IT IS NEVER TOO LATE FOR CARDIAC REPAIR STEM CELL ADMINISTRATION FOR CHRONIC ISCHAEMIC HEART INFARCTION (SCACIHI)

FINAL DEGREE PROJECT

CELL PRODUCTION UNIT

CARDIOLOGY DEPARTMENT

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1. LIST OF ABREVIATIONS

- Adipose tissuederived mesenchymal stem cells (AT-MSCs or ADSCs)
- Advanced therapy medicinal products (ATMP)
- Basic fibroblast growth factor (bFGF)
- Bone marrow-derived mesenchymal stem cells (BM-MSC)
- Bone marrow-derived mononuclear stem cells (BMMNCs)
- Cardiac stem cells (CSC)
- Cardiosphere-derived cells (CDCs)
- Cardiospheres (CS)
- Cardiovascular Regenerative Medicine (CRM)
- Case Report Form (CRF)
- Clinical Research Association (CRA)
- Coronary artery bypass grafting (CABG)
- Creatin kinase (CK)
- Embryonic stem cells (ESC)
- Endotelial progenitor cells (EPC)
- Growth Stimulant Factors (G-CSF)
- Heart falure (HF)
- Human pluripotent stem cells (hPSCs)
- Induced pluripotent stem cells (iPSC)
- Intention to treat (IT)
- Investigational medical product (IMP)
- Left Ventricular Ejection Fraction (LVEF)
- Magnetic resonance imaging (MRI)
- Major adverse cardiac events (MACE)
- Miocardial infarction (MI)
- Percutaneous coronary interventions (PCI)
- Stromal Cell-Derived Factor-1 (SDF-1)
- Stromal Vascular Factor (SVF)
- Wall Motion Score Index (WMSI)

2. ABSTRACT

BACKGROUND: myocardial infarction is a leading cause of death in humans around the world. Acute infarction leaves a print in heart walls (scar) that chronifies evolving to heart failure and probably death. This pathological mechanism accounts for million deaths just in Europe every year.

Despite all medical advances and new strategies for treating acute and chronic infarction, there's still a huge amount of morbility and mortality associated with infarction sequales. Regenerative Cardiac Medicine has emerged as an interesting concurrent therapy that could lead to healing and heart functionality improvement post infarction. Stem cell therapy has increased the therapeutic armamentarium in the figth against ischemic heart disease.

Fort he past 20 years lots of studies and different products have emerged and demostrated safety profiles on clinical trials. On the other hand, none of them has managed to excel and accomplish clearly positive results, improving functionality, morbidity and mortality of these patients.

Searching of the perfect solution it's still going on and advances on the field have been positive so far.

HYPOTHESES AND OBJECTIVES: cardiac resident cells have emerged as a promising product for ischemical patients. Interesting cardioprotective, immunoregulatory, and cardioregenerative properties have been demonstrated for human cardiac stem cells. They have already proved safety on "in vitro" and phase I and II clinical trials so medical community are looking forward to see safety and efficacy of this therapy on the clinical field.

Our objective is to asses possible beneficial effects for chronic ischemical patients and to bring out possible short and long term side effects.

DESIGN AND METHODS: this protocol has been designed as a prospective, double-blind, 2:1 randomized, controlled, and multicenter clinical trial that will evaluate safety, feasibility, and efficacy of intramyocardial delivery of allogeneic human cardiac stem cell in 150 patients with chronic myocardial infarction.
We will divide the sample randomly in two comparable groups and study cardiac stem cells effect on treated group.
A follow-up of 12 months will be performed to all patients, control and experimental group, with safety (serious adverse effects, MACE, death from any cause and cardiovascular death), clinical (complete blood analisis, NT-proBNP, PCR, inmunlogical assays, NYHA functional class, 6-min walking tests and Quality of life), ECO (and Doppler) and MRI parameters (Scar size, heart functionality and edema) .

KEYWORDS: Regenerative Cardiac Medicine, Chronic Ischaemic heart disease, Cardiac Resident Stem Cells.

3. INTRODUCTION

3.1 MYOCARDIAL ISCHEMIA

Heart is an aerobic organ that depends totally on the continuous oxygen supply for its operation; the cardiac metabolism must continuously produce high-energy phosphates, since in each heartbeat is consumed up to 5% of total ATP and creatine kinase (CK) stored in the myocardium. As the production of these substances due to anaerobic glycolysis is very limited, coronary circulation has to constantly supply the oxygen and necessary substrates. Ischemia is a situation caused by deprivation of oxygen to tissues and inadequate elimination of metabolites. In heart case, an ischemical procedure is the result of the imbalance between coronary supply and myocardial oxygen demand.[1]

Coronary atherosclerosis is one of the main causes of heart infarction. It's a chronic disease with stable and unstable periods. During unstable periods with activated inflammation in the vascular wall, patients may develop a myocardial infarction. Myocardial infarction may be a minor event in a lifelong chronic disease, it may even go undetected, but it may also be a major catastrophic event leading to sudden death or severe haemodynamic deterioration.[2]

3.1.1 EPIDEMIOLOGY:

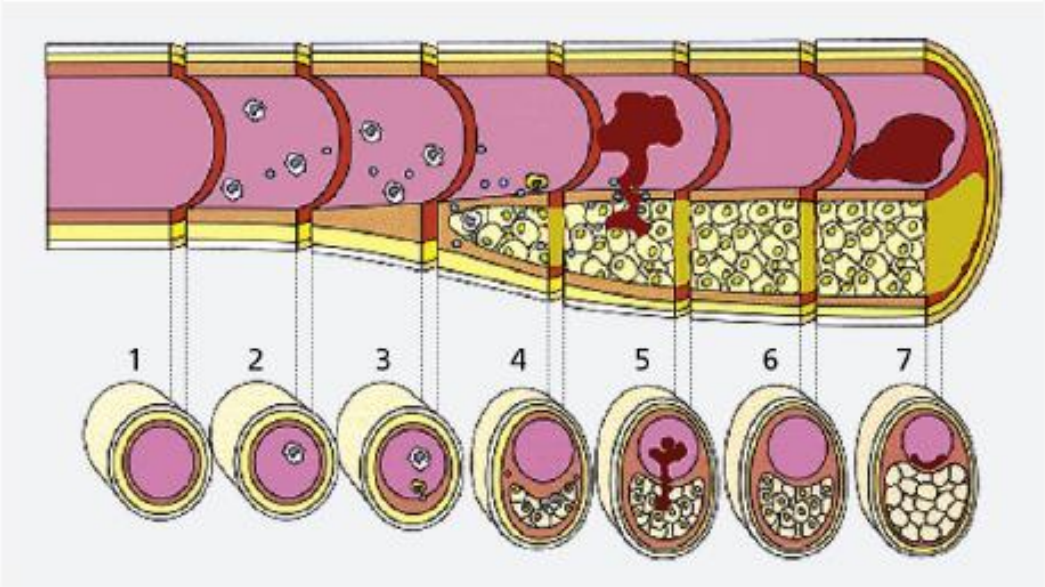
Cardiovascular disease is a leading cause of death in humans throughout the world. Myocardial infaction (MI) and its sequelae of ischemic heart failure (HF) account for mre tan 4 million deaths per year in Europe[3] and for one death every 40 seconds in the United States[4].

Despite major advances that have reduced early mortality of MI, 12% of patients die within 6 months postinfarction and 25% of survivors progressively develop HF[5], a condition that entails a mortality rate of 50% in 5 years.[6] One of the most powerful predictors of poor outcome after a MI is the extent of myocardial necrosis and the concomitant impairment of left ventricular function, both of which can be accurately and comprehensively assessed using magnetic resonance imaging (MRI).[7] In unselected populations with AMI, 1 out of 5 patients die or are hospitalized for HF in 1 year, despite receiving state-of-theart medical care.[8] This underscores an unmet clinical need for innovative therapies in high-risk patients with large infarctions who develop left ventricular adverse remodeling.

3.1.2 ETIOLOGY AND FISIOPATHOLOGY:

Causes of myocardial ischemia are multiple, but all of them act through two general mechanisms: reduction of blood flow by obstruction of coronary vessels and myocardial increased needs of oxygen. The most frequent causes in reduction of coronary flow are great coronary arteries progressive obstruction due to atherosclerotic lesions (Ilus. 1) in the epicardium and thrombotic coronary artery disease, which causes total or partial obstruction of arteries (In which we will focus basically). Other less frequent causes of coronary ischemia are coronary spasm, disease of small arteriolar vessels, arteritis, embolisms and spontaneous dissection of epicardial vessels.[1]

Ilustration 1: Atheroesclerosis Fisiopathology



Different steps of atheroesclerotic procedure, strat, progression and formation of vulnerable plaque. Longitudinal (top) and transverse (bottom) sections o fan coronary artery over time.[1]

3.1.3 CLINICAL APPROACH:

Ischaemia in a clinical setting can be identified from patients history and from the ECG. Possible ischaemic symptoms include various combinations of chest, upper extremity, jaw, or epigastric discomfort with exertion or at rest. Discomfort associated with acute myocardial infarction usually lasts at least 20 min. Often, discomfort is diffuse, not localized, not positional, not affected by movement of the region, and it may be accompanied by dyspnea, diaphoresis, nausea, or syncope.

These symptoms are not specific to myocardial ischaemia and can be misdiagnosed and attributed to gastrointestinal, neurological, pulmonary, or musculoskeletal disorders.

Myocardial infarction may occur with atypical symptoms, or even without symptoms, being detected only by ECG, biomarker elevations, or cardiac imaging.[2]

3.2 CARDIOVASCULAR REGENERATIVE MEDICINE ROLE:

The aim of cardiovascular regenerative medicine (CRM) is to find all potential diagnostic and therapeutic strategies aimed at restoring organ health.

It has been discovered that myocardium contains endogenous stem cells that are able to regenerate apoptotic or oncotic tissue, but regenerative capacity of adult human hearth is limited and insufficient to overcome a massive loss during acute damage or prolonged remodelling.

On the other hand, during human embryogenesis and in some animal species it has been detected an active cardiomyocite turnover. With this on mind, the paradigm that cardiomyocytes are terminally differentiated cells incapable of proliferation or renewal has shifted, and heart is recognized nowadays to be a self-renewing organ.[9]

Research has focused on acute myocardial infarction, chronic ischaemic cardiomyopathy, and, more sporadically, on dilated cardiomyopathy and other forms of non-ischaemic heart disease.

