent from these 3 patients included only participation in the 1-year follow-up, we could not include them in Study IV. Six patients refused to participate in the long-term study.

5.2 Perioperative safety of intramyocardial BMMC therapy (Study II)

In each group, patients received a median four (IQR 3 to 4) distal anastomoses ($P=0.41$). Measured at four time-points during the operation, all hemodynamic measurements (HR, MAP, CI, MPAP, CVP, and PCWP) showed a similar trend between these groups ($P>0.05$). Likewise, throughout their ICU stay, no dissimilarities between the groups were detectable either in their hemodynamics (HR, MAP, CI, SI, MPAP, CVP, or PCWP) ($P>0.05$) or in arterial blood gases, acid-base balance, lactate, blood glucose, or hemoglobin levels ($P>0.05$). Between groups, spontaneous rhythm recovery and the need for defibrillation after declamping or pacing during surgery were similar between groups. None of the patients needed an intra-aortic balloon pump.

In the morning of the first postoperative day, cumulative diuresis and total fluid balance showed no difference between the groups ($P>0.05$). Moreover, central body temperature displayed equivalent trends ($P>0.05$). Between the groups, the average need for inotropic agents (adrenalin, noradrenalin, milrinone) and insulin showed no differences ($P>0.05$) (Table 4). To detect perioperative myocardial damage and possible infarction, the level of CK-MBm was measured 24 hours after surgery. One patient in the control group had an elevated CK-MBm up to >100 µg/l; no BMMC-group patient showed any elevation ($P=0.47$).

5.3 Predicting myocardial function recovery after revascularization with SPECT and PET (Study III)

Of the 19 control patients, 15 had both MRI and PET-SPECT imaging data that were technically satisfactory: one patient refused postoperative studies, one patient received an ICD, and two patients’ PET-SPECT image quality was unsatisfactory. One patient showed signs of perioperative MI (CK-MBm >100 µg/l during the first postoperative day); for this patient, only preoperative imaging data underwent analysis. Thus, pre- and postoperative imaging studies of 14 patients were analyzed in Study III.

Of these patients, nine showed clinically significantly improved cardiac function (change in LVEF ≥5%). The remaining five showed less marked improvement, no change, or a slight deterioration in LVEF; no patients had severe deterioration in LVEF (<-5%). No preoperative parameters explained the variation of improvement in LVEF.

With the 10%-threshold method, for each coronary territory, the size of mismatch and match areas before surgery correlated significantly with preoperative ventricular wall thickening in the respective area, as evaluated with MRI (mismatch: $r=-0.33$, $P=0.03$; match: $r=-0.40$, $P=0.007$).

With the 50%-threshold method, only the preoperative match area showed a significant correlation with preoperative wall thickening in the respective coronary territory (mismatch: $r=-0.21$, $P=0.16$; match: $r=-0.45$, $P=0.002$). With the PET-only method, a significant correlation also emerged between preoperative defect size and preoperative wall thickening.
(r=-0.41, P=0.005). No significant correlation was observable for the preoperative total myocardial defect size (evaluated by any of the methods) and preoperative LVEF.

For analysis of overall recovery 1 year after CABG, we compared the preoperative mismatch and match areas (in 10% or 50% threshold PET-SPECT) and the area with reduced FDG (PET-only study) with global functional outcome (change in LVEF) and LV remodeling (change in EDV). No correlation appeared with any method. For analysis of local functional benefit after CABG, we compared local preoperative defect size evaluated by all three methods and local change in WT. Nor on this local level was any correlation observable (Table 3).

Table 3. Correlation between preoperative FDG-PET/99mTc-SPECT defect area in total myocardium and change in LVEF or LVEDV, and between preoperative FDG-PET/99mTc-SPECT defect area and change in wall thickening in the respective coronary territory for all patients.

<table>
<thead>
<tr>
<th>Method</th>
<th>Coefficient</th>
<th>P</th>
<th>Coefficient</th>
<th>P</th>
<th>Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVEDV</td>
<td>LVEF</td>
<td>Wall thickening</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10%-threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mismatch</td>
<td>-0.53</td>
<td>0.05</td>
<td>0.40</td>
<td>0.16</td>
<td>0.04</td>
<td>0.80</td>
</tr>
<tr>
<td>Match</td>
<td>0.16</td>
<td>0.60</td>
<td>-0.46</td>
<td>0.10</td>
<td>0.06</td>
<td>0.73</td>
</tr>
<tr>
<td>50%-threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mismatch</td>
<td>-0.45</td>
<td>0.10</td>
<td>0.30</td>
<td>0.30</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Match</td>
<td>0.21</td>
<td>0.48</td>
<td>-0.53</td>
<td>0.05</td>
<td>0.11</td>
<td>0.49</td>
</tr>
<tr>
<td>PET-only</td>
<td>0.14</td>
<td>0.63</td>
<td>-0.50</td>
<td>0.07</td>
<td>0.06</td>
<td>0.72</td>
</tr>
</tbody>
</table>

We also analyzed PET-SPECT data obtained 1 year postoperatively. According to these imaging studies, during follow-up, of the 14, cardiac viability had deteriorated in seven patients in three or more of the five defect-size parameters measured. Meanwhile, five of these very same patients showed considerably improved LVEF, and all showed reduced LVEDV, both evaluated by MRI, and NYHA I-II. When analyzing these five patients in more detail, we detected a trend implying that the patients with this discrepancy between deteriorated PET-SPECT data but with improved LVEF had preoperatively poorer LVEF and a more extensive mismatch area.

