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Refractory Angina Pectoris: Focus on Cell Therapy

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1. Introduction

Although medical and surgical treatments often provide adequate solutions for individuals with coronary artery disease, an increasing need exists to develop treatment modalities for those patients with angina who are unresponsive to medical therapy, have serious coronary atherosclerosis, and are not eligible for percutaneous techniques or bypass surgery, a condition known as *refractory angina pectoris*.
In recent years, alternative experimental therapeutic options (e.g. ranolazine, enhanced external counter-pulsation, shock waves) have been proposed in order to alleviate symptoms and improve the quality of life in these patients.
A promising therapeutic option arising from basic research is the use of autologous cells directly inoculated into the ischemic heart. Preliminary clinical trial results are encouraging.
Aim of this chapter is to review the current knowledge on refractory angina, focusing on cell therapy from a biological and clinical standpoint.

2. Definition of refractory angina pectoris

According to the European Society of Cardiology and the American College of Cardiology/American Heart Association refractory angina pectoris is defined as:
patients with stable angina pectoris, presence of coronary artery disease (CAD) on a recently performed coronary angiogram, who despite optimal conventional anti-anginal medical therapy (beta-blockers, calcium antagonists, short- and long-acting nitrates) have severe angina, functional class 3–4 according to the Canadian Cardiovascular Society classification (CCS). In addition, the patients are not accessible for conventional revascularization procedures such as coronary artery bypass grafting (CABG) or PCI (percutaneous coronary intervention).
A strong limitation of this statement lies however on the consensus of which is the patient not suitable for revascularization. Terms such as "patients who are not candidates for conventional revascularization intervention", or even less strict descriptions such as "patients who are suboptimal candidates for angioplasty or coronary bypass surgery", have commonly been used by investigators. The lack of standardized criteria not only may generate ambiguity but also make challenging to investigate the potential impact of new treatments. There are some criteria that allow to put patients into this definition: patients who are at high risk for invasive procedures (CABG or PCI) or those who we do not

expected to achieve significant and stable results for anatomical reasons, despite the recent advancements of bypass surgery (off-pump coronary by-pass surgery, total arterial revascularization, etc) and of PCI (drug eluting stents, interventional approaches to a degenerated saphenous vein graft using a wide range of distal embolic protection devices and potentially new generations of stent grafts). The keystone is probably to put particular attention on the individual patients' history and symptoms. In particular, there are two aspects that has to taken into an account: the lack of improvement after optimization of the standard medical therapy and the level of patient's discomfort (in terms of quality of life, angina chest pain, shortness of breath, etc).

DEFINITION OF REFRACTORY ANGINA PECTORIS BY THE JOINT STUDY GROUP

Refractory angina pectoris is a chronic condition characterized by the presence of angina caused by coronary insufficiency in the presence of coronary artery disease which cannot be controlled by a combination of medical therapy, angioplasty and coronary bypass surgery. The presence of reversible myocardial ischemia should be clinically established to be the cause of the symptoms. Chronic is defined as a duration of more than 3 months

3. Epidemiology

Available estimates suggest that refractory angina pectoris affects between 600,000 and 1.8 million people in the United States, with as many as 50,000 new cases each year. Approximately 30,000 to 50,000 new cases per year are also estimated in continental Europe. Canadian Community Health Survey (2000/2001) data suggest that approximately 500,000 Canadians are living with unresolved angina, but these data are limited by their reliance on self-report. The proportion of these patients living with true refractory angina is not known. *Despite wide variation in methods used to derive population estimates, there is a general consensus that the incidence and prevalence of this condition will continue to rise across countries as CAD-related survival rates continue to increase and populations age.*

The European Society of Cardiology (ESC) Joint Study Group on the Treatment of Refractory Angina has stressed the critical importance of systematic evaluation of the epidemiology of refractory angina pectoris to more accurately project disease burden and related health services demands. Such data point out the high relevance of the problem related to the refractory angina pectoris in terms of economic costs and human resource that are necessary to face this disease.

4. Features and diagnosis

4.1 Anatomo-physiological basis of myocardial ischemia

The anatomo-pathological base of the refractory angina pectoris is a well-known process called *atherosclerosis*, a chronic inflammatory disease of the artery – in this specific case the coronary arteries – that causes the progressive narrowing of the epicardial (and sometimes the intramural) coronary arteries by the development of a lesion called *atheroma* or *atherosclerotic plaque* causing the discrepancy between myocardial blood flow and myocardial energetic and oxygen demand, especially during exercise. The latter situation leads to the production of a series of biochemical signals such as potassium, lactate,

adenosine, bradykinin, and prostaglandins that could elicit some high threshold nerves ending in the myocardium mediating the typical symptomatology of the myocardial ischemia (i.e. fatigue, thoracic pain and shortness of breath). Recent evidences suggest that both α and β adreno-receptors are involved in the biochemical signaling of myocardial ischemia. In particular, while the β-receptor is mainly involved in the sympathetic activation during myocardial stress, the α-receptor seems to play a central role in the activation of the adaptive process. Furthermore, receptors localized on primary afferents sympathetic postganglionic neurones, and dorsal laminae of the spinal cord and of the brainstem are involved in analgesia and play a role in vasomotor control.

