INTRODUCTION
Refractory angina constitutes a serious clinical problem. The European Society of Cardiology has defined refractory angina as a chronic condition (> 3 months) characterised by the presence of angina caused by coronary insufficiency due to coronary artery disease which can not be controlled by a combination of medical therapy, angioplasty and coronary bypass surgery. 1

The prevalence of refractory angina is growing due to the improvement in the survival of patients with coronary heart disease. Patients with no options may account for up to 12% of those referred for diagnostic catheterisation. Reason for ineligibility include: the presence of diffuse coronary disease, disease in small distal vessels, recurrent in stent restenosis, chronic total occlusion and multivessel disease in patients with important co-morbidities. 2

Although mortality rate in this kind of patients is low the effect in the quality of life is very high 3,4. Patients with chronic angina present increasing levels of anxiety, depression and psychological stress that may worsen the degree of disability. Furthermore, it has been proved in large-scale clinical trials that the quality of life has prognostic value. The IONA study group found that basal severity of angina (Canadian Cardiovascular Society [CCS] grading) was a powerful independent risk factor of cardiovascular death or non fatal myocardial infarction. 5

There is no doubt that strategies aimed at reducing coronary disease progression, coronary events and angina rate (Table 1) are essential reducing both cardiovascular morbidity and mortality; however, other multidisciplinary treatments should be incorporated such as cardiac rehabilitation and psychological support. 6,7,8

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Level of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin/Clopidogrel</td>
<td>A/B</td>
</tr>
<tr>
<td>Statin</td>
<td>A</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>A</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>A/B</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>A/B</td>
</tr>
<tr>
<td>K-channel opener</td>
<td>A/B</td>
</tr>
</tbody>
</table>

Table 1: Medical management for patients with chronic angina

There are additional treatment possibilities for patients with refractory angina that are not considered as a standard therapy. These other options of treatment include enhanced external counterpulsation 9, spinal cord stimulation 10 or transmyocardial laser revascularisation 11,12,13. There are several randomised clinical trials and registries assessing the efficacy of these therapies as compared to placebo in patients with refractory angina. Although most of them have shown significant clinical improvement, inconsistent results about the effect on exercise capacity or myocardial perfusion are reported.
The regenerative medicine with stem cells has emerged as a promising field, especially in the clinical setting of refractory angina. In this regard, several studies have been published and others are ongoing. In this review we will focus on bone marrow derived stem cell transplantation as a novel option for the treatment of patients with refractory angina.

A change in the paradigm

The creation of new blood vessels involves three different processes that result in a better blood flow supply to ischemic tissues: angiogenesis, arteriogenesis and vasculogenesis. Arteriogenesis is the phenomenon in which a pre-existing arteriole of the resistance vessel class matures into a conductance vessel class. Angiogenesis is the formation of new vessels by sprouting of endothelial cells from pre-existing capillaries and vasculogenesis refers to in situ differentiation of endothelial precursor cells to form capillaries. Until recently, it was thought that vasculogenesis was only possible in the embryonic phase.

In this regard Ashara et al. in 1999 demonstrated that leukocyte fraction of peripheral blood contains a type of cells with CD 34+ expression that are able to differentiate into endothelial cells in vitro and to incorporate into sites of active angiogenesis in vivo. Thus, this study modified the old paradigm demonstrating vasculogenesis in adults. Others studies performed in this setting include the study conducted by Shi et al. using a canine model of bone marrow transplantation.

These authors demonstrated reendothelialisation of a dacron graft with cells that came from the transplanted bone marrow, confirming the bone marrow origin of endothelial progenitor cells. Moreover, Quaini et al. supported the theory of an extrinsic vascular repair system by using a chimera model (female heart transplanted into a male host). In this study Y chromosome + cells were found as a part of vascular structures in the heart showing the ability of extracardiac progenitor cells to engraft and develop vessels.

Transendocardial injection guided by electroanatomical mapping systems.

There are several methods to deliver stem cells to the heart. In patients with refractory angina the preferred delivery method is direct transendocardial injection guided by electroanatomical mapping. This method allows us to guide the stem cell delivery in a safe and fast fashion. The maps are constructed by combining and integrating information from intracardiac electrograms with the respective endocardial locations. The NOGA® XP System (Biosense-Webster, Diamond Bar, California) develops a low power magnetic field where catheters equipped with a location sensor provides real-time information on the location of the catheter.

The NOGA® XP System enables analysis of both global and local parameters that characterise mechanical, dynamic and electrical function of the mapped cardiac chamber. Among the global parameters provided in NOGA® XP maps are end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV) and ejection fraction (EF). Local functional analysis is based on local shortening data as tests of local mechanical function, and measurements of local intracardiac signals as tests of electrical functionality. Combined, these data provide information about the local electromechanical coupling.