3.2.1 MECHANISMS OF CARDIOVASCULAR REGENERATIVE RESPONSE:

Healing exact mechanisms are still in debate[9], but a combination of different molecular pathways(Ilus. 2) seem to be the key of the regenerative response. They include:

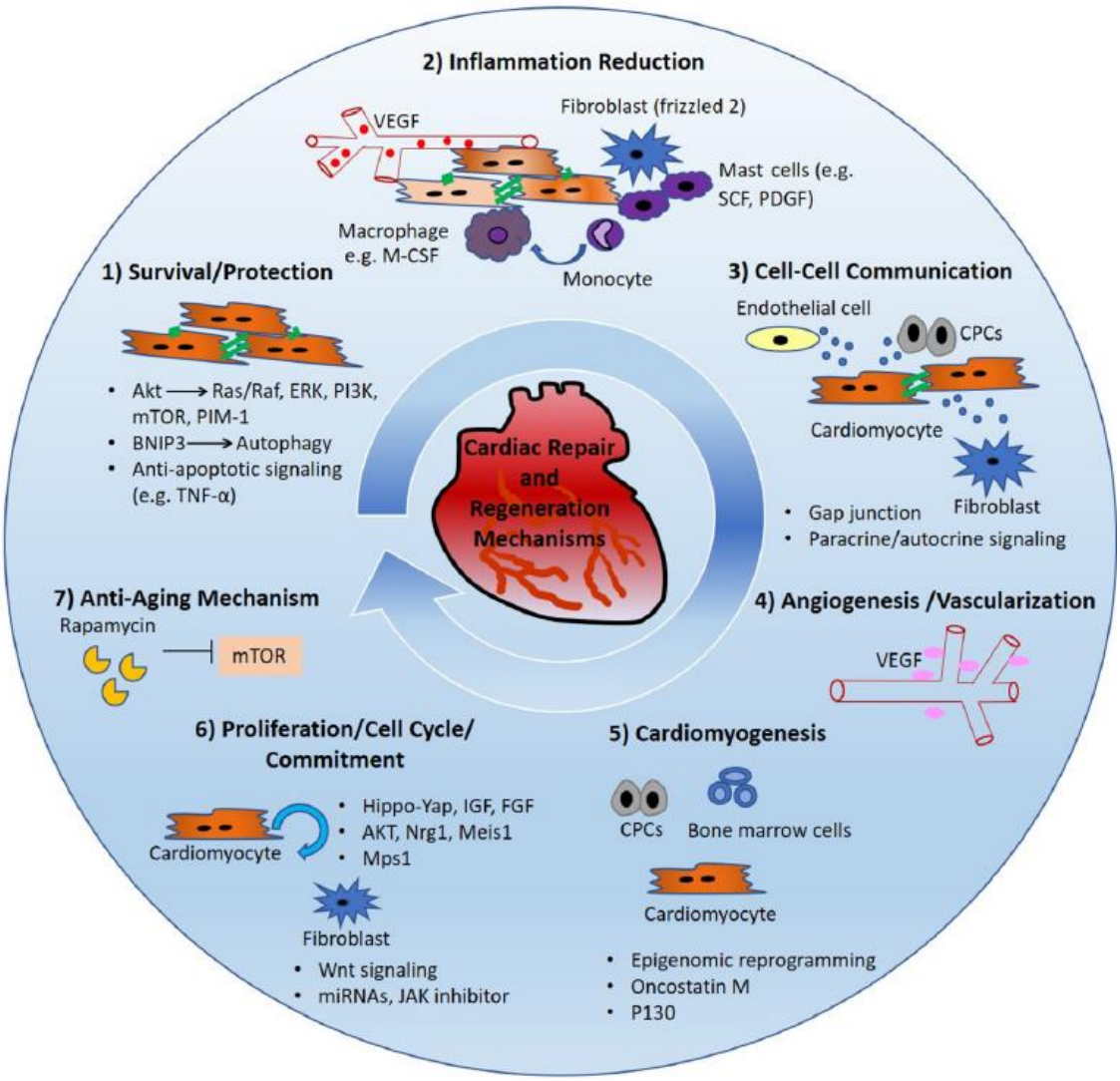
- Endogenous cardiac progenitor cells: 'cardiac stem cell niches', which have demonstrated their capacity to differentiate into several cardiac cell types under specific circumstances.

- Dedifferentiation, proliferation, and reprograming of pre-existing adult cardiomyocytes to produce new cardiomyocytes.

- Activation of cells from the epicardium as a reminiscence of its involvement in cardiogenesis during embryonic life.

- Paracrine effects have been recently discovered to have an important role on cardiac regeneration and could lead to a possibly definitive therapy.

Ilustration 2: Cardiovascular repair and regeneration multiple mechanisms



Mechanism work independently on a molecular level to collectivelly mediate concurrent celular actions of survival, repair and regenerative responses. Cardiovascular Regenerative Medicine aim is to activate and help this mechanisms in the most optimal way posible.[10]

3.2.2 REGENERATIVE STRATEGIES:

Cardiac regenerative medicine is a complex therapy in which many strategies and differents mechanisms are involved. Summarizing the main pathways we distinguish two main mechanisms by which we could be able to modify behavior in regeneration of cardiac scar:

(i) Exogenous regenerative responses, in which implanted products, cells, or tissues are expected to replace the structure of damaged or dysfunctional tissue. [11-15]

(ii) Stimulation of endogenous regenerative responses, in which the products delivered are aimed at enhancing the efficiency of endogenous reparative mechanisms.

We have not yet been able to fully understand any of these mechanisms, but, although they seem opposite, the paradigm seems to point out that by complementing them a definitive solution could be reached.

Nowadays, clinically speaking, only cell therapy has been used in clinical trials, with products like:

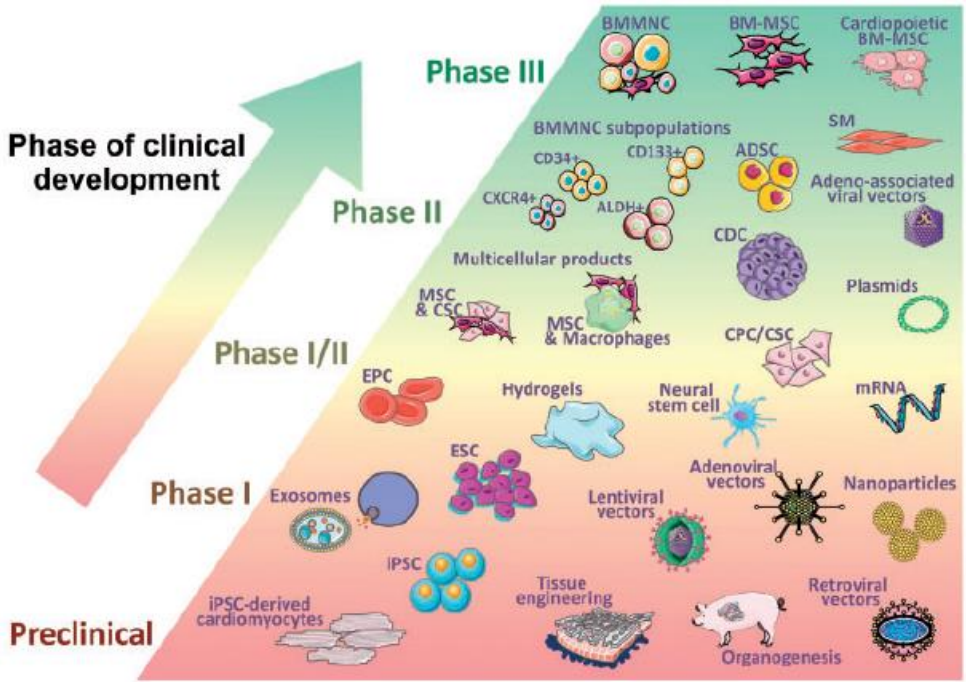
- **Adult stem cells**, including cells of cardiac origin [e.g. Cardiac Stem Cells (CSC) and cardiosphere-derived cells (CDCs)] and cells from other sources [e.g. bone marrow-derived mononuclear stem cells (BMMNC), bone marrow-derived mesenchymal stem cells (BM-MSC), adipose tissue derived stromal vascular fraction (SVF) and mesenchymal stem cells (MSCs)]. Excellent reviews summarizing their distinctive characteristics and outcomes have been published elsewhere.[16-17]

If we keep an eye on the future, new strategies have emerged with up to now good results in preclinical studies:

- **Injection of biological or synthetic factors** with active functions in endogenous regenerative processes, which emulate the benefits of cell therapy without the need for living cells. Products in this category include extracellular vesicles (microvesicles, nanoparticles, and exosomes) isolated from in vitro cell secretomes and synthetic growth factors. All these products can be generated in clinical grade and injected using various delivery strategies.[18-21]
- **Genetic and epigenetic** modifications that modulate the expression of genes and mRNA involved in the endogenous regenerative capacity of the heart and vessels. Increasing knowledge of the genetic pathways that govern cardiovascular generation and regeneration processes, which are active during the embryonic and neonatal stages, enables identification of factors that could be reactivated during adult life using genetic approaches. From the administration of mRNA produced in vitro to in vivo modifications of human DNA, the therapeutic regulation of gene expression and regeneration pathways may dramatically increase the possibilities of repairing the human cardiovascular system.[22-24]

With all this advances we can conclude that a probable future consists on the integration of all posible ways and strategies in order to achieve the perfect product.

Ilustration 3: Cardiac Regenerative Medical Products



Search for the most optimal product has not yet concluded. Nowadays more and more options and new product combinations are shuffled. Some of them already are in clinical phase III studies, while many others still in a preclinical phase. Regenerative medicine advances day by day with increasing force.[9]

3.2.3 WHAT DO WE NEED FROM FUNCTIONAL MULTIPOTENCY OF STEM CELLS? DIFFERENT SOURCES, AUTOLOGOUS VS ALLOGENIC:

"Stem cells" are undifferentiated self-replicating cells capable of generating, sustaining, and replacing terminally differentiated cells. In other words, they remain immature in an early stage of development, capable of dividing ad infinitum (self-renewable) leading to both identical cells and different cell lineages (potency), with capability for functional regeneration of tissues.

Broadly speaking, stem cells can be divided into two principal groups, embryonic stem cells (pluripotent) and adult stem cells (multipotent).[25]

Adult stem cells are the ones being used nowadays on clinical and preclinical studies. We can obtain this type of cells directly from the patient we are treating (autologous) or from a healthy donor (allogenic).

Autologous sources are nowadays the mostly used ones. Cells can be taken from the patient that is undergoing the procedure avoiding rejection issues and inmunological problems. The problem lies in the quality of these cells. Advanced age and comorbilities are not attended.

Allogeneic sources, on the other hand, may offer many advantages in cardiac regenerative medicine.

Firstly, allogeneic cells could be used as a scalable and reproducible cell product readily available "off-the-shelf" that could be administered in the setting of an ischemic cardiac event avoiding delays inherent to harvest and culture of autologous stem cells.

Secondly, this type of cells can be obtained from young and healthy donors, thereby avoiding the aforementioned impairment in autologous stem cells functionality observed with advancing age and comorbidities.

Most research in allogeneic cardiac regeneration has been done with mesenchymal stem cells (MSC) due to their immunoprivileged profile and immunosuppressive properties. MSCs lack expression of major histocompatibility class II antigens[26], down-regulate T cells response through direct contact and secretion of anti-inflammatory cytokines[27] and significantly affect the ability of dendritic cells to prime T-cell responses[28].

These findings raised interest in MSCs for allogeneic stem cell transplantation, but recent data have challenged this assumption suggesting that under certain inflammatory environment and during their differentiation process, MSCs can switch their immune phenotype towards an immune-enhancing pattern, limiting their long term survival and benefits.[29-30] However, and as pointed out before, autologous stem cell therapy faces the same problem, and if the advocated paracrine effect is responsible of stem cell regenerative capacity, survival and maintenance of cells in the transplanted heart could not be indispensable.[31]

3.2.4 DIFFERENT CELL TYPES ON THE FIELD:

-MYOBLASTS: The first type of stem cell thought to be useful for cardiac regenerative purposes were autologous skeletal myoblasts.