The role of the *vagus nerve* is still not well defined. As observed by *DeJongste & coworkers* while both vagal and sympathetic afferent fibers contribute to the increased activity of spinothalamic tract cells. Activation of vagal afferent fibers could modulate the processing of information of the thoracic spinothalamic tract cells receiving afferent input from the heart, by activating supraspinal pathways and nuclei. Abandoned the old idea that activation of vagal afferent fibers may lead to visceral pain, except in the neck and jaw regions, now we know that the vagal afferents may serve as an important rapid signaling pathway for communicating the immune changes from the periphery to the areas in the brain that respond to infection and inflammation. Depending on the integrity of the vagal afferent pathway, the release of inflammatory cytokines like interleukin (IL)-1, IL-6, IL-1b, and TNFα (tumor necrosis factor α), trigger several systemic responses. This reaction induces alterations in pain sensitivity and metabolism, hyperthermia, and increased release of adrenocorticotropin, glucocorticoids, and liver acute phase proteins. Furthermore, vagal afferent stimulation activates the hypothalamus–pituitary–adrenal axis. Finally, the activation of this vagal pathway to supraspinal structures, such as the hypothalamus and the amygdala, may activate descending antinociceptive pathways that may provide projections of a visceral organ against local inflammatory reactions.

In conclusion the vagus nerve seems to play an important role in the conduction of the pain stimulus from the periphery (i.e. ischemic myocardium) to the cortical network and in the visceral response to it mediated by different neuro-hormonal pathways.

4.2 Clinical features

There is a sort of threshold limit of ischemia that may start the typical complaints of a patient suffering from angina. This patient on exercise, or under other circumstances that can increase the oxygen demand from the myocardium (i.e. emotions, cold, smoking, etc) usually complains a characteristic thoracic pain, specifically precordial pain, or a more general thoracic discomfort (that may be constrictive, suffocating, burning) often irradiating to the left arm (ulnar side), neck, throat, jaw and upper abdomen (always above the umbilicus). This situation may be accompanied with autonomic reflexes: cold sweating, nausea, vomiting, ipotension, and in general with an automatic attempting of the patient to obtain relief from symptoms by immediately putting himself at rest e stop doing actions that was doing when the pain began.

The vagus nerve is implied in the afferent transmission to the brain (particularly to the *limbic system*) and in the efferent transmission to the visceral components. Also the sympathetic arm of the autonomous nervous system plays a role in the afferent and much more in the efferent response to ischemia, promoting the "*fight or fly*" response to stress by increasing the release of numerous stress-related hormones (epinephrine, norepinephrine,

glucocorticoid, etc). If the discomfort/pain is severe the patient may experience a terrifying sensation of "impending death" that could put him in a serious state of anxiety.
Typical pattern of a stable chronic angina in that the symptoms generally regress spontaneously at rest or by the aid of some medications in less of 20 minutes from the beginning. This is generally sufficient to not loose important quantity of myocardial tissue from the ischemic injury. *Typically, patients with refractory angina maintain a good left ventricular function.*
Generally speaking, patients with chronic refractory angina differ from the ordinary angina patient in three ways: first, patients with chronic refractory angina pectoris maintain their left ventricular function despite severe three vessel disease; second, they do not experience severe arrhythmias and therefore their mortality is only about 5%; and third, their angina is very debilitating.
Regarding quality of life, there are several considerations that a physician must take into an account. First of all, we know from the history of patients that this disease only allows limited activities, and thus his day-by-day life is severely restricted by symptoms. Secondly, the psychological impact of the angina is itself cause of stress on patient and on cure-givers. For these reasons, any attempt is welcome to improve quality of life in a patient with refractory angina. Moreover, any improvement in myocardial perfusion in these patients may have a beneficial impact on prognosis.

CLINICAL FEATURES OF CHRONIC REFRACTORY ANGINA PECTORIS
THORACIC PAIN OR DISCOMFORT ESPECIALLY ON EXERCISE
RELIEF OF SYMPTOMS BY REST AND/ OR MEDICATION (NITRATES)
SEVERAL EPISODES OF PAIN IN A DAY
SEVERELY RESTRICTED DAY-BY-DAY LIFE
NEGATIVE PSYCHOLOGICAL IMPACT TO THE PATIENT AND FAMILY
INCREASED SOCIAL COSTS FOR FREQUENT HOSPITALIZATIONS

4.3 Diagnosis

The clinical diagnosis of refractory angina pectoris is basically made on symptoms.
These patients have often a heavy clinical history of coronary artery disease, with several repeated percutaneous transluminal coronary angioplasty (PTCA) procedures or one or more operations of coronary artery bypass graft surgery (CABG).
A history of severe stable chronic angina (Canadian Functional Class 3–4) despite optimal conventional pharmacological therapy and ineligibility for conventional procedures of revascularization identify the patient with refractory angina. *Stress and rest imaging modalities (stress-echocardiography, SPECT, cardiac MR, PET) are also essential to identify location and extent of ischemia in regions of still viable muscle.*