5.4 Effects of intramyocardial BMMC therapy during 1-year follow-up (Study I)
During the first postoperative year, no mortality occurred. Considering cardiac rhythm disturbances, one patient in each group received a permanent PM, and one BMMC-group patient an ICD. One BMMC-group patient was diagnosed with a cerebral infarction and
another with a subdural hematoma; one control patient had a cerebral infarction postoperatively. None of these patients was receiving warfarin. No other hospitalizations caused by cardiovascular reasons followed during the 1-year follow-up in either group.

The BMMC patients received injections containing a median $8.4 \times 10^8$ (IQR 5.2 x $10^8$ to 13.5 x $10^8$) BMMCs.

Before surgery, the NYHA class median was 3 for controls and 2 for BMMC patients, dropping to 1 in each group 1 year postoperatively ($P$=0.59). Changes in levels of proBNP showed no differences between groups ($P$=0.08).

Because of a PM, an ICD, or of claustrophobia, four patients had contraindications for postoperative MRI. Thus, volumetric MRI analyses were successful for 17 patients in the control group and 18 in the BMMC group. Analysis of volumetric parameters revealed a beneficial change in LVEF (Figure 4) with a median 5.6% among controls (IQR 0.2 to 10.1), and 4.8% (IQR -0.5 to 8.2) in the BMMC group ($P$=0.59). Both groups showed reduced EDV and ESV, measured by MRI 1 year postoperatively, with no significant difference between groups (Table 4). In injected segments, WT rose by a median 4.5% among controls (IQR -18.1 to 23.9), and by 5.5% in the BMMC group (IQR -6.6 to 26.5) ($P$=0.68).

Figure 4. Change in LVEF during 1-year follow-up and during extended follow-up, by 2013 with individual values (circles, squares), medians (horizontal line), and IQR (whiskers).
Table 4. Left-ventricular volume and function (IQR) measured by MRI at the three timepoints: preoperatively, after 1-year follow-up, and at the late follow-up visit in 2013.

LGE-MRI images were technically satisfactory for 31 patients, for 15 controls, and for 16 BMMC, and were included in scar analyses. Scar volume in injected segments rose by a median 5.1% (IQR: -3.3 to 10.8) for controls, but was diminished by 13.1% in the BMMC group (IQR -21.4 to -6.5) (P=0.002) (Figures 5,6). For controls, the proportion of transmural scar remained unchanged with median change 0% (IQR -2.0 to 13.7) but shrunk by a median of 16.9% (IQR -29.3 to -5.9) in the BMMC group (P<0.0001).

Figure 5. Change in scar size after 1-year and extended follow-up with medians and IQR.
For 33 patients, SPECT-PET imaging follow-up during the first postoperative year was successful; 2 controls and 3 in the BMMC group had to be excluded due to insufficient image quality, and 1 control patient refused to participate in imaging before CABG. After follow-up, changes, as visually assessed by our two experienced nuclear medicine physicians blinded to treatments, showed no difference between groups: for controls, the median score for change was 4 (IQR 3 to 5) and for the BMMC group, 3 (IQR 3 to 4) \((P=0.40)\).

### 5.5 Effects of intramyocardial BMMC therapy during long-term follow-up (Study IV)

For the 30 patients willing to participate in the extended follow-up, the median follow-up time was eventually 60.7 months (IQR 45.1 to 72.6).

During this period, the difference in median change in plasma levels of proBNP was not significant when compared between groups \((P=0.36)\).

For the long-term follow-up, 25 patients (14 controls and 11 BMMC) were analyzed with MRI. One control and two BMMC-group patients had contraindications for MRI because of ICD or PM. Three control patients and five BMMC patients refused to participate in MRI for the long-term follow-up.

After the long-term follow-up, for controls, the median LVEF change was 4.9\% (IQR -2.1 to 12.3) and, for BMMC patients, 3.9\% (IQR -5.2 to 10.2) \((P=0.65)\) (Figure 3). LVEDV and LVESV showed reductions during follow-up in both groups; no difference in change was detectable \((P=0.77\) and \(P=0.65\), respectively). In injected segments, WT improved by a
median 17% (IQR -5 to 30) for controls and 15% (IQR -12 to 19) for BMMC patients ($P=0.43$). Scar size in injected segments increased by a median 2% (IQR -7 to 19) in the control group but diminished by a median change of -17% (IQR -30 to -6) in the BMMC group ($P=0.01$) (Figures 4,5). The fraction of transmural scar presented a similar effect from BMMC treatment: it remained unchanged (IQR -8 to 25) in placebo-injected segments and diminished with a median change of -23% (IQR -36 to -3) in BMMC-injected segments ($P=0.09$).