Their muscular phenotype and many other advantageous features including ease of isolation through muscle biopsy, rapid expansion *in vitro* and lack of ethical or immunological issues made the man attractive option.[32] In fact, their use in animal models and phase I non-randomized[33-35] human trials described their ability to form some cardiac structures and yielded promising results regarding improvement in cardiac performance after MI.[36-40]

Nevertheless, subsequent studies documented that myoblasts differentiate into skeletal myocytes instead of cardiomyocytes[41], and the first and larger randomized controlled trial in humans, the MAGIC trial, showed no benefits on cardiac function.[42]

-BONE MARROW DERIVED STEM CELLS:

- **BONE MARROW DERIVED MONONUCLEAR STEM CELLS (BMMNCs):** They represent a heterogeneous mixture of mononuclear stem cells including hematopoietic and endothelial progenitors and mesenchymal stem cells and are the most extensively used type of cells for cardiac regeneration. Many studies have shown a positive effect of BMMNCs in cardiac function after MI[43-45], both in the acute and chronic phases, althougth some other trials reported less evidente or even absent benefit.[46-48]
- **MESENCHYMAL STEM CELLS (MSCs):** also known as multipotent stromal cells, represent a subset of BMMNCs discovered more than 40 years ago.[49] They can be found within connective tissue in other organs, from which they can be easily isolated and cultured. They have awaken a big interest in recent years because they display a number of traits that made them an attractive cell product for cardiac repair.[50] MSCs are multipotent and have the capability to transdifferentiate into lineages of mesodermal tissues[51-52], including cardiomyocytes.[53]

-ADIPOSE TISSUE-DERIVED CELLS: represent a population of stem cells located in the adipose tissue that are able to differentiate into multiple cell lineages including cardiomyocytes and vascular cells.[54] They offer two major advantages over some previously mentioned types of cells: firstly, the easy and repeatable access that makes it possible to harvest large amounts of adipose tissue by a minimally invasive method and, secondly, their increased proliferative potential in culture. They represent two different groups of cells, adipose tissue derived Stromal Vascular Fraction (SVF) and Adipose Tissue-derived Stem Cells (ADSC). They have been tested in many preclinical and clinical trials and their safety profiles are as good as the other viable sources. They can be injected or grafted with tissue grafts.[55]

-CARDIAC STEM CELLS (CSCs): Strong evidence has supported the concept that turnover in the adult cardiomyocyte population is provided by cardiac stem cells (CSCs)[56-57], but the magnitude of this turnover and the exact underlying mechanisms remain unknown[58]. CSCs are rapidly activated after myocardial injuries or physiological stimulation[59-61], but, in fact, it is evident that self-renewal capacity of the adult human heart is unable to restore the large amount of cellular loss after MI.
Stem cell therapy with CSCs may offer some advantages over other extra-cardiac cells, because they are believed to be more related to differentiate towards cardiac lineages. The discovery of CSC has revolutionized regenerative medicine. As they are derived from the target organ, they are supposed to be more committed with a cardiac fate, and the understanding of the molecular mechanisms that steer their mode of action is providing new insights that extend application of cellbased therapies.

Procurement protocol of all this cell types are on annexes 1-3

3.2.5 HISTORICAL REVIEW OF THE REGENERATIVE MEDICINE TRHOUGTH THE YEARS

Stem cells were first used to prevent heart failure in clinical practice on 2002.[62-66] Ever since then, ischaemic heart disease (IHD) has been the most prominently evaluated disease, with more than 90 clinical trials carried out in the settings of acute myocardial infarction and chronic ischaemic infarction with heart failure, respectively.[67]

Ilustration 4: Summary of randomized clinical trials in cardiovascular diseases with regenerative products

Disease (patients treated)	Regenerative product	Safety	Overall efficacy[a] (surrogate endpoints)
Acute myocardial infarction (*n* = 2732)	BMMNC[48–63]	Favourable	Inconsistent
	BM-MSC[64]	Favourable	Inconsistent
	Specific BM cells[65–69]	Favourable	Inconsistent
	ADSC[70]	Favourable	Inconsistent
	CDC[71]	Favourable	Positive
	Growth factors[72–77]	Favourable	Inconsistent
Ischaemic heart failure (*n* = 2035)	SM[78–81]	Favourable[b]	Inconsistent
	BMMNC[82–85]	Favourable	Inconsistent
	BM-MSC[86–88]	Favourable	Positive
	Specific BM cells[89–96]	Favourable	Positive
	CSC[97]	Favourable	Positive
	Gene therapy[37,98–101]	Favourable	Inconsistent
Refractory angina (*n* = 353)	BMMNC[102–106]	Favourable	Positive
	Specific BM cells[107–109]	Favourable	Positive
	ADSC[110]	Favourable	Positive
Non-ischaemic heart failure (*n* = 166)	BMMNC[111,112]	Favourable	Inconsistent
	Specific BM cells[113,114]	Favourable	Inconsistent
	BM-MSC[115]	Favourable	Inconsistent
Peripheral artery disease (*n* = 1217)	BMMNC[116]	Favourable	Positive
	Specific BM cells[117–119]	Favourable	Positive
	Gene therapy[120–124]	Favourable	Inconsistent
Stroke (*n* = 95)	Neural stem cells[125]	Favourable	Inconsistent
	BMMNC[125]	Favourable	Inconsistent
	Specific BM cells[125]	Favourable	Inconsistent

Figure 4 is an extended summary of all types of different products that have been used for different pathologies with the aim of cardiac functionality regeneration. It includes 4 columns that represent: pathologies in which products have been applied and number of patients included in all tests carried out, the amount and specifications of products used in each group, safety profiles achieved in each case and effectiveness they have shown in all different trials.
As can be seen on the table, each and every one of products studied have a very high safety profile, however, efficiency in most of them has been inconsistent, so the search for the optimal product has not ended yet.[9]

As previously mentioned, first cellular product with which it was experimented were myoblastic cells. Their in vitro results were acceptable, but once it was demonstrated that this cell type could only differenciate into myocytes, they were discarded as a final product.

Although final results of therapy with myoblasts were not the best, they triggered all research groups into the searching of stem cells in different human body locations.

BONE MARROW DERIVED MONONUCLEAR STEM CELLS (BMMNC):

The myoblasts gave way to a new cell type, the BMMNCs. At that time, scientific community was so dazed by the new discovery that trials began to occur in an uncontrolled manner and without any kind of consensus among all different research groups.

This is how in several trials preclinical and clinical phases were produced in parallel.

The first step with bone marrow stem cells in our research team dates on 2004,[68] when a trial with two arms (preclinical and clinical) took place. The point was to:

-Seed Human BMCs (previously labeled) on top of cryoinjured mice heart slices, and cultured.

-Intracoronarily transplant Human Autologous BMMNCs in patients with acute heart infarction.

On that time, BMMNCs had proved to improve myogenesis, angiogenesis, function and surveillance in some trials with animals, so the point was to ensure safety and trying to figure out the pathofisiology in the regeneration procedure.

The results on preclinical trial were:

- (1) Observations that cardiac endogenous stem cells present in the normal myocardium and involved in the maintenance of the cardiac cellular homeostasis are also able to expand and regenerate myocytes and microvasculature in the infarcted myocardium.
- (2) Evidence that cardiomyocyte repopulation by extracardiac progenitors of hematopoietic origin can take place in the human.
- (3) Demonstration that it is possible to increase the efficiency of the intrinsic cardiac regeneration capacity in animals with acute MI by both local delivery of BMCs and bone marrow mobilization with cytokines, resulting in a reduction of infarct size and clear improvement in LV performance and survival.

It was proved that stem cells had the quimiotactic power to migrate to the injured zones trying to regenerate scared tissue.

Clinical trial results were encouraging too, with an improvement of the end-systolic volume and the ejection fraction. Regional contractility improved, as demonstrated by a significant decrease of the number of abnormal ventricular segments and a better wall motion score index of the infarcted wall.

Alongside with our group of research more spanish groups have been trying to find the best regenerative products and testing their safety and efficacy. A mention to make is on the job of Suárez de Lezo's team which was the first to compare in a trial different regenerative alternatives.[69]

- First group were treated with autologous mononuclear cells.
- The second group was treated with growth stimulant factors (G-CSF).
- The final group was the control, treated with standarised methods for IAM

The results were promising with a 20% increase on the EF was observed in group I, on the other hand only a 4% increase was observed in the second and a 6% increase on the third one.

Considering all the information that we had on that moment, initial reports in human beings were encouraging and established the safety of these therapies. But their efficacy had been subject of continuous debate, since robust evidence is lacking due to inconsistency in benefits observed in clinical trials.

For this reason a new bigger project was presented and carried out comparing different products. The TECAM study was a comparison, first time done, between the efficacy of BMMC injection, G-CSF mobilization, and the combination of both with standard treatment.

TECAM was a multicenter study that observed the evolution of 64 patients treated between 2002 and 2005 with bone marrow stem cells and a follow up of 58 months.

A total of 120 patients were randomized in groups of 30 patients each into BMMNC injection, G-CSF infusion, a combination of G-CSF+BMMNC and standard therapy.[70]

In the Gregorio Marañón Hospital the follow up of their 29 patients included a RM and heart functional proves, heart contractibility, volumes, mass and ejection fraction was analiced. Clinical, laboratory and angiograms were also performed on al patients.

A significantly improvement on the ejection fraction was observed and mantined on the 58 months later. On the other hand the heart volumes and the contractibility suffered no variation.[71]

But, in conclusion, no statistical significant difference in LVEF and cardiac volumes between treated and control groups were found.[72]

The results of the TECAM study were not as expected, but research community was far away of giving up.

More studies were published at the same time with equal results:

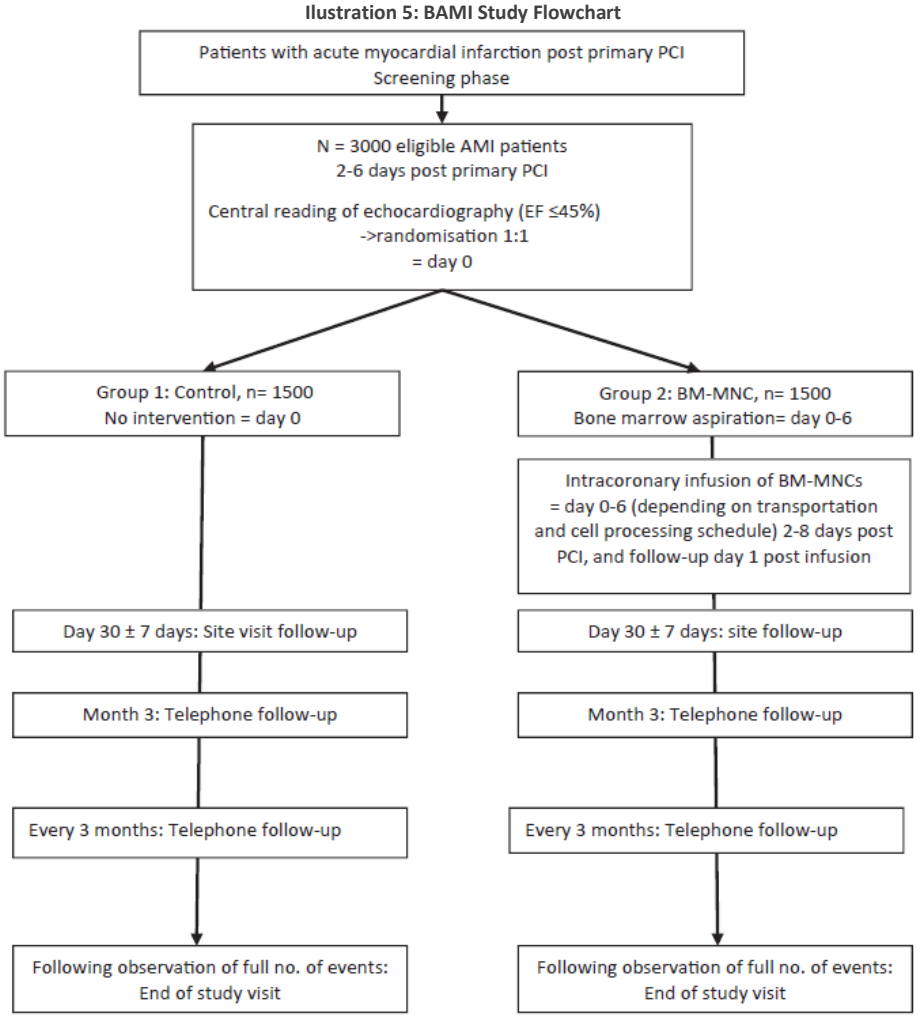
- BOOST1 (Bone marrow transfer to enhance ST-elevation infarct regeneration) showed a significally improvement on the FEVI with RM in patients treated 6 months later to the treatment. But within 18 months the differences disappeared.[73-74]
- REPAIR-AMI5 (Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study) within a follow up of 12 months with RM, it was observed an improvement on the ventricular function and the heart remodelate but only in patients with low basal FEVI.[75]
- ASTAMI7 (Autologous Stem cell Transplantation in Acute Myocardial Infarction) a 3 year follow up showed no difference between groups.[76]
- Cao et al8 study, with a 4 years followe up with ECO, showed asignifical improvement of FEVI and heart remodelating parameters.[77]

As aforementioned, different research groups did not follow any type of quality control terms or standardized procedures. In order to control and asses terms on clinical trials concerning regenerative medicine in 2012 the meeting[78] of several European groups involved in research led to the largest clinical trial to date. The first phase III clinical trial, the BAMI trial.[79]

BAMI was designed as the first Phase III controlled clinical trial with autologous bone-marrow-derived stem cell injection as part of standard treatment for AMI with the aim to finish recruitment by October 2017. The trial was led by academia, funded by the European Commission and had a 2-year follow-up with a mortality endpoint that compared current best practice to best practice and autologous cell injection.[80]

The objective of the trial is simple with a single primary endpoint aiming to detect a 25% reduction in all-cause mortality with BM-MNC therapy. The results of the BAMI trial are highly anticipated, as the field of regenerative therapy will finally have an appropriately sized trial to provide an answer for the most important clinical outcome of all.