DIAGNOSIS OF REFRACTORY ANGINA PECTORIS
HISTORY OF MYCARDIAL ISCHEMIA
PREVIUOS CABG AND / OR PTCA
EVIDENCE OF ISCHEMIA IN VIABLE MYOCARDIUM
SEVERE STABLE ANGINA PECTORIS (CCS III / IV) ALTHOUGH OPTIMAL CONVENTIONAL PHARMACOLOGYCAL THERAPY
INELIGIBILITY FOR FURTHER REVASCULARIZATION

5. Conventional medical management

The conventional pharmacological treatments for patients suffering from chronic stable angina pectoris are aimed to either reduce the oxygen demand by the myocardium and improve myocardial perfusion, all of this expecting to lead an improvement in cardiac function and relief from symptoms.
Changes in lifestyle (stop smoking, weight loss if needed and treatment of comorbidities such as diabetes, ipertension) are also warranted. Additive measures, such as lipid lowering, inhibition of platelet aggregation, and interference in the renin-angiotensin system have also become established treatments for stable angina pectoris.

5.1 Nitrates

Nitrates are the first-line option to treat angina and work to promote vasodilation, thus decreasing preload and myocardial oxygen demand. They are subject to tachyphylaxis phenomenon, so they should be discontinued for at least 8-12 hours a day (generally during the night) to maintain their therapeutic effectiveness. Side effects are hypotension, headache, metahemoglobin, stomachache. An important contraindication is cerebral ischemia; caution should be done in case of assumption of alcohol and some medications (sildenafil). Nitrates have not to be suddenly suspended (rebound effect).
They are administered by different routes of administration: sublingual, spray, IM and IV.

NITRATES
NYTROGLYCERINE, ISOSORBIDE MONONITRATE, ISOSORBIDE DINITRATE

5.2 β – Blockers

Together with nitrates are first-line choice, if no contraindications exist. By virtue of their inotrope and chronotrope negative effect they reduce the heart work and oxygen demand from myocardium. Net evidence from different clinical trials have demonstrate the improvement in the survival rate in patients with coronary artery disease treated with β-blockers agents; those patient have also decreased the relative risk to suffer from another myocardial infarction. Contraindications are severe heart failure, COPD and heart block.

B- BLOCKERS
ATENOLOL, METOPROLOL, BISOPROLOL, CARVEDILOL

5.3 Calcium channel blockers

This category of drug cause vasodilation by blocking the calcium ions flow in the smooth muscular cells of the arteries and less of the veins. They are the first-line option in the Prinzmetal's angina caused by coronary vasospasm; they are also employed in chronic stable angina when other agents are contraindicated or ineffective. Some of these agents cause tachycardia (niphedipin) while others induce bradycardia (verapamil, diltiazem).

CALCIUM CHANNEL BLOCKERS
NIPHEDIPIN, AMLODIPIN, NICARDIPIN, VERAPAMIL, DILTIAZEM

5.4 Other medications

Antiplatelet agents are useful in the secondary prevention of myocardial infarction. Statins are employed in the treatment of dyslipidemia to reduce LDL-C serum levels and improve HDL-C ones in order to reduce the progression of the atherosclerotic plaques; they also possess antiproliferative and antioxidant properties. ACE inhibitors and ARBs (angiotensin receptor blockers) modulate the renin-angiotensin-aldosterone pathway and so they improve the left ventricular function reducing the ventricular remodeling.

OTHER AGENTS
ASPIRIN, CLOPIDOGREL, TICLOPIDINE, ROSUVASTATIN, PRAVASTATIN, SIMVASTATIN, CAPTOPRIL, RAMIPRIL, LISINOPRIL, ENALAPRIL, FOSINOPRIL, VALSARTAN, LOSARTAN, IRBESARTAN, TELMISARTAN

5.5 Ranolazine

Ranolazine, approved by the Food and Drug Administration in 2006, was the first specific novel medical therapy available for the treatment of chronic stable angina after the introduction of calcium channel blockers, in the 1980s . Ranolazine is a proven antianginal agent that, unlike beta-blockers, nitrates, or calcium channel blockers, does not affect either heart rate or blood pressure. Its mechanism of action is primarly due the ability to influence the Na+ and Ca2+ homeostasis in cardiomyocytes. In particular, ranolazine's mechanism of action primarily involves inhibition of the late Na+ flux. By this effect, ranolazine prevents intracellular calcium overload and its subsequent deleterious electrical and mechanical effects. Ranolazine attenuates the abnormally prolonged and dysfunctional myocardial contraction that increases myocardial oxygen demand and, at the same time, is thought to improve coronary blood flow and myocardial oxygen supply by optimizing diastolic function. Randomized clinical studies have been performed to test ranolazine's ability to reduce angina symptoms.