This system has been used as a new technique for myocardial delivery of grown factors, recombinant genes and stem cells in both human and animal models. In patients with chronic ischemia, viable myocardium is selected as the target zone for stem cell injection. These areas are defined as zones with low local shortening and an unipolar voltage > 6.9 mv (ref Perin). This method allows us to inject at the target zone thanks to the real time three dimensional catheter position. Safety is assured after proper needle length adjustment according to myocardial thickness.

Preclinical studies in chronic ischemia

Improvement in the knowledge of stem cell biology has allowed us to develop preclinical and early clinical studies that have shown encouraging results. There are several preclinical studies showing the safety and the feasibility of stem cell transplantation in animal models. Li et al. showed that bone marrow stem cell transplantation into the normal myocardium was not associated with a local or systemic inflammation response or the development of cardiac tumours. In the same way, Goodchild et al. demonstrated that bone marrow-derived cells are not arrhythmogenic. Seven weeks after the treatment, an electrophysiological study showed no inducible ventricular arrhythmias.

Early several preclinical trials have proved the efficacy of bone marrow derived cells in models of chronic ischemia. In these studies the type of cells more frequently used in are those from the bone marrow, specially the mononuclear fraction. However, there are authors that have used an enriched fraction of endothelial progenitor cells: CD 34 + cells, or CD 31+ cells. In this regard, Kamamoto et al. demonstrated that NOGA-based transmyocardial injection of CD 31+ cells freshly isolated from the peripheral blood decreased the percentage of ischaemic area measured by NOGA, increased capillary density and improved of left ventricular ejection fraction in a pig model of chronic ischemia. On the other hand, Fuchs et al. showed that transendocardial injection of bone marrow mononuclear cells into the ischemic myocardium of pigs significantly increased myocardial perfusion as well as local contractility in the treatment group as compared with the control. No safety issues were derived from these two studies.

Clinical trials in no-option patients

Based on these preclinical observations, preliminary clinical studies have been performed in no option patients. The majority of them are non randomised, uncontrolled studies and have used the mononuclear fraction of the bone marrow. The delivery method, in all but one of them, was the NOGA guided transendocardial injection platform. These studies have shown the safety and the feasibility of the procedure and very promising results in terms of efficacy.

Four randomised trials have been performed in no option patients. The FOCUS trial has been conducted by the group at the Texas Heart Institute, Houston. This study has not been published yet; however, Dr. E. Perin presented the preliminary results in the Fourth International Conference on Cell Therapy for Cardiovascular Diseases. In this trial, 30 patients were included in a 2:1 randomised fashion to receive 30 million bone marrow mononuclear cells or placebo. At three months follow-up a significant reduction in angina functional class and an increase in perfusion measured by SPECT were observed in the treatment group; whereas no significant changes were reported in the control group.
In 2007, Losordo et al. published a randomised, dose escalating controlled trial that enrolled 24 no option patients. The treatment consisted of an enriched selection of CD 34+ cells collected from the peripheral circulation. The study showed the safety and feasibility of the procedure and clinical improvements (angina frequency, nitroglycerine use, exercise time, CCS class). The Canadian class was reduced in both groups but the cell treated experienced a greater magnitude of reduction. In contrast, the SPECT showed inconsistent findings.

Tse et al. included 28 patients, 19 were assigned to receive bone marrow mononuclear cells in two different doses or control (n= 9). In this study, the total exercise time and the left ventricular ejection fraction significantly improved in the cell group whereas no differences were observed in the control group. The most recent study was conducted in the Netherlands, in which 50 patients with chronic myocardial ischemia were included. The patients were randomly assigned to receive intramyocardial injection of autologous bone marrow-derived mononuclear cells or placebo solution. After 3-month follow up, single photon emission computed tomography-summed stress score improved from 23.5 to 20.1 (p<0.001) in the bone marrow cell group, compared with a decrease from 24.8 to 23.7 (p=0.004) in the placebo group.

A 3% absolute increase in left ventricular ejection fraction, measure by magnetic resonance imaging, was observed only in the bone marrow cell group. CCS angina score improved significantly in the bone marrow cell group compared with no significant improvement in the placebo group. Quality-of-life score (measure by Seattle Angina Questionnaire) increased from 56% to 64% compared with a smaller increase in the placebo group 57% to 61%. The improvements in CCS class and quality of life score were significantly greater in bone marrow cell-treated patients than in placebo-treated patients (p = .03 and p = .04, respectively).