Ilustration 5: BAMI Study Flowchart



BAMI trial has been designed as a clinical trial fase III with a sample syze of 3000 patients. A control group of 1500 patients and an experimental group of 1500 aptients will take part on the study with a follow-up of 36 months.[85]

BAMI trial is nowadays still ongoing and their results have not been published yet.

MESENCHYMAL STEM CELLS:

Mesenchymal stem cells derived from bone marrow have also been used in some trials, but their represenation on the field is way lower tan BMMNCs.

As an example on the applicatin of these type of cells we have Chen team work date don 2004.[81] In this study, sixty-nine patients who underwent primary percutaneous coronary intervention within 12 hours after onset of acute myocardial infarction were randomized to receive intracoronary injection of autologous bone marrow mesenchymal stem cell or standard saline. Several imagining techniques demonstrated that bone marrow mesenchymal stem cells significantly improved left ventricular function.

An spanish group is also actually working with this type of cells. Ricardo Sanz-Ruiz team is working on a project called MYOCITE. It is a randomized, double blind and placebo-controlled trial that will enroll 70 patients with Idiopatic Dilated Cardiomyopathy. In a first pilot phase, 10 patients will receive 15 transendocardial injections in the anterior wall of left ventricle. In a second phase, 60 patients will be randomized in a 3:1 ratio to receive MSC or placebo.[82]

So far, six patients have been included by now with no MACE, adverse and arrhythmic events or procedure related complications.

CARDIO DELIVERED MESENCHYMAL BONE MARROW CELLS:

Only a few trials have studied so far mesenchymal stem cell results on myocardal ischaemic disease, and most of them delivered cells intracoronarly. CHART-1 was one of the first studies to test endomyocardiac delivery.

CHART-1 study was a prospective, multicentre, randomized, shamcontrolled, patient- and evaluator-blinded clinical trial. Investigators at 39 centres in Europe and Israel participated.[83]

Patients with symptomatic ischaemic heart failure on guideline-directed therapy (n= 484) were screened; n = 348 underwent bone marrow harvest and mesenchymal stem cell expansion.

The primary endpoint was neutral, with safety demonstrated across the cohort, but using markers of heart failure severity, the CHART-1 trial identified a clinically relevant patient population characterized by severe heart enlargement (LVEDV 200–370mL) that appeared to derive consistent benefit from cardiopoietic cell treatment as regards the primary endpoint.

This results were atonishing, for the first time a trial was able to locate a targered group of patients that could have a major percentatge of benefit with regenerative therapies.

ADIPOSE TISSUE-DERIVED CELLS:

In 2009 a new source of stem cells was discovered and seemed to have some advantages in front of bone marrow ones. Assessed by Bai X. team trial in mices,[84] adipose tissue-derived stem cells proved their safety.

On this study myocardial infarction was experimentally induced in severe combined immunodeficient mice and they were treated with Stromal Vascular Factor (SVF) and human adipose tissue-derived mesenchymal cells (ADSCs) or phosphate-buffered saline.

The results were a significantly improvement on myocardial function in mice treated with ADSCs or SVF 4 weeks after the infarction. Immunofluorescence revealed that grafted ADSCs and SVFs underwent cardiomyogenic differentiation pathway. Human adipose tissue-derived stem cells survived in injured hearts up to 4 months, as detected by luciferase-based bioluminescence imaging. Vascular density was significantly increased, and fewer apoptotic cells were present in the peri-infarct region of cell-injected mice.

This report demonstrates that freshly isolated adipose tissue-derived stem cells (ASCs) transplanted into ischaemic hearts after acute myocardial infarction promote cardiac function as well as do cultured ASCs. Secondly, it indicates that transplanted cells could act through differentiation and paracrine effects and are able functionally to engraft and proliferate in the infarcted heart.

Finally, it demonstrates that injected cultured ASCs can survive in injured hearts up to 4 months after myocardial infarction without migrating to other organs. With this results the clinical trials were next step.

Gregorio Marañón Hospital in colaboration with the Thoraxcenter (Erasmus MC, Rotterdam) designed a clinical trial with adipose derived cells, the PRECISE.[85]

The PRECISE was the first-in-man experience with ADSCs in patients with advanced coronary artery disease. A number of 36 patients were divided in 3 different cohorts of 12 patients each. In each one 3 patients will be infused with placebo while the rest will be infused with different ADSCs concentrations ($0'4 \cdot 10^6$ on the first cohort, $0'8 \cdot 10^6$ on the second and $1'2 \cdot 10^6$ on the third). A posterior follow-up of 36 months were carried out with clinical, imaging and laboratory assesments.[86]

In 2013, PRECISE trial conclusions and results were presented. It was the first randomized, placebo-controlled, double-blind trial to examine the safety and feasibility of the transendocardial injections of ADSCs in no-option patients with ischemic cardiomyopathy.

Procedural, postoperative, and follow-up safety end points were monitored up to 36 months. Liposuction was well tolerated, ADSCs were successfully prepared, and transendocardial injections were feasible in all patients. No malignant arrhythmias were found. The ADSC-treated patients showed significant improvements in total left

ventricular mass by magnetic resonance imaging and wall motion score index. Singlephoton emission computed tomography results suggested a reduction in inducible ischemia in ADRC-treated patients up to 18 months.

Isolation and transendocardial injection of autologous ADRCs in no-option patients were safe. The results suggested that ADRCs may preserve ventricular function, myocardial perfusion, and exercise capacity in these patients.[87]

Studies are still being carried out nowadays with adipose tissue derived cells with new techniques. As a representative example we count with the AGTP-II trial. In this study their team used the adipose graft transposition procedure (AGTP), a dissection of a vascularised flap of autologous pericardial adipose tissue is taken and positioned over the myocardial scarred area.

It will be an investigator initiated, prospective, randomised, controlled, multicentre study to assess the efficacy of the ADSCs in 108 patients with non-revascularisable MI. Patients will be assigned to standard clinical practice or the AGTP and results are being expected for next year.[88]

CARDIAC STEM CELLS (CDCs):

It is now accepted that the most important documented source of regenerating cardiac myocites is a small population of cells distributed throughout the atria and ventricles (i.e., the "cardiac stem cell niche"), that show stem cell characteristics: they are self-renewing, clonogenic and multipotent, and are able to differentiate into the three cardiogenic cell lineages of the normal adult heart.

After the first report of these cells, at least eight cell populations have been identified according to different membrane markers and transcription factors. Due to evident overlap of markers used for the characterization, it has been hypothesized that these populations may represent different phenotypic variations of only one multi-potent resident cardiac stem cell, with the capacity to differentiate in vitro and in vivo into cardiac myocites, smooth muscle cells and endothelial cells. [89]

In the field of preclinical and clinical research, resident cardiac stem cells have been generically grouped under three denominations: cardiac stem cells (CSC), cardiospheres (CS) and cardiosphere derived cells (CDC). CSC (also "cardiac progenitor cells") refer to all cardiac progenitors that are isolated from heart biopsies, are about 12–15 mm in diameter and are positive for stem cell markers (such as C-kit and Sca-1) and cardiac markers (such as Isl1, NKX2.5 and GATA4). Human CSC can migrate out of in vitro cultured human myocardial biopsies and form spheroids in suspension conditions.

Those spherical clusters are termed CS (50–200 mm in size, thus precluding their administration through the intracoronary route), and can be subsequently dissociated to obtain CDC (on average 20 mm in diameter). Interestingly, in these CS, C-kit+cells are localized in the center of the spheroids. Only these cells in the center are maintained in an undifferentiated state, whereas the cells at the surface layer are continuously undergoing differentiation. CDC have shown superior cardiomyogenic/vasculogenic differentiation and paracrine potential compared with bone marrow-derived, mesenchymal and adipose-derived cells in mice.

Cardiac stem cells offer several advantages over the other products presented so far where almost all of them have to be administered from the same patient undergoing the procedure (autologous source) while cardiac stem cells could be obtained from healthy and younger patients.

The theoretical advantages over autologous sources are many:

- Cardiac progenitor cells have a robust safety profile and have been reported to promote the regeneration of the cardiac tissue, improve cardiac function, and reduce the adverse remodeling process.
- Can be strictly quality-controlled and manufactured in large quantities to be immediately available as an off-the-shelf product for urgent applications, including MI.
- Allogeneic CSCs seem to activate the endogenous regenerative cardiac process (ie, the recruitment of resident CSC to the border zone of the infarction) by secreting a variety of growth factors and cytokines.
- They have a distinctive allogeneic immune behavior, inducing a PD-L1–dependent T cell immunomodulation and anti-inflammatory natural killer cell activity, that is likely to contribute to repair inflamed myocardium.
- Induce paracrine-mediated protection of cardiac myocytes and other cardiac cells at risk of dying during the recovery from ischemia.
- Allogeneic CSCs are small (on average 12.5 μm in diameter), allowing safe intracoronary infusion early after MI without ischemic/thrombotic complications or epicardial flow disturbances.
- These cells induced a low immune response, which did not trigger acute rejection of CSC after intracoronary administration. Thereafter, a more slowly developing host immune response eliminates nonself cells at a time when they have mediated their reparative function, thus, avoiding eventual side effects (ie, tumorigenesis).

There are already many trials studying this type of cells, their safety and posible use as a beneficial therapy in infarction patients. Some of them are still on going:

- SCIPIO and the CADUCEUS trial showed the safety of the procedures and had moreover some interesting beneficial results.[90-92]
- ALCADIA was another trial base don the previous trials results that addressed the safety and efficacy of the intramyocardial delivery of autologous CSC together with a controlled release formulation of basic fibroblast growth factor (bFGF), in the form of a biodegradable gelatin hydrogel sheet. The results have not been published yet.[93]
- Several clinical trials with allogeneic CSC are on their way: ALLSTAR[94], DYNAMIC, SCORT.

As an example, a mention to make is the CAREMI trial. It was one of the firsts trials designed to test the safety of the allogeneic cells in order to show the advantages over autologous sources and results have been published recently. [95]

CAREMI is a phase I/II multicenter, randomized, double-blind, placebo-controlled trial in patients with ST-segment-elevation myocardial infarction, left ventricular ejection fraction ≤45%, and infarct size ≥25% of left ventricular mass by cardiac magnetic resonance, who were randomized (2:1) to receive AlloCSC-01 or placebo through the intracoronary route at days 5 to 7.[96]

Forty-nine patients were included (92% male, 55±11 years), 33 randomized to AlloCSC-01 and 16 to placebo. No deaths or major adverse cardiac events were reported at 12 months. One severe adverse events in each group was considered possibly related to study treatment (allergic dermatitis and rash). AlloCSC-01 elicited low levels of donor-specific antibodies in 2 patients. No immune-related adverse events were found, and no differences between groups were observed in magnetic resonance–based efficacy parameters at 12 months. The estimated treatment effect of AlloCSC-01 on the absolute change from baseline in infarct size was −2.3% (95% confidence interval, −6.5% to 1.9%).

Efectiveness has not been so high but, AlloCSC-01 can be safely administered in ST-segment–elevation myocardial infarction patients with left ventricular dysfunction early after revascularization. So, in conclusión, low immunogenicity and absence of immune-mediated events will facilitate adequately powered studies to demonstrate their clinical efficacy in this setting.