We also analyzed changes occurring in the period between the first follow-up visit at one year and the late follow-up visit. During this period, WT in the injected segment increased by a median of 9% (IQR 2 to 24) for controls and 7% (IQR -16 to 19) for BMMCs ($P=0.35$). Scar size in the injected segments increased by a median of 1% (IQR -14 to 6) in the control group, and diminished by a median of 5% (IQR -16 to 8) in the BMMC group ($P=0.886$) (Figure 5); the proportion of transmural scar in the injected segments changed 0% (IQR -25 to 5) and - 1% (IQR -16 to 8), respectively ($P=0.931$).
6 Discussion

6.1 Selection of patients

After pooling previous BMMC studies, we had decided to aim at strict inclusion criteria, the main limit’s being an LVEF of 15 to 45%. We hypothesized that this more markedly reduced LVEF would reveal a clearer benefit from cell therapy, a hypothesis now supported by other studies (Yerebakan et al. 2011; Jeevanantham et al. 2012).

Patients with coronary artery disease and ischemic heart failure are an interesting and also important patient group, because the treatment strategy, especially the role of CABG, has yet to be clearly established. Hence, studies in search of alternative treatment options are warranted. Recently, a randomized trial assessed CAD patients with LVEF <35% assigned to groups receiving either medical therapy and CABG or medical therapy alone (Velazques et al. 2011). They concluded that, between the treatment groups, death from any cause did not significantly differ, although patients allocated to CABG, when compared to patients receiving medical therapy alone, had lower rates of death from cardiovascular causes and of death from any cause or less frequent hospitalization for cardiovascular causes.

Although CABG seems to be a beneficial choice for treatment of patients with ischemic heart failure, we also wanted to treat our patients with optimal pharmacotherapy, which is often neglected (Fang et al. 2011). As a consequence, in the screening echo repeated after a period of 4 to 12 weeks with optimal, standardized pharmacotherapy, many of our initially selected 104 patients were no longer eligible because of their improved LVEF. Furthermore, our patients presented extensive LV remodeling in terms of LVEDV than did those in similar earlier trials (Patel et al. 2005; Hendrix et al. 2006; Mocini et al. 2006; Ahmadi et al. 2007; Stamm et al. 2007; Zhao et al. 2008), probably reflecting a more congruent group of patients with no further benefit achievable from conservative pharmacotherapy and with less endogenous recovery-capacity.

For the extended follow-up, selected patients became fewer due to unavoidable limiting factors. As three patients had died after the one-year follow-up, and six patients refused to participate in the extended follow-up for personal reasons, the patient number fell to 30. Unfortunately, since three of these patients had also received an ICD or a PM, contraindicating MRI, assessing cardiac function and scar changes during the long-term follow-up was possible for only 25 patients, making power calculations challenging. This patient number is a limitation of our study and demands relative caution when interpreting results.

6.2 Safety

We wanted also to invest in studying the safety of the cell-injection procedure. Although no major concerns had arisen in earlier trials, we had noticed that many trials report safety-related parameters quite poorly. Safety issues are not of minor importance, since studies have demonstrated arrhythmogenic adverse events associated with bone marrow-derived cell therapies (Chang et al. 2006; Fukushima et al. 2007). One experimental study also reported detecting intramuscular calcification in rats receiving intramyocardial bone marrow cell
injections (Yoon et al. 2004). No signs of these kinds of adverse events have appeared in clinical trials.

Currently, cell therapy trials are also aiming at including more severely ill patients, since evidence suggests that they may gain more benefits (Yerebakan et al. 2011; Jeevanantham et al. 2012). For studies using CABG, this procedure is known to elevate the complication risk: low LVEF and triple-vessel disease are linked to more frequent complications during and after CABG (Hillis et al. 2011). A few years ago, one clinical trial studying intracoronary BMMC injections after PCI to treat patients with a large acute anterior myocardial infarction due to occlusion of the proximal left anterior descending artery (LAD) raised concerns of cell therapy safety. The reason was that the trial had to be prematurely terminated when investigators detected an increase in serious complications in BMMC-treated patients (Penicka et al. 2007). Fortunately, none of the complications were related to the injection procedure, since most complications occurred before BMMC injections. The study, however, underscores the necessity of appropriate patient selection by, for example, excluding patients at very high risk for complications from any interventional procedure.

Concerning peroperative hemodynamics and the need for inotropics, we regarded our study procedures as safe. We recorded a vast array of hemodynamic parameters during the surgery and in the ICU with no difference between the groups being detectable. Since we included no control group treated with CABG only, our results do not elucidate the effects of injection of fluid into failing heart muscle but concern only the adverse events of the BMMCs themselves. Throughout the follow-up, no tumor formation was observable in any injection area. Naturally, to assess longer-term safety of the treatment, Holter recordings for detection of arrhythmias would have also been necessary, and this is a limitation of our study.