There are several trials ongoing. The FOCUS trial is an ongoing randomised study that will assess the effect of autologous bone marrow mononuclear cells delivered transcendocardially to patients with left ventricular (LV) dysfunction and symptomatic heart failure or angina. They plan to recruit 87 patients (58 treated and 29 controls) and will have myocardial perfusion, LV contractile performance and maximal oxygen consumption as primary outcomes. Patients will be followed for at least five years. The Baxter ACT 34 CMI is a phase II randomised trial that will included 150 patients with refractory angina. The patients will be treated by transcendocardial injection of an enriched fraction of CD34+ cells or placebo. Finally, the PROGENITOR trial is a multicentre, randomised and double-blind study conducted by our group in Spain.

There is no official sponsor of this trial and has been supported by a Spanish Health Ministry Grant and a Mutua Madrileña Foundation Grant. The PROGENITOR trial is still in recruitment phase. The main inclusion criteria are patients with demonstrated myocardial ischemia in a certain territory without any option of revascularisation (percutaneous or surgical) and symptomatic for angina despite optimal medical treatment (refractory angina) and will be randomised to received transcendocardial injection of an enriched fraction of CD133+ cells isolated from the peripheral blood cells or placebo. CD 133+ is a surface antigen with unknown functional relevance and is an early haematopoietic stem cell marker, that is not expressed in mature endothelial cells and therefore, CD 133+ defines a primitive population of cells with the capacity to differentiate into endothelial cells. Furthermore, there is a subpopulation of these cells that are CD 34 – which are functionally more potent than CD34+ CD133+ positive cells.

To the best of our knowledge, this is the first-in-man trial with transcendocardial injection of peripheral selected CD133+ cells in no-option patients.

### Table 2: Non randomised trials assessing stem cell treatment in no-option patients

<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>Nº of patients</th>
<th>Controlled</th>
<th>Delivery method</th>
<th>Type Cells</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamano et al., 2001 (35)</td>
<td>5</td>
<td>No</td>
<td>CABG</td>
<td>MNC</td>
<td>Improved perfusion.</td>
</tr>
<tr>
<td>Tse et al., 2003 (36)</td>
<td>8</td>
<td>No</td>
<td>NOGA</td>
<td>MNC</td>
<td>Improved perfusion, wall motion and symptoms.</td>
</tr>
<tr>
<td>Perin et al., 2003 (37)</td>
<td>21</td>
<td>Yes</td>
<td>NOGA</td>
<td>MNC</td>
<td>Improved perfusion, LV function, and symptoms.</td>
</tr>
<tr>
<td>Fuchs et al., 2006 (38)</td>
<td>10</td>
<td>No</td>
<td>NOGA</td>
<td>MNC</td>
<td>Improved perfusion and symptoms.</td>
</tr>
<tr>
<td>Briguori et al., 2006 (39)</td>
<td>10</td>
<td>No</td>
<td>NOGA</td>
<td>MNC</td>
<td>Improvement in symptoms and quality of life.</td>
</tr>
<tr>
<td>Beeres et al., 2006 (40)</td>
<td>20</td>
<td>No</td>
<td>NOGA</td>
<td>MNC</td>
<td>Improved perfusion, LV function, exercise capacity and symptoms.</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Refractory angina is a chronic and deteriorating syndrome caused by myocardial ischaemia where patients are still symptomatic despite receiving complete medical therapy and when all revascularisation options have been ruled out. Only secondary prevention with blood pressure reduction, glycermic levels control, statins and aspirin use has been shown beneficial in terms of mortality and mobility reduction. Stem cell therapy has proved to be a feasible, safe and effective method of inducing therapeutic angiogenesis in experimental models.

However, to date the evidence only supports the safety and feasibility of this procedure. Data derived from clinical trials, most of them uncontrolled and with a small number of patients, are still insufficient to state the clinical efficacy of stem cell therapy. There are several ongoing trials involving many number patients and using new type of cells. The results of this study will shed light on the treatment of this subset of patients.

**Figure 1:** An example of an electromechanical mapping in a no-option patient. The patient has angina and an occlusion of the circumflex artery. The percutaneous revascularisation of this artery was failed twice. The maps show an area of viability at the lateral wall that was the target zone to treat with endothelial progenitors cells. A) An unipolar voltage map showing normal voltage at the lateral wall B) An local shortening map showing low values at the lateral wall.

**REFERENCES**


2. Mukherjee D, Bhatt DL, Roe MT, Patel V, Ellis SG. Direct myocardial revascularization and angiogenesis—how many patients might be eligible? Am J Cardiol. 1999;84;598-600.


REFERENCES (Continued)