Finally, after all this brief introduction, our protocol will be aimed to a non explored field with CSC products, chronic ischaemic patients.

A BRIGTH FUTURE:

A new generation of stem cells are under testing nowadays and lots of different new products and cell sources has been discovered.[97]

Genetic and pharmacological priming together with the discovery of new sources of cells led to a "second generation" of cell products that holds an encouraging promise in cardiovascular regenerative medicine.[98]

Since the first clinical trial in 1998, more than 200 cardiovascular gene therapy studies have set the principles for an evidence-based modification of gene expression to improve angiogenesis, protect the myocardium or regenerate dead tissue.

Successful delivery of genetic material to the myocardium is paramount to achieve therapeutic efficacy and can be done using viral and non-viral vectors.

Chung and collegues reported the results of a double-blind phase II clinical trial to assess the safety and efficacy of plasmid stromal cell-derived factor-1 (pSDF-1) in patients with CHF. Ninety-three patients with end-stage IHD were randomized 1:1:1 to receive transendocardial injections of 15 mg or 30 mg of pSDF-1 or placebo. The primary endpoint was a composite of 6-min walk distance and quality of life [Minnesota Living with Heart Failure Questionnaire (MLWHFQ)] at 4 months, which was safely improved in the high-dose group. This clinical benefit was extended to 12-months follow-up and was accompanied by improvements in ventricular remodelling, especially in those patients with the most severe ventricular dysfunction.[99]

The results of the STOP-HF trial suggest that a single dose of pSDF-1 is enough to exert a beneficial effect on left ventricular performance in advanced stages of IHD.

Alongside with it, genome editing on human pluripotent stem cells (hPSCs) together with the development protocols for organ decellularization opened the door to the generation of autologous bioartificial hearts.

Decellularization of a whole heart can lead to hundreds of acellular slices ready to use as scaffolds that allows the generation of cardiac grafts showing enhanced electrophysiological properties in a relatively short time period (24 days), avoiding time consuming coculture techniques (i.e: bioreactor, perfusion system, among others), and anticipating that such procedure can be immediately applied in laboratories with special focus in heart bioengineering and cardiac disease modeling.[100]

Ilustration 6: "Road-map" of investigated stem cell types

Stem cell type	Tissue of origin/cell subtype	Phase of clinical research	Overall results (safety/efficacy)
First generation			
Skeletal myoblasts	Skeletal muscle	II	Arrhythmogenic issues/positive results
Bone marrow mononuclear fraction	Bone marrow	III	Safe/divergent results
Specific bone marrow-derived cells	$CD34^+$	III	Safe/positive results
	$CD133^+$	II	Safe/positive results
Circulating peripheral blood cells	Endothelial progenitor and bone marrow-derived cells	II	Safe/divergent results
Adipose-derived stem cells	Adipose tissue (stromal vascular fraction)	II	Safe/positive results
Mesenchymal stem cells	Bone marrow	III	Safe/positive results in phase II (phase III ongoing)
	Adipose tissue	II	Safe/positive results
	Cord blood	II	Safe/divergent results
	Dental pulp	II	Safe/divergent results
Epicardial stem cells	Developing heart	NA	NA
Second generation			
Cardiac stem cells	Cardiac biopsies	II	Safe/positive results
Cardiosphere-derived cells	Cardiac biopsies	II	Safe/positive results
Embryonic stem cells	Blastocysts	NA	NA
Induced pluripotent stem cells	Adult somatic tissues	NA	NA
Third generation			
Phenotypically modified stem cells	Cardiopoietic mesenchymal stem cells	III	Safe/positive results in certain subpopulations
Allogeneic stem cells	Mesenchymal stem cells	III	Safe/positive results in phase II (phase III ongoing)
Pluripotent cell-derived stem cells	Cardiac stem cells	II	Safe/positive results
	Embryonic-derived $Isl1^+/CD15^+$ progenitor cells	I	No results available yet

This table represents different “generations” of products. It has to be commented that different generations differ depending on the autor, so we are calling “second generation” to genetic, pharmacological and paracrine effect products while in this table they are third one. But, in conclusión, it reflects the evolution of stem cell products.

While the new generation is going forward cells studies are still being carried with the final aim of repairing heart with cell products of cardiac lineage or with cardiomyogenicpotential:

- C-CURE and CHART studied study implantation of BM-MSC. And now the CHART-II is on its way.[101-103]
- CONCERT-HF is studying the combination of CSC and BM-MSC. [104-105]
- Cell-free therapies: the identification of the growth factors andcytokines secreted by transplanted CSC that activate, expand anddifferentiate endogenous CSC should make possible the designof specific therapies based on those principal effector molecules. This CPC-derived secretome and exosomes migth be the future of regeneration therapies or maybe a combination between them and cell products.[106]
- We are still searching for the best administration dose, and secuencial administrations seem to have a more powerful beneficial effect with no side effects.[107-108]

THE NEED OF STANDARIZATION:

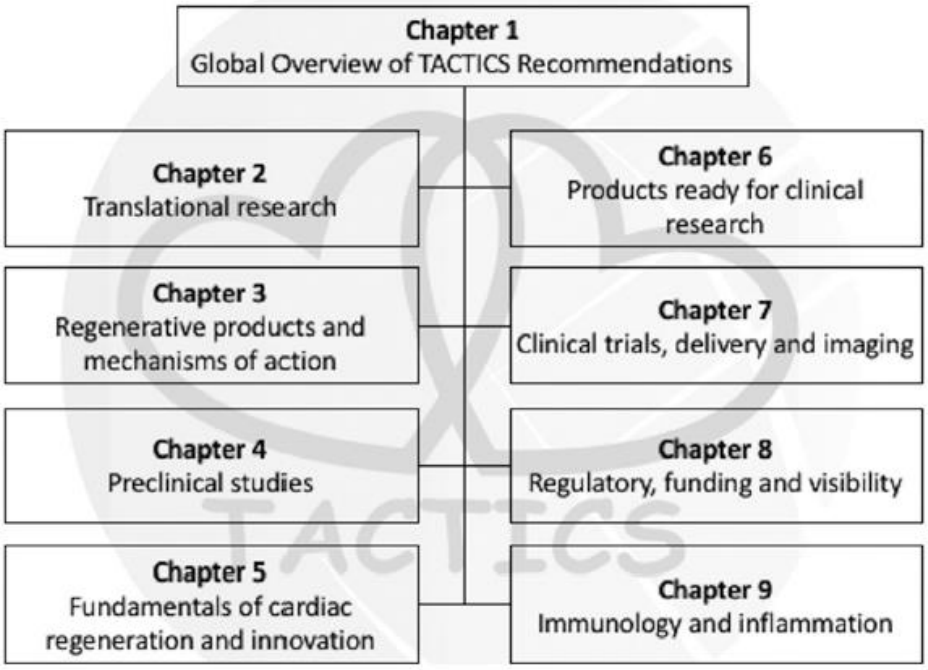
If we are discussing about cell therapy, we have to mention the TACTICS foundation. TACTICS is a transnacional Alliance between european, asian and american (North and South) different medical investigation groups which was founded in 2015 to promote the regenerative therapies as a technique able to avoid the limitations of the clinical practice to deal with some cardiovascular syndromes.

The most prestigious clinical and basic researchers in the field are working coordinately to boost the advances in cardiovascular regenerative therapies.[109]

Every year a congress is carried on with the aim of uniting and presenting all new product changes that have been discovered over the year. It's aim is also trying to unify and reach a consess between different groups standarizacing procedures in order to compare results.

Finally, this year, TACTICS have published an essay with their latest recommendations and challenges for the field. The main objective of this series of articles is to describe and reflect on the priorities of cardiovascular regenerative research from a critical point of view, promoting an open discussion about the lights and shadows of the field, increasing its credibility and facilitating the advance of CRM to definitely repair the failing cardiovascular system.[110]

Ilustration 7: TACTICS schematic diagram of all research chapters



This is the diagram presente don the last TACTICS essay and their latest recommendations.[110]

4. JUSTIFICATION:

The choice and elaboration of this protocol have been inspired by two main reasons:

The first one are the numerous benefits that an effective treatment could provide to the large number of patients suffering from this disease. We have already been able to verify all the consequences of suffering from this pathology and the need to find an effective and curative treatment is evident.

Myocardial ischaemic disease is still a huge problem worldwide. It's morbility and mortality is so high and keeps increasing with illness time.

High prevalence of this disease makes it an special case, not only for personal, but also social and economic impact that it represents.

The degrees of disability and support that these patients need are very variable but, in the long run, a large part will end up developing heart failure and possible death.

It is not only mortality rates that frightens, but also the morbidity ones. In many cases, a myocardial infarction is associated with a certain degree of disability, patient life will no longer be the same, many activities will be restricted and, in some cases, he will even need help to carry out daily basic activities.

The economic impact for society it's also important. In a clinical trial performed on the Hospital Universitario Marqués de Valdecilla (Santander, Cantabria) a group of 584 patients younger than 65 years old were studied in a period of four years.[111]

For those who later went back to work, the duration of time off work after the IAM was a function of the age and the labor sector, so that we can simplify affirming that the number of days of discharge are the result of multiplying the factor 3.15 by age in years, 54.6 more days should be added if the patient work in the field or industry. If we apply this formula in a patient of 50 years old we get the amount of 472'5 days of discharge that in some cases could result on the dismissal of the patients casue only 56.6% of patients who worked before the IAM rejoined their activity.

In second place, there are personal motivations. The field of research is a fundamental and necessary part of medicine. Everything we know and use in our daily practice has been part of a research project, from the physical examination to every pill we prescribe. Therefore, the motivation to participate in a research group, in a medical specialty of my liking and the oportunity of learning from such qualified professionals has led me to choose the Cell Production Unit of Gregorio Marañón as my destiny for developing my TFG. Thanks to them and to the cooperation of the Girona's University, the elaboration of this protocol has been possible.

So, if we rely on this reasons, the elaboration of a fase II clinical trial which aim is to evaluate safety and efectiveness of ***CSC product II*** administration seems to be the answer.

5. FOUNDATIONS AND HYPOTHESIS:

During the last decades a new approach to myocardial scar tissue has emerged as a promising therapy wich aim is to restore cardiac tissue functionality once an aggresion has been established.

Lots of products have been tried on the field with promising results. In research for new and more efficient cellular regenerative products, interesting cardioprotective, immunoregulatory, and cardioregenerative properties have been demonstrated for human cardiac stem cells. Moreover, allogeneic cells show several advantages over autologous sources: they can be produced in large quantities, easily administered off-the-shelf early after an acute myocardial infarction, comply with stringent criteria for product homogeneity, potency, and quality control, and may exhibit a distinctive immunologic behavior. So, for all this reasons CSC are the product I have chosen for this protocol.

SCACIHI pretensions are to evaluate safety and efectiveness of the ***CSC Product 2*** assesed by intramyocardial administration (NOGA). The specific protocol hypotheses are:

· **Hipótesis 1:** Cardiac resident stem cells are benefficial in chronic myocardial infarction.

· **Hipótesis 2:** ***CSC Product 2*** use is safe.

· **Hipótesis 3:** Find a targered population for the treatment and the perfect dose in order to achieve the utopical background with the aim for the treatment to be the most benneficial.

·**Hipótesis 4:** ***CSC Product 2*** administration produces positive effects in terms of:

- Better regional contractibility
- Increased LVEF
- Less remodelling (assessed by MRI)
- Lower levels of biomarkers (NT-proBNP and high sensitive troponin T)

6. OBJECTIVES OF THE STUDY AND END POINTS:

Our protocol is a fase II clinical trial which aim is to evaluate safety and effectiveness of ***CSC Product 2*** administration. In SCACIHI pacients will be included in a randomized, double-blind way in order to analize:

·**PRIMARY OBJECTIVE:** asses safety of the treatment with a long follow-up of all patients. This follow-up will include clinical, analytical and imaging tests that will ensure adverse effects detection.