6.3 Assessing myocardial function, morphology, and viability with imaging methods

6.3.1 Predicting benefits from revascularization with SPECT and PET
The main problem with imaging hibernating myocardium, the primary target of revascularization, is that none of the available techniques seem to be sufficient alone (DiCarli et al. 2002), probably due to their inability to detect different impairments in complex disease. Traditional rest SPECT is mainly designed to detect irreversible perfusion defects, further refined with stress-SPECT detection of provocation-induced ischemia. FDG-PET identifies ischemic shift in metabolism. MRI is the gold standard for function measurement, with applicable refinements of LGE-MRI for myocardial scar assessment and low-dose dobutamine MRI (LDD-MRI) for assessment of contractile reserve; dobutamine-induced stress is also useful for stress echocardiography. Thus, combining different methods to benefit from their different advantages and to minimize their disadvantages is a logical approach.

Some studies have combined SPECT and PET, aiming to obtain even more useful information for evaluation of the heart (Zhang et al. 2001; Yamakawa et al. 2004). The purpose of this combination is to detect any mismatch in perfusion and metabolism, representing the area of viable tissue, tissue presumably benefiting from revascularization.
As a sub-study of our cell therapy trial, we also assessed techniques using both SPECT and PET imaging for measuring preoperative hibernating but viable tissue and predicting benefit from CABG. One technique used PET only, two other techniques combined information from both imaging methods with different thresholds. This study included only control patients. To gain more feasibility and quantitative accuracy for the traditionally subjective, analyzer-dependent evaluation method, we used computer software, the Emory Cardiac Toolbox. MRI served as the gold standard, measuring change in local and global contractile function during the first year after surgery. None of the SPECT-PET methods detecting viable hibernating myocardium showed correlations with alteration in functional changes at a global or local level.

Ischemic heart-failure patients are known as a challenge for clinicians trying to choose an appropriate treatment (Velazquez et al. 2011). One consensus is that patients with >10% of dysfunctional but viable LV myocardium may show more benefit from myocardial revascularization, than would patients with ≤10%; this hypothesis, however, remains debatable (Desideri et al. 2005; McMurray et al. 2012). Guidelines imply the usefulness of several non-invasive imaging techniques in selecting patients for revascularization, although without naming any single method as their preference (McMurray et al. 2012). Evidence suggests that imaging techniques for detection of viable myocardium that will lead to revascularization do improve survival (Bonow et al. 1995; Allman et al. 2002; Bax et al. 2003).

Reports of three prospective randomized trials, the Heart Failure Revascularisation (HEART) Trial (Cleland et al. 2011), the PET And Recovery following Revascularisation (PARR-2) trial (Beanlands et al. 2007), and the Surgical Treatment for Ischaemic Heart Failure (STICH) trial (Bonow et al. 2011; Velazquez et al. 2011) have recently appeared addressing the question of the need for preoperative imaging. They all challenge the use of non-invasive imaging methods, as they failed to find any benefit for guiding patient selection for revascularization or any influence on mortality outcome.

A cautious attitude towards imaging techniques seems justified. We detected a clear discrepancy when we compared pre- and postoperative PET- and PET-SPECT-derived data to functional changes in MRI: many patients showed an increase in defect size, although their LVEF improved significantly, and symptoms were alleviated.

Many factors account for this heterogeneity between different imaging modalities. All of our patients had three-vessel disease, known to complicate analysis of at least perfusion data (Aarnoudse et al. 2003; Diamond et al. 2008). A study by Lima et al. (2003) reported that SPECT demonstrated neither a significant perfusion defect nor a single-vessel pattern of disease in 54% of patients with three-vessel disease as shown by coronary angiography. This led to a severe underestimation of the level of compromised perfusion. Another study, by Melikian et al. (2010), showed that in patients with multivessel CAD, SPECT tended to underestimate or overestimate the significance of coronary stenosis detectable in coronary angiography when compared to intracoronary pressure-derived fractional flow reserve (FFR), a perfusion gold standard. These discrepancies are likely to be, at least in part, due to unreliable distribution of tracers, because of the compromised circulation in three-vessel
CAD. In addition, both SPECT and PET show difficulties in detection of subendocardial scar (Klein et al. 2002; Wagner et al. 2003). However, as transmural myocardial function depends on subendocardial blood flow (Edwards et al. 1992), this may cause confounding effects.

Considering the different patterns between MRI and nuclear-medicine imaging methods in detecting suffering cardiomyocytes, studies have also shown a discrepancy between the methods in favor of PET: a higher fraction of cardiomyocytes was reportedly necessary to maintain contractile reserve than to achieve radiotracer uptake (Baumgartner et al. 1998; Gunning et al. 1998). This implies that the same number of cardiomyocytes fails to show viability in function, although showing viability in nuclear imaging studies. Which parameter is clinically more relevant is not yet established.