The MARISA (Monotherapy Assessment of Ranolazine in Stable Angina) investigation, a double-blind, multicenter, randomized trial in which were involved 191 patients, evaluated improvements of stress-induced angina. Exercise testing was performed at the conclusion of each treatment phase, during both peak (four hours after dosing) and trough (12 hours after dosing) plasma ranolazine concentrations, to assess the sustainability of a clinical response and establish a dose-response relationship. The MARISA investigators found that ranolazine 500 mg, 1000 mg, and 1500 mg twice daily incrementally increased exercise duration relate to the assumption of placebo. These improvements were all significant when compared with placebo. Furthermore, dose-related increases in exercise duration at peak, as well as trough, and peak times to 1 mm ST segment depression, and times to angina onset were also demonstrated ($P < 0.005$). *The use of ranolazine is recommended in patients with chronic angina in combination with standard therapy.*

6. Shock wave therapy

Cardiac shock wave therapy (CSWT) is a novel, noninvasive intervention that may ameliorate myocardial ischemia and improve cardiac function. Early clinical trials performed showed that CSWT alleviated angina symptoms and improved cardiopulmonary performances in patients with myocardial ischemia. More and more evidences indicate that CSWT may reduce the ischemic burden and provide angina relief by promoting

angiogenesis and revascularization in ischemic myocardium. Earlier in vivo animal studies and human clinical studies demonstrated that low-energy pulse waves produced by CSWT induced a sort of ''cavitation effect'' (micron-sized violent bubble collapse within and outside cells), exerting a mechanical shear force on myocardial and vascular endothelial cells. Furthermore, improved regional myocardial blood flow and capillary density were also observed.
Clinical studies corroborated these early findings, as myocardial perfusion in ischemic regions was enhanced following CSWT. In a recent study by *Yu Wang* and colleagues good clinical outcomes are reported. Investigators described improved regional cardiac systolic function and imaging studies demonstrated increased myocardial blood flow in ischemic myocardium, supporting the notion of CSWT-mediated promotion of angiogenesis. In that study CSWT procedure was well tolerated, performed without anesthesia, and allowed for concurrent monitoring of ECG, blood pressure, and blood oxygen saturation. One important limitation of current clinical experience with CSWT is the small number of patients enrolled and the relatively short follow-up period. Further larger clinical trials are expected to more reliably evaluate the outcomes of this new approach to chronic myocardial ischemia.

7. Cell therapy in refractory ischemia

The clinical limitations of the efficiency of conventional approaches justify the search for new therapeutic options. Regenerative medicine can be considered the next step in the evolution of organ replacement therapy. It is driven largely by the same health needs as transplantation and replacement therapies, but aims further than traditional approaches (*Daar, 2007*). In fact, its purpose is not just replacing the malfunctioning organs, but providing the elements required for *in vivo* repair, to devise replacements that seamlessly integrate with the living body, and to stimulate and support the body's intrinsic capacities to regenerate and to heal itself (*Greenwood, 2006*). Tissue ischemia is a promising platform for cell-based therapies. Ideally, the induction of vascularisation into an ischemic region might decrease the lesion size, prevent loss of cells through apoptosis and might inhibit the development of organ failure.
The increasing knowledge on the role of circulating cells deriving from the bone-marrow, called endothelial progenitor cells (EPCs), on cardiovascular homeostasis in physiologic and pathologic condition, have prompted the clinical use of these cells to relieve ischemia (*Krenning, 2009*). The biological rational and the initial clinical results of the use of EPCs in refractory ischemia will be hereafter discussed.

7.1 Endothelial progenitor cells

7.1.1 Biology

The discovery of bone marrow(BM)-derived endothelial progenitor cells (EPCs) circulating in the blood vessel system by *Asahara et al*. in 1997 has resulted in a new paradigm for endothelial regeneration and introduced a potential new approach to the treatment of cardiovascular disease. EPCs are adult progenitor cells, which have the capacity to proliferate, migrate and differentiate into endothelial lineage cells but they have not yet acquired characteristics of mature endothelial cells (ECs) (*Urbich, 2004*).
These cells induce neo-vascularization through paracrine stimulation (*Yoon, 2005*) and became incorporated in the wall of newly formed vessels when injected into animal models of hind limb ischemia (mouse and rabbits). EPCs cells can be localized in the adult BM