·**SECONDARY OBJECTIVES:** prove efficacy and find a population group of patients that are going to have optimal benefits from this treatment. For this purpose we need to find the perfect treatment dose.

·**END POINTS:**

-PRIMARY END POINTS:

1. All-cause death at 30 days.
2. Other safety events:
   a. In the dose-escalation phase: all-cause adverse events from MRI acquisition (patient inclusion) until 7 days after Investigational medical product (IMP) administration.
   b. In the randomized phase: MACE (major adverse cardiac events) during the first 30 days after IMP administration, defined as all-cause death, reinfarction, hospitalization because of HF, sustained ventricular tachycardia, ventricular fibrillation, and stroke.

-SECONDARY END POINTS:

1. Safety follow-up:

   a. In the dose-escalation phase:
   — All-cause adverse event during the first 30 days after cell administration.
   — MACE at 6 and 12 months after treatment.
   — All-cause death during the clinical trial (12 months).
   — Cardiovascular death during the clinical trial (12 months).
   — General monitoring of all-cause adverse event (monthly for the first 6 months, quarterly thereafter).

   b. In the randomized phase:
   — MACE at 6 and 12 months after treatment.
   — All cause death during the clinical trial (12 months).
   — Cardiovascular death during the clinical trial (monthly for the first 6 months, quarterly thereafter).
   — General monitoring of all-cause adverse event (monthly for the first 6 months, quarterly thereafter).

2. Efficacy assessments (for both phases):

    a. MRI analysis. The following parameters are compared between the placebo and CSC-treated groups: evolution of infarct size (as percentage of left ventricular mass and the changes at 6 and 12 months versus screening), evolution of biomechanical parameters (end-systolic and end-diastolic volumes indexed by body surface and percentage of changes in both volumes at 6 and 12 months versus screening), wall motion score (normal/hypokinesia/akinesia/dyskinesia in a 16-segment model), sphericity index, systolic thickening by segment, absolute change of LVEF, and evolution of edema (percentage of change in the edema volume at 1 month versus screening, being this MRI at 1 month optional).

    b. Clinical and laboratory parameters, including: NT-proBNP (N-terminal probrain natriuretic peptide) or BNP curve (before treatment and at 6 and 12 months after treatment), C-reactive protein (before treatment, at discharge, at 7 days, and 1 month after treatment), the 6-minute walking test (at discharge and at 6 and 12 months after treatment), the New York Heart Association class (at discharge and at 3, 6, and 12 months after treatment), and the Minnesota Living with Heart Failure Questionnaire (at discharge and at 6 and 12 months after treatment).

3. Immunologic surveillance (for both phases): because allogeneic CSC are administered, cellular and humoral immunologic parameters are also to be evaluated for safety and efficacy (immunomodulatory) purposes. These include HLA typing of both donor cells and recipient, cross-matching between cells/patient serum, screening and characterization of anti-HLA class I and class II antibodies pre- and post-treatment, and cytokine profiling in blood samples.

7. MATERIAL AND METHODS:

7.1 STUDY DESIGN:

This study has been designed as a prospective, double-blind, 2:1 randomized, controlled, and multicenter clinical trial that will evaluate the safety, feasibility, and efficacy of intracoronary delivery of allogeneic human cardiac stem cell in 150 patients with chronic myocardial infarction.

7.1.1 RANDOMIZATION PHASE:

A sample of aproximately 150 patients will be taken and divided in two groups, the first one will receive the cell injection and the second one will recieve a saline solute with 5% Human Albumin Serum (HSA) solution injection.

Randomization will be performed with a 2:1 formula in favor of the treated group.

7.2 STUDY DISCUSSION:

7.2.1 CONTROL TREATMENT SELECTION:

Patients in control and experimental groups will undergo same procedures in order to avoid placebo effect. The difference between those groups will be that control group patients will not get cell infusion during cateterism procedures, they will get injected with salines with 5% HSA.

Both groups will continue wit their usual medication. In order to the most recent guides chronic infarction patients are treated with medical support as betabloquers, ca-antagonist, diuretics, AINES, Aldosteron antagonists, digoxine… in order help them to control symptoms of hearth failure.

7.2.2 STUDY DURATION:

This study will be a long term prospective study with a follow-up of patients in order to asses safety and possible efficacy of our treatment.

Enrollment is anticipated to be completed 18 months after the first patient will be treated. SCACIHI complete duration is stimated to be two and a half years.

7.2.3: RISK-BENEFIT ANALYSIS:

Lots of studies have assesed safety of cardiac stem cells in patients with acute miocardial infarction and it’s possible benefits. Patients with chronic heart infarction trend is to evolve into heart failure and possible death. This treatment could be benneficial in this type of patients in which medical care is not able to stop the evolution of the disease.

The aim of the treatment is trying to restore scared tissue functionality and improve heart parameters in order to stop or at least slow down heart deterioration.

7.3 STUDY POPULATION:

Spain is a country with aproximatetly 46 million people in which life expenctancy at birth is 80(male)/86(female) years old.[112]

Myocardial ischaemic disease is a leading cause of mortality, morbidity, and health care cost in Spain. In 2013 there were 115 752 stimated cases of myocadial infarction (95% confidence interval, 114 822-116 687). Within 28 days, 39 086 of these patients died and 85 326 were hospitalized.[113]

7.3.1 PATIENT POPULATION:

Men or women aged ≥18 years, with a settled cardiac scar that is anatomically non-amenable to surgical revascularisation will be included and randomised after they agree to participate and provide signed informed consent.

7.3.2 SAMPLE SIZE:

We took left ventricular ejection fraction as the variable with which we were gonna stimate our sample size.
Assuming an Alpha risk of 5%, with 80% statistical power and calculating a drop out of 10% the sample size calculated was 149 patients.

7.3.3 INCLUSION/EXCLUSION CRITERIA:

Inclusion criteria include every patients aged >18 years with any of the following criteria that meets the diagnosis for chronic myocardial infarction:

- Development of new pathological Q waves with or without symptoms.
- Imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract, in the absence of a non-ischaemic cause.
- Pathological findings of a healed or healing myocardial infarction.

Exclusion criteria includes severe valvular disease that could be repaired surgically, candidacy for surgical ventricular remodelling, contraindications for MRI, extracardiac disease with a vital prognosis of <1 year, severe renal or hepatic failure, previous cardiac surgery, pregnancy or breastfeeding.

7.3.4 PATIENT COMORBILITIES:

Patient comorbidites present before myocardial infarction are factors that we should keep in mind when recolecting and analyzing patients results on our tests. They represent posible risk factors for treatment failure and, generally, they represent this five entities:

-Diabetes mellitus: Diabetes is a major risk factorin western europe for myocardial infarction. The prevalence of diabetes mellitus (DM) is on the rise as a direct consequence of the progressive aging of the population and changes in lifestyle, which favor obesity and sedentary lifestyle. The increase in morbidity and mortality from

myocardial infarction in diabetic patients is explained by the worse baseline risk profile and higher rates of reinfartion and cardiac failure in these patients.[114]

-Obesity: Epidemiological evidence suggests that overweight and obesity have been associated with acute myocardial infarction (AMI). It is a risk factor that could be avoided and it's absence represents a high benefit fr this type of patients. [115]

-Hypertension: Both systolic and diastolic hypertension increase the risk of a myocardial infarction and the higher the pressure, the greater the risk. Even when other major risk factors are absent, the increased risk still exists. Almost 40% of patients with ischemic heart disease who die suddenly have a history of hypertension.[116]

-Age and Sex: Sex and age are two non modificable risk factors that have a high significance in morbidity and mortality of this type of patients.

Some studies have reached the conclusión that women are generally older than men at hospitalization for myocardial infarction (MI), they present less frequently with chest pain/discomfort and have higher mortality than men within the same age group, but sex differences in clinical presentation without chest pain and in mortality were attenuated with increasing age. However, few studies have taken age into account when examining sex differences in clinical presentation and mortality.

We can resume that while sex is not a determining factor, patients more aged have higher rates of morbidity and mortality.[117]

7.3.5 PATIENT SELECTION:

All patients who attend the cardiology outpatient service and who meet the inclusion and exclusion criteria of the study will be offered the option to enter SCACIHI trial.

8. TREATMENT:

8.1 RESEARCH PRODUCT:

CSC product 2 is a suspension of allogeneic human CSC in saline solution with 5% human serum albumin (final volume 18 mL). Cells are isolated from human heart biopsies (right atrial appendages), donated by patients undergoing cardiac surgery (usually valve replacement procedures) after providing informed consent, and are sent to Hospital Gregorio Marañón Manufacturing Practice facility for processing of all trial cell products.

Cell procurement methods are explained in Annex 3.

Quality control of the final product is made by Flow cytometry. The expression of cell surface markers is analyzed using the following monoclonal antibodies: purified SSEA-1 (Millipore), CD166, fluorescein isothiocyanate-conjugated CD45, CD90 (Thermo Scientific), phycoerythrin-conjugated CD11b (AbD Serotec), CD34, and CD44 (eBioscience). Indirect staining is performed with phycoerythrin-conjugated rat anti-mouse immunoglobulin G (CD166) or IgM (SSEA-1) antibodies.

8.2 PLACEBO:

All patients will undergo all phases of SCACIHI clinical trial. Treated group will be infused with cardiac stem cell solution while the control group will be infused with 5% HSA solution in order to prevent placebo effect.

Placebo effect is based on the aspect of healing that is produced, activated or enhanced by the context of the clinical encounter, as distinct from the specific efficacy of treatment interventions, is contextual healing.

Factors that may play a role in contextual healing include the environment of the clinical setting, cognitive and affective communication of clinical personnel, and the ritual of administering treatment.

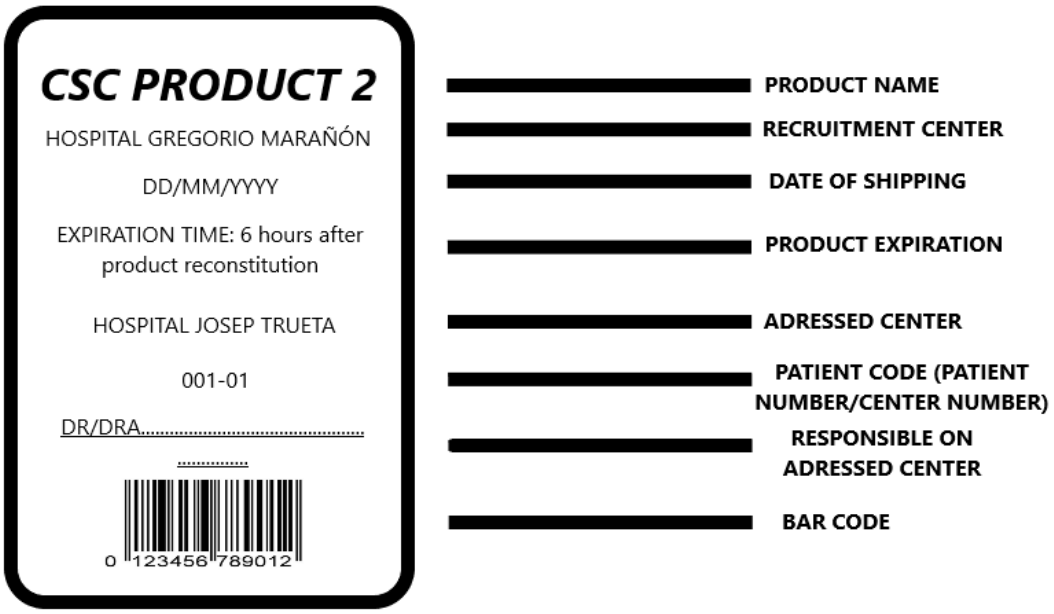
On the other hand, all patients will be treated as european society of cardiology guidelines stipulate and they will receive all medical treatments that are necessary for the condition.

Finally, if any of the patients infused primarily with placebo wish to be treated with the cellular product once the trial has demonstrated beneficial effects, the corresponding dose may be administered.