Today, as recommended by most guidelines, it is mainly patients with three-vessel CAD, often accompanied by heart failure, that undergo CABG. Based on our results, it seems clear that conventional imaging methods need to evolve as well, since preoperative assessment of this patient group is challenging, even with the use of two combined imaging modalities. More sophisticated methods are under investigation. One of the potential candidates is PET perfusion, at rest and at stress, praised for yielding better image quality (Bateman et al. 2006) and identifying more functionally significant stenoses also in three-vessel disease patients with balanced ischemia (Beller et al. 2011; Romero et al. 2012). High hopes are also directed toward MRI (Hausmann et al. 2004; Gerber et al. 2012; Greenwood et al. 2012). LGE-MRI in combination with LDD stimulation is suggested to be the most accurate technique for viability detection (Schuster et al. 2012). With their limited availability, for the time being, these remain for research purpose only.

Meanwhile, it is noteworthy that today, despite evolving non-invasive cardiac imaging techniques, patients with ischemic heart failure and three-vessel CAD should not be excluded from the potential benefits of revascularization solely dependent on imaging-test results (Velazquez et al. 2012).

### 6.3.2 Evaluating effects of BMMC therapy with MRI

Our cell therapy study was designed to have as its primary endpoint the change in LVEF after one-year follow-up measured by MRI. As secondary endpoints we also had MRI-derived parameters: changes in LV volumes, in WT, and in scar size.

All studies combining intramyocardial BMMC injections with CABG (Patel et al. 2005; Mocini et al. 2006; Ahmadi et al. 2007; Stamm et al. 2007; Ang et al. 2008; Zhao et al. 2008; Akar et al. 2009), except for two (Hendrikx et al. 2006; Nasseri et al. 2014) used some other imaging modality for their primary end-point, most commonly echocardiography. For us, however, MRI was an obvious choice because for function and volume analyses it is the gold standard. Its high spatial resolution permits localization and later relocalization of the LV segments of interest for analyses. MRI should also be superior to PET in resolution capacity, especially in assessment of subendocardial scar tissue (Klein et al. 2002); this might also explain the failure of PET to identify the fairly small difference in scar change between our groups.
One critical appraisal pointed out one of the reasons for mixed results in BMMC studies to be their heterogeneity in imaging techniques (Wollert et al. 2010). Two independent recent publications also suggest that MRI had given more doubtful results (Abbasi et al. 2011; Jeevanantham et al. 2012). When using echocardiography or left ventriculography, trials more often report significant improvement in LVEF in BMMC-treated patients, and also a trend toward improvement when using SPECT. In contrast, the improvement was insignificant with MRI. Infarct scar size diminished significantly according to both SPECT and left-ventriculography, but not MRI (Jeevanantham et al. 2012). A similar effect emerged in a study analyzing trials with a follow-up of more than 2 years: two of the studies there analyzed reported a positive BMMC effect on LVEF, neither of which used MRI, whereas three studies reporting negative long-term results all had used MRI for assessment of cardiac indexes (Abbasi et al. 2011).

As also noted, attention should be directed at detection also of moderate functional changes with state-of-the-art imaging (Wollert et al. 2010), for example, regional WT changes with MRI. LVEF may be too rough a variable, especially since its prognostic significance weakens with values >45% (Jeevanantham et al 2012). Furthermore, with evolving software, WT should be measured truly quantitatively, as in our study, rather than with approximate semi-quantitative, more subjective scales. Today, insufficiency in technology of automated WT tools still requires involvement of an MRI-analysis expert in the data evaluation but software under development should provide a solution.

For future cell therapy studies, another improvement from our study might involve including stress-imaging studies. For example, Beller et al. (2011) suggested stress perfusion PET as a useful tool for assessing perfusion in new therapies that are supposed to increase myocardial blood flow. Moreover, after successful revascularization, viability may improve, although rest LVEF remains unchanged; stress LVEF improves similarly between patients with or without any positive change in resting LVEF in dobutamine stress echocardiography (Rizzello et al. 2005). Thus, evaluation of cell therapy effect on viability in future trials might, for example, also benefit from even more accurate LDD-MRI. To even further improve evaluation of the effects of BMMCs with assessments at a histological level, a tempting option would be myocardial biopsies taken after follow-up – an improvement unfortunately not feasible for our study.

### 6.4 Effects of intramyocardial BMMC injections as an adjunct to CABG

Our randomized, double-blinded study showed that, as compared to placebo injections, BMMC therapy as an adjunct to CABG had no effect either on LVEF - our predefined primary endpoint measure – or on local LV wall thickening of the injected segments. Nor was any effect on myocardial viability revealed by PET studies. Yet, during the one-year follow-up, when compared with placebo injections, with BMMC therapy, the amounts of local scar, in MRI, diminished statistically significantly. Intriguingly, the difference in change in scar size was sustained also in the long-term follow-up.