(*Peichev, 2000*), in the peripheral blood (PB) (*Asahara, Matsumoto, 2000*) and in the human umbilical cord blood (UCB) (*Pasino, Naruse, Ma, Liew*).
In the adult life, EPCs, are supposed to derive from the hemangioblasts and can be expanded ex vivo from CD34+/CD133+/KDR+/CD45+/- cells. EPCs are distinguished in "early" and "late" based on the different timing of their appearance and differences in the clones shape (*Hur, 2004*). Yoon and colleagues demonstrated that the induction of neo-vascularization by early EPCs in vivo occurs through paracrine stimulation, while late EPCs directly contribute to formation of novel vessels. Another distinction between early and late EPCs has been established with the finding that early EPCs, also named colony forming unit-endothelial cells (CFU-ECs), originate from CD34+/CD133+/KDR+/CD45+ cells in the MNCs cellular fraction while late EPCs, also named *endothelial colony forming cells* (ECFCs) originate from CD34+/CD133-/KDR+/CD45- cells (*Timmermans, Ingram, Prater*). Other cell types present in BM and PB mononuclear fractions are considered EPCs. For example, it has been shown that CD14+ monocytes have angiogenic activity (*Pujol*) and that certain subsets of T-lymphocytes also behave as EPCs (*Asahara, Gehling*).
Stem cells that can be differentiate into EPCs exist in a quiescent state associate with bone marrow niches. In the microenvironments EPCs can either remain in an undifferentiated and quiescent state or differentiate. Under physiologic conditions only a small number of these cells are maintained in peripheral circulation, where they contribute to endothelial and vascular homeostasis. In response to vascular injury or physiological stress, EPCs can be mobilized from the BM and recruited to the damage area (Pesce). Increase of peripheral blood EPCs can be induced by a variety of signal from the periphery, including angiogenic growth factors (VEGF-A, SDF-1, G-CSF) cytokines (GM-CSF), hormones (EPO, estrogen) or drugs (statins) and home to areas of ischemic injury, where they integrate into growing vessels (*Zammaretti, 2005*). In fact, EPC levels are generally low in healthy subjects, decrease in chronic vascular disease and transiently increase during acute vascular damage (*Barsotti, 2009*).
There is evidence that patients with risk factors (diabetes, hypertension, high cholesterol, smoking, obesity and metabolic syndrome) have dysfunctional endothelial progenitors; in fact their numbers are reduced in the circulation, they have a reduced migratory activity, impaired clonogenicity and survival and, thereby, a reduced in vivo neo-vascularization capacity. Similar function alteration have been reported in EPCs isolated from aged and/or male individuals and from patients with coronary artery disease or ischemic cardiomyopathy (*Hung, 2009*).
EPC reduction may have different causes, such as an exhaustion of the pool of progenitor cells in the bone marrow, a reduced mobilization, survival or differentiation (*Barsotti, 2009*).

7.1.2 Role in ischemia

The advantage of EPCs therapeutic use depends on their ability to integrate into newly forming vessels (ECFCs) or to activate neo-vascularization by pararcine mechanisms (CFU-ECs). The two distinct, direct and indirect, ways of human EPC types participation to neo-vascularization process may represents two different modalities for biologically treating ischemic disorders at the heart or peripheral levels. In fact, while CFU-ECs have a predominantly paracrine angiogenic activity, ECFCs have a modest paracrine effect and may be thus useful for long term engrafting into ischemic tissues or promote re-endothelization of injured vessels (*Young, 2007*). The positive contribution of EPCs to adult neo-vascularization has been considered an useful approach in order to attenuate myocardial ischemia in coronary artery disease. For example, when EPCs were delivered in

animal models of myocardial ischemia via either systemic administration or direct intramyocardial injection, they were found in the infarcted tissue and contributed to neo-vascularization, thereby diminishing the infarct size (*Kawamoto,2006*). An important feature of EPCs is their ability to promote rapid re-endothelialization of carotid vessels denuded as a consequence of balloon-injury (*Griese, 2003*). One of the principal mechanisms in this framework appears to be the release of vasculoprotective molecules, such as nitric oxide (NO). In particular, the endothelial-specific NO Synthase (eNOS) exerts pleiotropic cytoprotective effects in the vessel wall, reduces oxidative stress, modulates vascular tone and platelet adhesion, and impairs the development of atherosclerosis. It has been shown that EPCs overexpressing eNOS have an enhanced antiproliferative in vivo effect that significantly reduced the neointimal hyperplasia (*Kong*). A study by Werner at al showed that blood levels of CD34+ KDR+ EPCs are inversely correlated with cardiovascular events and death from cardiovascular causes. These findings implied that EPCs support the integrity of vascular endothelial cells (*Werner, 2005*). EPCs also exert a significant reduction in collagen deposition, apoptosis of cardiomyocytes and cardiac remodeling (*Itescu, 2003*). Finally, Hinkel and co-workers, showed that Embryonic EPCs (eEPCs) exert post-ischemic cardioprotection by paracrine factors activating the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway in cardiomyocytes in vitro and in vivo (after ischemia and reperfusion in a preclinical pig model). eEPCs were capable of reducing the amount of adhesive inflammatory cells. In particolar they found that Tβ4, one of the most highly expressed AKT-activating factors in their eEPC population, is indeed responsible for cardiomyocyte protection.

7.2 Choice of cell type and source for clinical use

The observation that bone marrow elements contribute to cardiac repair in the ischemic heart served as the rationale for adult bone marrow cell therapy after ischemic event. This evidence that precursors of endothelial cells exist within the mononuclear cell fraction of adult bone marrow forms the basis for the use of *bone marrow mononuclear cells* (BMMNCs) in clinical trials (*Oettgen,2006*).