8.3 RESEARCH PRODUCT LABELING:

In order to establish the correct management of the product and avoid posible complications in delivery or administration fases all products will include the following labeling:

Ilustration 8: Product Identification Label



Every product sample will be identified with a label that will include all information on the representation

8.4 RESEARCH PRODUCT MANAGEMENT:

As this celular product can be produced on a large scale, Hospital Gregorio Marañón will be the one producing and shipping doses to all destinations.

Hospital counts with all installations necessary for the large production and has already trained technicians. In addition, one of the advantages of centralizing the product is the standarization in the obtention and delivering of this type of cells.

Cardiovascular regenerative medicine products have special characteristics that differentiate them from classic pharmacological treatments in terms of production, shipping, delivery, tracking, and assessment. The manufacturing of these advanced therapy medicinal products (ATMP) includes multiple step from the acquisition of biological samples to the delivery of a personalized product for each patient. Given the heterogeneity present in the generation of most biological cardiovascular regenerative medicine products, the process of manufacturing and delivery technology need to be considered as part of the cardiac regenerative medicine product itself.

The functionality of a cell-based product is influenced by multiple factors, including the initial source, harvesting and isolation techniques, and manufacturing. Standardization of these procedures and methods is especially important, as lack of uniformity in cell manufacturing may influence clinical outcome.

8.5 INVESTIGATION PRODUCT SHIPPING:

This medical product needs to accomplish several safety parameters in order to ensure that treatment will be properly administered and its results will be valid. During manufacturing process, ***CSC product 2*** cells have to accomplish several quality standards, regarding number of population doublings, doubling times, genomic stability (by comparative genomic hybridization), and sterility testing, which are done in each batch according to the European Medicines Agency Guidelines.

The manufacture and transfer of the product must be given under certain levels of purity passing through several phases of decontamination.

Regarding the manufacturing process, Gregorio Marañón Hospital has a specialized laboratory with four different levels of decontamination and personnel trained in the field. The finished product is stored frozen in cryopreservation containers with liquid nitrogen until the release of each cell batch for clinical use.

Images of specialized stem cell laboratory on the Gregorio Marañón Hospital

8.6 CELL DOSE:

In the initial open-label escalation phase, 6 patients will receive increasing doses of CSC: 10×10^6 in the first 2 patients, 20×10^6 in another 2, and 35×10^6 in the last 2 patients. A mandatory follow-up of 3 days after cell administration of the first patient of each specific cohort is scheduled, as well as a mandatory follow-up of 7 days between cohorts. After evaluation by the data and safety monitoring board, and if no safety/toxicity events are observed after 7 days, the second double-blind phase of the study will be initiated, in which 35×10^6 is the target dose to be used in all patients.

8.7 PRODUCT RECONSTITUTION FOR CLINICAL PURPOSE:

When a potential patient is available for the trial, the recruited center will request the pre-established dose of product to be administered. Since the product will be stored in Gregorio Marañón Hospital, the specialized personnel in this center will be in charge of preparing the necessary sample volume.

The product will be sent cryopreserved and once in the recruited center a previously trained team will proceed to reconstitute and prepare it for clinical administration.

8.8 CELULAR PRODUCT ADMINISTRATION:

Delivery methods identified use catheter systems to accurately deliver stem cells or other therapeutic substances to the myocardium. Biologics can be delivered via different routes: intracoronary (IC), intramyocardial (IM), and IV.

There are advantages and disadvantages of each route and variable retention rate of cells in the myocardium.

The main objective of delivery technologies is to achieve the optimal dosage of biological material needed to provide benefits in the region of interest of the host tissue. Although all available modalities of regenerative product delivery display the four desired characteristics (safety, ease of use, clinical utility, and low cost), after 20 years of research we can conclude the following:

Percutaneous catheter-based delivery has been the most extensively used modality for cardiac diseases. Intracoronary infusion of regenerative products has been the mainstay in the setting of acute coronary syndromes, whereas more sophisticated catheters (with or without navigation platforms) for endomyocardial delivery have been specifically used in HF and refractory angina.

The procedure will be assessed intramyocardialy with cathethers and NOGA system will assist doctors finding the scared and targered zone.

The Myostar Injection Catheter (Biosense Webster) is a multi-electrode, percutaneous catheter with a deflectable tip and injection needle designed to inject soluble agents or cells transendocardially into the myocardium. The tip of the injection catheter is equipped with a location sensor and a retractable, hollow, 27-gauge nitinol needle for fluid and/or cell delivery.

The catheter interfaces with the NOGA (Biosense Webster) 3D electromecanic cardiac mapping system for navigated local agent delivery into the myocardium. The NOGA map is a 3D reconstruction of the left ventricle from points obtained by the mapping catheter. The NOGA map discriminates between areas of MI, the border zone, and normal myocardium. With the combination of echocardiography and low voltage, one can identify the area of MI and thin wall.

8.9 TREATMENT ASSIGNMENT:

In the first phase, all patients will receive CSC. Once on the randomization phase assignment to placebo or CSC is to be performed by an automated interactive voice-response system in blocks of 3 (2:1 for CSC and placebo, respectively). The designated storage/reconstitution service team is aware of the assignment of the patients throughout the study. The presence of cells in the final product will be masked for the interventional team with translucent sterile dressings in the syringe.

8.10 PREMEDICATION:

Regenerative medicine products goals are to reduce necrotic residual área extension in order to improve ventricular disfunction and coronary disease.

Chronic ischaemic patients are usually treated with farmacological support after infarction. This treatment consists in: antiaggregants, anticoagulants, beta-blockers, angiotensin-converting enzyme inhibitors, aldosterone antagonists, nitrates and calcium antagonists which final aim is to avoid complications, heart failure symptoms and posterior infarctions while myocardial insult chronifies.

This treatment assigned to this patients will be the same during this trial, and no farmacological treatment will be removed or changed unless patient cardiologist says otherway.

8.11 TREATMENT FULFILLMENT:

While entering SCACIHI clinical trial all patients agree in informed consent to fulfill all treatments assigned by the searching team or, in case of refuting, they will be discarted from the trial but continue farmacological treatment assigned before it.

8.12 VISIT SCHELUDE AND FOLLOW-UP:

Once all procedures have been performed, scheduled visits for clinical, biochemical, immunologic, and imaging follow-up will include assessments at 1 week and 1, 2, 3, 4, 5, 6, 9, and 12 months.

- Safety evaluations will be assessed at 7 days and 1, 2, 3, 4, 5, 6, 9 and 12 months post-infusion. They will include any serious adverse events, Major Adverse Cardiac Effect (MACE), death from any cause and cardiovascular death.
- Clinical parameter will be analised on: complete blood análisis including blood cell count, biochemical parameters and liver/kidney function (1, 3, 6 and 12 months), NT-proBNP (6 and 12 months), PCR (7 days and 1 month), Immunological assays (1, 3, 6 and 12 months), NYHA functional class (3, 6 and 12 months), 6-min walking test (6 and 12 months) and Quality of life (MLHFQ) (6 and 12 months).
- Magnetic ressonance will be performed in order to look for: Scar size (%) (1, 6 and 12 months), Heart functionality (LVEF, volumes, WMSI, sphericity and wall thickening) (1, 6 and 12 months) and Edema (1 month).
- Ecocardiogram and Eco-Doppler will be performed in all medical visitations.

8.13 MEASUREMENT METHODS:

-ANALYTICAL METHODS:

·Weight (kg) and Length (m):

When the two parameters are calculated we can obtain the Body Mass Index (BMI). BMI is a value derived from the mass (weight) and height of an individual that attempts to quantify the amount of tissue mass (muscle, fat, and bone) in an individual, and then categorize that person as underweight, normal weight, overweight, or obese based on that value.

WHO regards a BMI of less than 18.5 as underweight and may indicate malnutrition, an eating disorder, or other health problems, while a BMI equal to or greater than 25 is considered overweight and above 30 is considered obese.

BMI	Nutritional status
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Pre-obesity
30.0–34.9	Obesity class I
35.0–39.9	Obesity class II
Above 40	Obesity class III

WHO table for BMI measurement

·Complete blood analisis:

-Complete blood count:

·Number of red blood cells measured in millions/mm3, hematocrit (proportion between red blood cells and plasma volumes) in percentage (%), hemoglobin measured in g/dL, mean corpuscular volume in fl, mean corpuscular hemoglobin concentration in g/dL, mean corpuscular hemoglobin in pg.

·Number of leukocytes per mL, number of neutrophils per mL and %, eosinophils per mL and in %, basophils per mL and in %, lymphocytes per mL and in %, monocytes in %.

·Platelets per mL

-Glucose in blood measured in mg/dL

-Sodium in blood measured in mEq/L

-Potassium in blood measured in mEq/L

-Calcium in blood measured in mg/dL

-Liver function parameters:

·GOT/GPT and GGT measured in UI/L

·FA measured in UI/L

·Direct and Indirect Bilirubin measured in mg/dL

·Albumin measured in g/dL

-Kidney function parameters:

·Creatinin measured in mg/dL

·FG measured in ml/min

·Urea measured in mg/dL

-NT-Pro BNP measured in pg/mL

-PCR measured in mg/L

-Immunological assays

·Ecocardiogram: an ecocardiogram and an eco-doppler will be performed in all visitations because it is an inexpensive, non-invasive and easy to interpret test. Macroscopic imaging and flux changes will be measured.

·Magnetic ressonance parameters:

-Scar size: late gadolinium enhancement (LGE) using cardiac magnetic resonance (CMR) has emerged as the gold-standard technique for imaging of myocardial scar. The basic principle is inversion-recovery imaging after a 5- to 10-min delay following intravenous administration of gadolinium contrast. With appropriate settings, normal myocardium appears nulled or black, whereas nonviable regions appear bright or enhanced.

-Heart functionality parameters:

·Left Ventricular Ejection Fraction (LVEF)

·Volumes

·Wall Motion Score Index (WMSI)

·Sphericity

·Wall thickening

-Edema

-CLINICAL METHODS:

-Age: this variable will be recorded in years and will be expressed as a quantitative discrete variable.

-Gender: this variable will be presented as a qualitative dichotomous variable (male or female).

-**Toxics:** the consumption of tobacco and alcohol will be measured in cigarette packets/year for the first variabe and grams of alcohol/day for the second.

-**Serious adverse events:** defined as any untoward medical occurrence that at any dose results in death/ is life-theatening/ requires inpatient hospitalization or causes prolongation of existing hospitalation/ results in persistent or significant

disability/incapacity/ may have caused a congenital anormaly/birth defect/ requires intervention to prevent permanente impairment or damage

-MACE: is defined as a composite of nonfatal stroke, nonfatal myocardial infarction, and cardiovascular death.

-**DEATH:** description of the cause of death divided in:

-Non cardiovascular

-Cardiovascular

-NYHA functional class: provides a simple way of classifying the extent of heart failure. It classifies patients in one of four categories based on their limitations during physical activity; the limitations/symptoms are in regards to normal breathing and varying degrees in shortness of breath and or angina pain.

1. Class I - No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
2. Class II - Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
3. Class III - Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20-100 m). Comfortable only at rest.
4. Class IV - Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

-6-min walking test: is a submaximal exercise test that entails measurement of distance walked over a span of 6 minutes. The 6-minute walk distance (6 MWD) provides a measure for integrated global response of multiple cardiopulmonary and musculoskeletal systems involved in exercise.

The 6 MWT provides information regarding functional capacity, response to therapy and prognosis across a broad range of chronic cardiopulmonary conditions. Main strengths of the 6 MWT stem from its simplicity in concept and performance, low cost, ease of standardization, and acceptance by test subjects, including those who are deconditioned, elderly, or frail.

-Quality of life: is a valid instrument for measuring quality of life across patient groups and is conceptually different from health status or other causal indicators of quality of life.

A FLOW CHART RESUMES ALL METHODS ON ANNEX 5

9. RESULTS MANAGEMENT:

9.1 DATA RECOPILATION, QUALITY, GESTION AND DATA BASE CONTROL:

Data of this study will be collected in a Case Report Form. A CRF is an electronic questionnaire specifically used in clinical trial research. The CRF will contain all the data refering to each patient and all variables studied. The personnel in charge of transferring data in each cooperating center will be previously trained in the use of the questionnaire.