Similar studies have suffered from short follow-up times (Donndorf et al. 2011), and even in studies using other delivery routes, longer-term follow-ups are few (Beitnes et al. 2009; Meyer et al. 2009; Yousef et al. 2009; Leistner et al. 2011; Assmus et al. 2014). This can be
disadvantageous, because after revascularization, recovery of hibernating myocardium may need several months to evolve; premature assessment may thus rely too much on chance and lead to misinterpretation of results of functional recovery. Haas et al. (2001) reported that myocardial injury is usually present in various degrees, both stunned and hibernating, in the same patient. In their study, after one year, 31% of stunned segments showed total functional restoration, whereas only 18% of hibernating segments had similarly recovered. For postoperative LGE-MRI analysis, after too short a follow-up, postoperative edema after successful revascularization and recovered blood flow could presumably compromise scar measurement accuracy and hamper detection of scar reduction. Thus, recovery and outcome analysis even after our one-year follow-up, but especially after our extended follow-up, may be more optimal and reliable than analysis in previous studies.

Three major meta-analyses concerning BMMC therapy in CAD have emerged recently (Donndorf et al. 2011; Delewi et al. 2012; Jeevanantham et al. 2012). Donndorf et al. analyzed data derived from studies similar to ours, combining BMMC injections with CABG. They concluded that LVEF improved with a mean difference of 5.4% between BMMC patients and control patients in favor of cell therapy. As noted earlier, when comparing our study to studies analyzed in the Donndorf meta-analysis, it seems that our patients had more extensive cardiac remodeling in terms of LVEDV. Although LVEF did improve in both our groups postoperatively, this difference from earlier studies could be relevant in explaining why no difference was detectable between the groups. Since extensive LV remodeling can prevent improvement in LVEF after revascularization, even despite the presence of viability (Bax et al. 2004), the case might be that our patients had a significantly more severe degree of heart failure, without an inherent capacity to recover beyond the effects of CABG.

Our protocol for this cell therapy study was thoroughly considered before the start of the trial. To improve objectivity, we used prospective, controlled, randomized, double-blinded techniques. We wanted to study intramyocardial injections, as they have been regarded as the most efficient method of delivery, enabling direct visualization and offering better engraftment (Li et al. 2009, Mäkelä et al. 2009). Combination with CABG was logical because of the invasive nature of our intramyocardial injection technique.

Applying this treatment to patients with chronic ischemic heart disease was also a topic of deliberation, since, in a stabilized condition, the heart would probably no longer be influenced by the acute inflammatory response prevailing after acute myocardial infarction. It would have gained integrity in its structure, thus serving as a reliable platform for cell therapy. In addition, the longer the period after AMI, the smaller the likelihood of misinterpreting spontaneous functional recovery occurring as part of the natural healing process as being a benefit from the cell therapy (Behfar et al. 2014). However, this timing of cell injections may also affect the potentiality of the treatment. Because the cells might exert at least some of their effects through immunomodulation and paracrine effects (Yeghiazarians et al. 2009), administering the cells soon after AMI might cause a more pronounced positive outcome. Unfortunately, as we also noted when reading through our patients’ medical history, many patients suffer from asymptomatic MIs that are detected only when the heart is already extensively remodeled, sometimes many years after the infarction (Mann 1999). Thus, for these patients, acute treatment immediately after MI is impossible. At this chronic stage, the
injected cells are more likely to alter the remodeling process through their effects on scar and extracellular matrix (Figure 7) (Goumans et al. 2014).

The various policies concerning the procedure of harvesting and processing bone marrow-derived cells for cell therapy studies have also been under discussion (Wollert and Drexler 2010). As the bone marrow consists of numerous differing cells, the unfractionated BMMC sample to be used in cell therapy may contain variable numbers of hematopoietic progenitor cells (CD34+ or CD133+), mesenchymal stem cells, and endothelial progenitor cells, as well as more lineage-committed subsets of cells (Young et al. 2014). Thus, the cell therapy product may vary enormously between trials using different cell-processing methods. Handling of the harvested cells before transplantation is also suggested to affect their characteristics, as culture media may differ, and incubations times may range from 4 hours to overnight, no recommendations for these are yet clear (Behfar et al. 2014). A study comparing differences in cell isolation and storage protocols between two clinical trials, REPAIR-AMI (Schachinger et al. 2006) and ASTAMI (Lunde et al. 2006), showed that the protocol applied had a major impact on cell functionality, potentially explaining the differences in outcomes (Seeger et al. 2007). Further analyses by REPAIR-AMI investigators further underline emphasis on the

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**Figure 7.** Phases of the healing process after myocardial infarction and potential effects of bone marrow cell therapy when applied in different phases (based on data from the review article of Goumans et al. 2014). MI, myocardial infarction.
protocol used: they concluded that contamination of the bone marrow-derived cell therapy product with red blood cells was associated with poorer bone marrow cell viability and poorer recovery of LVEF (Assmus et al 2010).

We utilized a BMMC isolation protocol that is in widespread, decades-long use in stem-cell transplantation in clinical practice. Hence, the protocol was highly reproducible and feasible. Processing of BMMC transplants during CABG was efficient and reliable, and patients could avoid undergoing two separate procedures. Our cell-processing protocol yielded unfractionated BMMCs, which are used also in many other trials. Some trials have preferred to use one specific subgroup, for example CD34+ or CD133+. However, little evidence implies that in clinical trials single-cell populations would have a greater effect than would a mixed cell population (Tendera et al. 2009). On the contrary, an interesting finding in one of the subgroup analyses in the meta-analysis by Jeevanantham et al. (2012) was that reduction in scar size was significantly greater with BMMC therapy than with CD133+/CD34+ cells.