Many studies were in agreement that administration of autologous cells in the heart is safe, and it causes an improvement, although modest, in some clinical endpoints such as left ventricular function and clinical status.

Because the numbers of autologous EPCs from peripheral blood or cord blood are limited, a great amount of attention has been directed to autologous whole bone marrow mononuclear cells (*Zammaretti, 2005*). Several investigators have chosen to deliver unfractionated bone marrow–derived cells, a technique that has the advantage of minimizing extensive ex vivo manipulation of the cells to isolate and expand a selected population of cells (*Oettgen, 2006*). The potential disadvantage of delivering a mixture of cells is that the percentage of cells that are therapeutically useful may be small. Moreover, because whole mononuclear cell preparations contain monocytic cells, it remains to be determined whether the improvement is in part aided by the monocytic cell fraction. A concern in using whole bone marrow mononuclear cells is potential unwanted side effects such as growth of bone or fibrosis from mesenchymal and stromal stem cells contained in this population (*Zammaretti, 2005*). An alternative strategy is to isolate purer populations of cells that express specific antigens.

These was clearly demonstrated by comparing in a rat model the administration of total MNCs, $CD34^+$ cells, and a higher dose of total MNCs containing the same number of $CD34^+$ cells of the stem cell treatment group. The $CD34^+$ cell receiving group was the best in terms

of capillary density, fibrosis area, shortening fraction and echocardiographic measurements (Kawamoto). Douglas et al. showed in a randomized trial in patients with intractable angina, feasibility, safety and bioactivity of intramyocardial injection of autologous CD34+ cells. Despite this, a growing body of evidences suggests that CD133 could be a useful marker that identifies a more primitive human progenitor subpopulation compared to CD34. Moreover, in addition to haematopoiesis, $CD133^+$ cells have been shown to possess endothelial capacity (*Bhatia, 2001*). Other reports of different groups, (*Stamm et al., Pompilio et al., Losordo et al.*) showed that intramyocardial delivery of purified CD133+ cells is safe; if associated with coronary artery bypass grafting (CABG) surgery, it provides beneficial effects and if used for refractory myocardial ischemia improves heart perfusion.
Another study by Freund and coworkers directly compared $CD34^+$ and $CD133^+$ cells isolated from 10 individual healthy donors. Although they did not find differences in terms of cell expansion properties, they found a greater subpopulation of more committed cells in the $CD34^+$ group and a lower long term colony-forming units (LTC-FU). Moreover, $CD34^+$ cells contained a higher proportion of erythroid colony-forming cells, whereas the highest content of myeloid colony-forming cells were in the $CD133^+$ selected cells (*Freund, 2006*). From all these results we can conclude that CD133 could be a useful antigen to select progenitor cells for a therapeutic purpose.
Many trials focused the attention on mobilizing cells from bone marrow by different regimens of growth factors stimulation. While many cytokines have been used in preclinical models, at the clinical level only G-CSF received sufficient priority. Use of this factor in patients is facilitated by its already available clinical approval to mobilized and collect HSCs for hematologic transplantation by apheresis (*Pesce, 2011*).

7.3 Route of administration

The optimal delivery route with regard to safety and efficacy remains to be established. Three main route of cell administration of have been described: retrograde via the coronary sinus, anterograde intracoronary, intramyocardial (endocavitary/epicardial injections). In 2005, *Vicario et al* reported good outcomes with retrograde delivery catheterizing the coronary sinus via the brachial vein. However given the scarcity of clinical experiences with this technique, its role in therapeutic angiogenesis is unclear.
Intracoronary delivery (*Wang, Lasala*) is performed by the direct injection of a suspension of cells into the coronary artery of the ischemic (target) area and it is most often used after MI and reperfusion attempts rather than in a context of chronic myocardial infarction.
Direct intramyocardial (IM) injection appears to be the most promising technique due to its ability to more closely target the ischemic territory of interest and, potentially, achieve the greatest local concentration of the therapeutic solution. Preliminary experiences reported IM administration via the epicardial route under direct mini-thoracotomic surgical access after an accurate study of the electrophysiological properties of the myocardium to assess the target area of ischemic but still viable myocardial tissue (*Babin-Ebell, Van Ramshorst J, Gowdak, Briguori, Reyes, Hossne, Pompilio*).

ROUTE OF DELIVERY ADMINISTRATION OF THE CELLS
CORONARY ARTERIES (ANTEROGRADE FASHION)
CORONARY SINUS (RETROGRADE FASHION)
DIRECT INTRAMYOCARDIAL INJECTION

Subsequently, the era of percutaneous direct IM injection was advanced by the introduction of an electromechanical mapping and injection catheter using the NOGA system. This approach has the potential to be as precise as the direct surgical injection technique, while avoiding the risks of general anesthesia, surgery, and painful postoperative recovery (*Hung-Fat Tse, Beeres, Losordo*).

Concerns have been raised about arrythmogenicity of cell therapy. Available trials did not show an increased risk of developing serious ventricular rhythm disturbances related to direct injection of cells in the myocardium.

7.4 Overwiew from clinical trials

On the basis of encouraging results of preclinical studies, various clinical trials have been carried out in order to evaluate safety and efficacy of cell therapy in patients with refractory ischemic cardiomyopathy, as shown in the table below.

The clinical experience of cell therapy in a setting of refractory ischemia encompasses up to now about 250 patients, 120 involved in phase I/II and 130 in randomized controlled trials (RCTs).

Hung-Fat Tse et al. conducted the first in-human study to evaluate the safety of intra-myocardial transplantation of autologous BM-MNCs for eight patients with intractable angina. Immediately before bone marrow cell injection, NOGA system was used to perform electromechanical mapping of the left ventricle and then to guide the BM-MNCs injections to the area of ischemia. The absence of any acute procedural complications or long-term sequalae, including ventricular arrhythmia, myocardial damage, or development of intra-myocardial tumour provided a strong foundation for performing larger and more definitive trials. In most trials, EPCs were isolated from the total MNCs population via magnetic positive selection of CD34+ or CD133+ cells (Losordo, Babin-ebell, Kovacic, Pompilio, Wang). The safety, feasibility, and efficacy of intra-myocardial CD133+ cell transplantation have also been established for patients with refractory ischemia as a sole therapy in the absence of bypass surgery (Babin-Ebell, Kovacic, Pompilio).

Although the limited number of patients included in the early trials, there are evidences suggesting an improvement in therms of clinical benefits and myocardial perfusion and almost all reports has demonstrated acceptable safety profiles.

Following these reports, four randomized, multicenter trials were performed to evaluate the safety and efficacy of different type of bone marrow derived cells compared to placebo or best standard care. To our knowledge, there are no published data comparing the effect of cell therapy to specific drug for refractory angina.

Losordo et al. performed a phase I/IIa, double-blind, placebo-controlled, dose-ranging trial to evaluate the intra-myocardial transplantation of G-CSF-mobilized CD34+ cells in 24 patients with intractable angina. Patients were enrolled into 1 of 3 cohorts ($5X10^4$, $1X10^5$ and $5X10^5$ CD34+ cells/kg) versus placebo. Patient-specific procedures included G-CSF injection, leukapheresis for cell harvesting, and NOGA-mapping-guided cell injection, all of which were well tolerated with no severe adverse events reported. Favorable trends in angina frequency, nytroglicerin usage, exercise tolerance and perfusion defect were observed in patients administered CD34+ cells compared with patients who received placebo. They reported few and evenly distributed serious adverse events. Following these outcomes, a phase IIb study is under way in the United States. A recently published trial randomized (1:1) 150 patients to receive intracoronary transplantation of autologous bone marrow derived CD34+ cells. The target population included patients with class III and IV angina refractory to medical treatment and not amenable to revascularization. Serious adverse

Authors	Study design	Delivery	Cell type	Mean FU period	Safety	Results
Hung-Fat Tse et al. 2003	Phase I (8)	IM^{endo}	BM-MNCs	3 months	no AEs reported	↓ angina episodes ↑ perfusion
Vicario et al. 2005	Phase I (14)	IV	BM-derived CD31^{+} cells	6 months	chest pain during procedure (x2)	↑ perfusion ↓ CCS class ↑ collateral vessels ↑ QoL
Briguori et al. 2006	Phase I (10)	IM^{epi}	BM-MNCs	1 year	acute AF 7 days after procedure (x1)	↓ CCS class ↑ LVEF ↑ QoL ↑perfusion
Losordo et al. 2007	RCT phase II (18/6)	IM^{endo}	mPB-derived CD34^{+} cells	12 months	SAEs evenly distributed	↓ CCS class ↓ angina episodes
Tse et al. 2007	RCT phase II (19/9)	IM^{endo}	BM-MNCs	19 months	carcinoma of the urinary bladder (x1)	↑ exercise time ↑ LVF ↓ angina episodes

Table 1. Clinical trials of stem cell therapy in refractory angina

Authors	Study design	Delivery	Cell type	Mean FU period	Safety	Results
Babin-Ebell et al. 2008	Pilot (6)	IM^{epi}	BM-derived CD133^{+} cells	6 months	no AEs reported	↓ CCS class ↑ LVEF
Gowdak et al. 2008	Phase I (8)	IM^{epi}	BM-MNCs	6 months	no AEs reported	↓ CCS class ↑ perfusion
Kovacic et al. 2008	Phase I and II (36)	IC	mPB-derived CD133^{+} cells *vs* MNCs	3 months	cardiac ischemia (x4), thrombocytopenia(x2) and gout(x1)	↓ angina episodes ↑ perfusion
Pompilio et al. 2008	Pilot (5)	IM^{epi}	mPB-derived *vs* BM-derived CD133^{+} cells	24 months	no AEs reported	↓ CCS class ↑ perfusion ↓ angina episodes
Jan van Ramshorst et al. 2009	RCT phase II (25/25)	IM^{epi}	BM-MNCs	3 - 6 months	pericardial effusion after procedure (x1)	↓ CCS class ↑ LVEF ↓ SSS ↑ QoL