That meta-analysis was additionally interesting in its findings. It contained pooled data from 2625 patients including studies with either intracoronary or intramyocardial injections and patients with either AMI or chronic ischemic heart disease with the aim to assess efficacy of cell therapy and reasons for the mixed results. In line with earlier hypotheses, BMMC injections for patients with more reduced preoperative LVEF were associated with superior improvement in LVESV and LVEDV when compared to patients with near normal preoperative LVEF. Subgroup analyses also showed that although efficacy showed no clear difference in treating patients with either chronic ischemic heart disease or AMI, the LVESV ($P=0.01$) and infarct size ($P=0.06$) seemed to improve – or remodel – more after chronic ischemia. BMMC effects on diastolic or autonomic function, unfortunately neither studied in our trial but proposed as elements for BMMC-derived improvement (Yao et al. 2008; Piepoli et al. 2010; van Ramshorst et al. 2014), went unanalyzed in the meta-analysis. Eventually, no clear explanation for result heterogeneity in BMMC clinical trials emerged. The results, however, indicated that, when compared to patients who received traditional therapy, BMMC-treated patients benefitted from the therapy, with a significant reduction in the number of recurrent MI, and stent thrombosis. Importantly, BMMC therapy had a statistically significant impact on both all-cause and cardiac mortality, suggesting an overall improvement in survival.

Our primary endpoint, change in LVEF, was negative. The discovery of sustained scar diminishment with BMMC injections should, however, not be ignored. Detection of reduced scar is in line with many study findings (Jeevanantham et al. 2012). Although we found no trend towards a better prognosis for BMMC-treated patients during our long-term follow-up, scar size has proven an important prognostic parameter after myocardial infarction (Kwon et al. 2009; Boye et al. 2011), one even more essential than LVEF (Roes et al. 2007). Although heterogeneity in previous trials limits drawing unambiguous conclusions from our interesting findings, BMMC therapy seems promising, it challenges standard therapies, and it proves to be a potential future treatment alternative for patients with ischemic heart failure.
Summary and conclusions

This study was a prospective, controlled, double-blinded clinical trial evaluating the safety and efficacy of bone-marrow cell injections as an adjunct to CABG in the treatment of ischemic heart failure. Our study group received intramyocardial BMMC injections, whereas a control group received vehicle medium as placebo injections. Study follow-up was originally one year, and continued into an extended follow-up with a median follow-up of 5 years. Effects were analyzed with state-of-the-art imaging. We also studied the role of preoperative nuclear medicine imaging in predicting outcome of revascularization in our ischemic heart-failure patients.

1. The effects of intramyocardial BMMC injections combined with CABG were studied in a clinical trial with 1-year follow-up. The effects were measured with MRI, PET, SPECT, and clinical parameters. The primary endpoint, change in LVEF measured by MRI, was negative but, as compared to placebo injections, BMMC therapy led to a reduction in local scar size.

2. During the cell-therapy surgery and the postoperative stay in the ICU, we recorded a wide array of safety-related parameters, including hemodynamics, acid-base balance, and fluid balance. No differences were detectable between the two study groups.

3. Of the 104 patients originally evaluated for the trial, 39 remained eligible after the pharmacotherapy-optimization period. Thus, when evaluating new therapies for treatment of heart failure in clinical trials, for patient selection to be optimal, standardization of pharmacotherapy is essential.

4. Patients included in the original study were invited to an extended follow-up in 2013. After this long-term follow-up, differences between the groups in changes in cardiac function measured by MRI remained insignificant. However, in the BMMC-treated segments, when compared to controls, the significant reduction in scar size was sustained.

5. We evaluated preoperative FDG-PET and $^{99m}$Te-SPECT data by three different analysis methods, two of which combined data from both studies, and one used FDG-PET data only. In ischemic heart-failure patients with three-vessel CAD, we measured with each technique the size of viable and of non-viable myocardium, and compared each to revascularization-induced change in global and local systolic cardiac function measured by MRI. None of the methods seemed to predict the benefit received from CABG.

This study suggests BMMC therapy as an adjunct to CABG as safe but with no detectable effect on cardiac function. At the site of BMMC injection, myocardial scar size diminished significantly, diminishment evident at both one-year follow-up and at the long-term follow-up. Thus, for patients with ischemic heart failure, BMMC therapy seems to present an intriguing potential future-treatment option.

In this study, preoperative myocardial area identified as viable but hibernating in PET-SPECT imaging failed to correlate with the change in global or local cardiac function after revascularization. Along with earlier studies, this result further challenges the role of PET viability- and SPECT perfusion studies in excluding ischemic heart-failure patients with three-vessel disease from the potential benefits of revascularization procedures.
Acknowledgements

This study was performed at the Department of Cardiac Surgery, Heart and Lung Center, Helsinki University Central Hospital and the University of Helsinki. I thank Professor Ari Harjula for allowing me to participate in this important research project, trusting me to take the responsibility of coordinating the project, and for placing excellent research facilities at my disposal.