Table 2. Clinical trials of stem cell therapy in refractory angina

Authors	Study design	Delivery	Cell type	Mean FU period	Safety	Results
Reyes et al. 2009	Phase I (14)	IM^{epi}	BM-MNCs	7 months	no AEs reported	↓ CCS class
Hossne et al. 2009	Pilot (8)	IM^{epi}	BM-MNCs	12-18 months	no AEs reported	↓ CCS class ↑ perfusion
Wang et al. 2010	RCT phase II (56/56)	IC	BM-derived $CD34^+$ cells	6 months	no AEs reported	↓ angina episodes ↓ CCS class ↑ perfusion
Lasala et al. 2011	Phase I (10)	IC	BM-MNCs *vs* BM-MSCs	6 months	no AEs reported	↑ LVEF ↑ perfusion ↑ QoL

FU: follow-up; IV: intra-venous; BM: bone-marrow; QoL: Quality of Life; IM^{endo}: endocavitary intra-myocardial injection; BM-MNCs: bone marrow-derive mononuclear cells; AEs: adverse events; IM^{epi}: epicardial intra-myocardial injection; LVEF: left ventricle ejection fraction; RCT: randomized controlled trial; SSS: summed stress score; AF: atrial fibrillation; LVF: left ventricular function; mPB: mobilized peripheral blood; SAEs: serious adverse events; BM-MSCs: bone marrow-derived mesenchymal stem cells.

Table 3. Clinical trials of stem cell therapy in refractory angina

events were distributed evenly between cell and placebo group. CCS class, exercise tolerance and angina frequency appear to be improved in both groups at 3 and 6 months follow-up. However, the CD34+ stem cell-treated group experienced greater reduction of sytomps. Tse et al. randomized 28 "no option" patients, class III or IV angina refractory to medical therapy to receive low-dose ($1X10^6$ cells/0.1 mL) or high dose ($2X10^6$ cells/0.1 mL) autologous bone marrow cells or placebo via a direct endomyocardial injection guided by electromechanical mapping. Compared with controls, there was a significant increase of total exercise time, left ventricle function and a lower NYHA class at 6-month follow-up, but CCS class was reduced similarly in both groups. There were no acute or long-term complications associated with bone marrow cell implantation.

More recently, a randomized, double-blind, placebo-controlled trial investigated the effect of intra-myocardial bone marrow cell injection on myocardial perfusion and LV function. The study population consisted of patients with severe angina pectoris despite optimal medical therapy and myocardial ischemia in at least 1 myocardial segment as assessed by SPECT and all patients were ineligible for conventional revascularization as determined by an independent expert. The intra-myocardial injections of $100X10^6$ autologous bone marrow-derived mononuclear cells or placebo (randomly assigned in a 1:1 ratio) were delivered after electromechanical mapping using NOGA system. In this trial, bone marrow cell injection resulted in a significant improvement in angina symptoms, quality of life, and exercise capacity, in line with precedent trials (Van Ramshots J).

The Safety and Efficacy of Autologous Endothelial Progenitor Cells CD133+ for Therapeutic Angiogenesis (PROGENITOR) trial is currently ongoing in Spain and will provide more information regarding the potential benefit of CD133+ to produce a clinically meaningful angiogenic response (see also www.clinicaltrial.com).

7.5 The road ahead of cell therapy

The current state of therapeutic angiogenesis certainly still leaves many questions unanswered. It is of paramount importance that the treatment is delivered safely. Direct IM and IC administration have demonstrated acceptable safety profiles in these early trials, and may represent a major advance over surgical thoracotomy. Once the treatment is administered, assessing the benefit remains a critical issue. Exercise testing, evaluation of angina parameters, and myocardial perfusion are routinely used to assess for bioactivity, but which most reliably endpoint reflects efficacy remains unknown.

While therapeutic angiogenesis is not ready to become part of routine therapy for refractory angina, it is crucial that we continue to learn from both encouraging and disappointing clinical and preclinical studies. The combined efforts of bench and clinical researchers will ultimately answer to the question whether cell therapy will be a suitable strategy for patients with refractory angina.

8. Conclusions

Refractory angina is still a very debilitating condition, with a negative impact on patient's prognosis and social costs. Recent advancements in pharmacological and non-pharmacological therapy open new perspectives for these patients. If promising results recently achieved by different approaches will be confirmed in the near future, it is likely that the next generation of physicians dealing with such a debilitating illness will have more effective strings in their bow.

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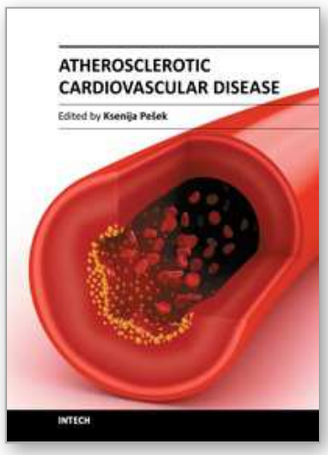

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