This project has been long, full of surprises, colorful, rewarding, frustrating, exciting, and constructive. In November 2009, when for the first time I discussed taking on this project with my supervisors, Tommi Pätilä, MD, PhD, and Docent Antti Vento, I had no clue as to what was ahead. Yet, despite a few moments of despair, it was worth it. I have to express my sincerest gratitude to these supervisors for helping me along the journey, Antti especially during the first years, and Tommi during the last years. Antti, despite his increasingly tightening timetable, always found time if necessary for a short meeting; my warmest thanks for that flexibility. I want to thank Tommi for his calming down-to-earth attitude throughout this challenge and his earnest interest in supporting both my scientific and clinical career. With his endless patience, we find a solution to every problem.

I thank Esko Kankuri, MD, PhD, both for his essential contribution to the scientific content of this study and for his detail-deep expertise for all the bureaucracy and for the moot points that this thesis project brought up.

I am also grateful to my three co-authors, Docents Juha Sinisalo and Mika Laine and Professor Markku Kupari, from the Department of Cardiology, Heart and Lung Center. They made valuable contributions and provided welcome differing points of view to further improve this study.

I owe my warmest gratitude also to Professor Kirsi Lauerma, from the Division of Radiology, Helsinki Medical Imaging Center, who was a key member of this study as an expert in cardiac imaging, sharing her knowledge of the wonderful world of cardiac MRI. Whatever the misfortune or conflict faced, she always took my side and encouraged me in my efforts.

I thank also Docent Reino Pöyhä, from the Department of Anesthesiology and Intensive Care, for his enthusiasm and energy in teaching me the secrets of anesthesia – and dancing.

I want to also thank Aapo Ahonen, MD, PhD, Jukka Schildt, MD, and Päivi Nikkinen, PhD, from the Division of Clinical Physiology, for teaching me precious lessons in cardiac nuclear medicine imaging. Furthermore, my co-author, Mia Holmström, MD, PhD, as well as Sari Kivistö, MD, PhD, from the Division of Radiology, Helsinki Medical Imaging Center, deserve my gratitude for their continuing interest and compassion in the twists and turns of this project and their valuable tips for my occasional struggles with MRI. Special thanks go also to Docent Raili Suojäranta-Ylinen, for introducing me to every-day life in the Cardiac Intensive Care Unit, an important postoperative venue for our study patients. I thank also my co-authors Docent Riitta Alitalo and Anne Nihtinen, MD, PhD, for their incomparable expertise in the most important ingredient in this study, bone marrow mononuclear cells.
Crucial for the success of this project have also been Heidi Syrjä and Elina Lappi. I express my warmest gratitude to them for helping with all the practical details and handling both patient data and the patients with expertise. I also thank Carol Norris, PhD, for her valuable and quintessential contribution to the texts of this thesis and of my article manuscripts.

During the last months of this PhD project, I received indispensable support and inspiration from Professor Kenneth Chien and his group members, my colleagues at Karolinska Institute, Sweden – thank you all for that. This support accelerated me through the last steps of the process.

I express my deepest gratitude to my parents, Mauno and Marketta, who, despite the overwhelming medical jargon jungle have listened to my worries throughout this process and supported me with patience and empathy. Special thanks go also to my sister Maija, with whom I made my plans to become a cell therapy scientist more than a decade ago. I have not yet received a Nobel Prize, which was part of our original plan, but I promise to be on my way to it!

I must also thank my girlfriend gang from Tuusula, Emmi, Iida, Johanna, and Satu, for organizing the crucial occasional vacation without any connection to the scientific world; it has made the roller-coaster ride of this long journey easier to withstand.

But since, in both the darkest scientific despair and the greatest scientific triumph, it is necessary to receive compassion that only a fellow young scientist can give, it has meant a world to me to have the friendship of two colleagues, for which I am eternally grateful. Riikka, I thank you for your ability to continuously encourage me forward with your energizing Tampere-temper. And Suvi, it has been a relief and strength to be able to rely on you, as a senior scientist, through these years, as you have paved the way for me with your more established knowledge of the scientific world, supported and defended my rights, always ready to discuss any matter and find time for an emergy pva-meeting. I certainly hope that we both will have many rewarding years ahead in the field of science.

And then, special thanks also have to be expressed to Nikolas. Thank you for putting up with me throughout these crazy years. The amount of support from you for making this project possible has been outstanding. It has been refreshing to debate scientific questions with your non-medical approach and to share my research issues, which you somehow always managed to convert from extremely complex and cumbersome to simple and straightforward with your ingenious engineer wit.

This thesis work was financially supported by the Paavo Ilmari Ahvenainen Foundation, the Society of Angiology, the Klingendahl Foundation, the Society of Transplantion Surgery, the Finnish Foundation for Cardiovascular Research, the Aarno Koskelo Foundation and the Paulo Foundation.

Miia Lehtinen
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