Research coordinator will supervise itself all data collected supported by a Clinical Research Assotiation (CRA) that will supervise and review that all the data entered is valid and do not contain errors. In case of finding an error, the case will be reported to the corresponding center in order to be corrected.

10. STADISTYCAL METHODS:

10.1 GENERAL METHODS:

After all data have been recorded, the statistical analysis will begin. This study is a Phase II clinical trial where safety and efficacy of ***CSC Product 2*** will be evaluated.

Baseline characteristics patients will be summarized. These characteristics will include age and other demographic data, cardiovascular diseases, cardiovascular risk factors, weight categories, tobacco and alcohol consumption and other data of medical relevance. All study participants who have given their consent and have met the inclusion criteria will be included in the analysis.

The allocation of events to the primary variable will be carried out by the team of researchers that forms the Independent Committee for Safety and Monitoring of Drug Efficacy. For the rest variables (mainly the efficacy data) an exploratory analysis will be carried out per protocol. The results will be recorded using the patient codes and will be sent to the statistical team.

The qualitative variables will be shown using absolute and relative frequencies. The quantitative variables are shown as mean and standard deviation (SD) or mean and interquartile range (IQR) in the case of asymmetry. In all cases, the distribution of the variables will be verified with respect to the theoretical models.

The quantitative variables that follow a normal distribution (expressed as mean and standard deviation) will be compared through the means (Test of the T-Student). When a normal distribution (Kolmogorov - Smirnov test) cannot be assumed, nonparametric methods (Mann-Whitney test U or median test) will be used. On the second case, it will be expressed as median (p50) and interquartile range (IQR p25-p75).

The qualitative variables will be compared using the chi-square test or Fisher's test. The null hypothesis of equality between two groups (pre- and post-treatment, with each patient having their own control) is rejected when p value is <0.05. A significance level of 0.05 will be used for all comparisons.

The interactions in the multivariable models will be evaluated according to the type of efficacy variable (linear, logistic or Cox regression). In the initial analysis, the covariates that do not have a homogeneous distribution among groups will be considered for inclusion in these models.

Security: safety analysis will include all patients who have received the treatment.

Intention to treat (IT): intention to treat will include all randomized patients and will be used for the analysis of efficacy results. In order to take into account incomplete data and analyze the efficacy results, longitudinal analyzes of visits will be used. Under the assumption of "missing at random", longitudinal analyzes will provide partial estimates even when data are missing and/or there are early withdrawals and deaths.

Therefore, no imputation of missing data will be required for the analysis of the efficacy data for IT.

Analysis by protocol: Patients with large deviations in the protocol or premature termination of treatment due to reasons unrelated to the study treatment will be excluded from the per-protocol analysis. Large deviations from the protocol will be defined as a failure to satisfy the inclusion or exclusion criteria, or the impossibility of applying the treatment.

The group included in analysis by protocol will be reviewed and finalized at the blind review meeting that will take place once all the data has been collected and cleaned from the database and before revealing the treatment of the study.

The results of selected patients that have not been included in the study and patients included in the study who have not received the treatment under study will be excluded from the statistical analysis. For those participants in the clinical trial who have been lost on follow-up, those patients who do not have traceability, or other reasons to interrupt it, the analysis will include all the results up to the point at which the latest data have been collected. The reason for the interruption will be recorded and summarized using the descriptive statistics (counts, percentages).

10.2 SUBGROUP ANALISIS:

The following subgroups will be analyzed:

-LVEF: Patients with big differences in left ventricular ejection fraction will be analised in subgroups in order to possibly finding an optimal patient population that would benefit from the treatment.

-Scar Size: with scar size we will also perform a subgroup analisis between patients with big differences. The aim of this subgroup analisis is the same as the previous.

10.3 SAFETY STADISTYCAL ANALISIS:

Safety assessment will be based mainly on frequency of adverse events and on the number of laboratory values that fall outside the predetermined ranges. Other safety data (electrocardiogram, vital signs, special tests, etc.) will be considered appropriate.

11. WORK PLAN AND CHRONOGRAM:

The reference points of our study are commented below, and a chronogram is presented after the explanation for easier visualization of the whole process. The following sequence of activities will be carried out by the main investigator and its team.

- **PHASE 1**. Preparation and coordination (4 months):

  ·Activity 1.1. Protocol consideration and elaboration. Once ready, the protocol will be evaluated by the Ethical Committee of Hospital Gregorio Marañón in Madrid. The objectives and covariates chosen for our study are the result of a clinical need detected in clinical practice around the world.

  ·Activity 1.2. Coordination and preparation of the research team. 10 training days will be necessary, 8 hours a day, to ensure the well performing in obtaining, proceduring, conservation and shipping of cell products. During this phase also a pilot study will be carried out to check that methods are perfectly understood and assessed. Every recruited center personnel will be trained by a Gregorio Marañón Team.

  A research coordinator will be in charge of maintaining the team, solving the problems that could appear and supervise the whole process.

  Laboratory personnel will be trained in the cell reconstitution process and clinical teams will be assessed in delivering methods with NOGA system.

  The interpreter will ensure that all the information in the study is comprehended by the participants.

- **PHASE 2**: Field work and data collection (1-1’5 years):

  ·Activity 2.1. Patient recruitment and cell infusion procedure. Patients with chronic heart ischaemic disease will be recruited in all different cooperating centers and cell infusion procedures will take place.

·Activity 2.2. Data collection. Based on all visits patients have to attend. A visit schelude will be held to all participating patients in order to avoid confusion and visitation absense or breach. Data collected from all visits will be registered and send to a research coordinator which function will be to receive, collect and classify all availabe data from all patients and register i ton a data base.
Laboratory and magnetic ressonance data will also be send and registeres by the research coordinator.

- **PHASE 3**: Data analysis and final evaluation (3 months):

·Activity 3.1. Statistical analysis. A statistician will be involved in the study, so that, statistical analysis is performed with excellent guarantees. Once the results in all tests are registered the statistician will perform the analysis of all variables.

·Activity 3.2. Results interpretation and final report. The coordinator of the team will prepare a final report with the results, discussion and conclusion of the study.

- **PHASE 4**: Publication and dissemination of the results (5 months):

·Activity 4.1. Project reviewing. The results obtained by the statistical analysis must be confirmed by a steering committee and a statistician.

·Activity 4.2. Scientific publication and congress assistance. Finally, when data is obtained and processed, results can be published and promulgated in written publications and international congresses.

		2019												2020												2021					
PROJECT ACTIVITY	**PERSONNEL**	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
PHASE 1: PREPARATION AND COORDINATION																															
1.1 Protocol consideration and elaboration	**Research Coordinator (IiSGM)**																														
1.2 Coordination and preparation	**All research team**																														
PHASE 2: FIELD WORK AND DATA COLLECTION																															
2.1 Patient recruitment and cell infusion procedure	**All research team**																														
2.2 Data collection	**All research team**																														
PHASE 3: DATA ANALYSIS AND FINAL EVALUATION																															
3.1 Statistical analysis	**Statician**																														
3.2 Results interpretation and final report	**Research coordinator**																														
PHASE 4: REVISION, PUBLICATION AND DISSEMINATION																															
4.1 Project revision	**"Unidad Central de Investigación Clínica y ensayos Clínicos" (UCAICEC)**																														
4.2 Scientific publication and congress assistance	**Research coordinator and statician**																														

12. ETHICS AND GOOD MEDICAL PRACTICE:

This study will be carried out taking into account the basic principles established by:

- The World Medical Association in the 'Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects' (last reviewed in October 2013) which is universal and everybody has to follow.
- The Standards and Operational Guidelines for Ethical Review of Health-Related Research with Human Participants. World Health Organization, WHO (Geneva, 2011).
- The International Ethical Guidelines for Biomedical Research involving Human subjects. Council for International organization of Medical Sciences, CIOMS (Geneva, 2016).

Note that our study will also follow the "Ley de Autonomía 41/2002, de 14 de noviembre" about the basic regulation of the autonomy of the patient and of rights and obligations in the matter of information and clinical documentation. (latest update on 22/09/2015)

According to "Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal", personal and clinical information of participants will be confidential and only used for the purpose of the research. Moreover, all data will be analyzed anonymously. The participant (or the responsible person of the participant) will always be allowed to modify or destroy any of their collected data. (latest update 31/06/2018)

The study will also have to follow the "Ley 14/2007, Real Decreto 1716/2011", that shall apply to the use of biological samples for the purposes of scientific and technical research, which includes innovation and development as the principal or secondary purpose of obtaining, storing or transferring them. The rights of the subjects must be respected whenever their biological material is used to obtain new scientific knowledge, confirm hypotheses, or carry out activities of technological adaptation, quality controls, teaching, and others. (latest update 02/12/2011)

Two recent laws will also be followed by our study, the first one is "Real Decreto Legislativo 1/2015" that refers to the law on guarantees and rational use of medicines and health products. This law pretends to regulate human medication use, it's investigation, evaluation, autorization, register, fabrication, elaboration, quality control, storage, distribution, circulation, comercialization, information, import and exportation, prescription y dispensal.

The second law we are refering to is the "Real Decreto 1090/2015" which regulates clinical trials with drugs, the Research Ethics Committees with drugs and the Spanish Registry of Clinical Studies.

INFORMED CONSENT IS PRESENTED ON ANNEX 4

12.1 TREATMENT CEASE:

Patients may voluntarily leave the study at any time without giving any reason and with no consequences for their medical care. They will also be withdrawn if any exclusion criteria is detected after informed consent is obtained, if they are lost on follow-up or fail to collaborate. However, regardless of the withdrawal reason, investigators will make all efforts to perform the end of study visitations and collect all available information from the patient.

13. STUDY LIMITATIONS:

This protocol has been planned and structured with the aim that its results can be as representative as possible of the study population. However, like all experimental studies, it has certain limitations.

The main limitations that a study of these characteristics could have are limitations attributable to the intervention itself. It is clear that since it is a study that will include several centers, there may be parameters or some phases of the procedure that can be carried out in different ways depending on the place. In order to mitigate this problem, we have a training before the trial even takes place.

This study also needs a good hemodinamic equipment, laboratory team and clinical personnel used to carry out cell administration procedures. Even if the recruited hospital team has experience, they will be instructed again in the study procedures.

A second limitation that we can found in this type of study is the external validity of its results since its results may not be extrapolable to the entire target population.

The loss of subjects, suspension of the intervention or lack of cooperation by the study participating patients are limitations to consider too.

Patients, due to different factors, may also need other types of co-interventions that could alter the results of the study.

Sample size is another factor to take into account, since it is almost impossible to know and determine exactly the perfect sample size to draw maximum extrapolable conclusions.

Finally, a very important factor is the identification of all adverse effects by the different centers integrated in the study and the correct collect of all the variables that should be known in order to monitor each patient.

14. FEASIBILITY:

This study could not be carried out without the help of many other centers. It is almost impossible for a single center to be able to take care of the entire sample necessary to carry out the whole process, so, cooperation and understanding between all centers that are included in the project is necessary.

The possibility of being in contact with other research centers and teams and being able to establish a good feed-back during the whole process is fundamental for this project to move forward with the highest possible success rate.

Contact persons will be established in all the centers that want to be included in this project and they will be in charge of coordinating the collaboration with the Gregorio Marañón Hospital in order to facilitate all phases of the study.

Those professionals will be paid for their expected extra-work caused by taking part of this study, and will also have meetings at the start to make team building and to ensure that all that has to be done is understood.

Also a statistician will be needed for our work, to ensure the best information analysis.

Hospital Gregorio Marañón already has several agreements with different hospitals, both Spanish and European, together with those that have already been carried out and several tests and projectors in the past, so establishing a new connection should be a possible task.

15. BUDGET:

Public personnel working in this study (IiSGM) will have their own remuneration with no added costs as our study is academic with no Farmaceutic implication.

Based on “Instituto de Investigación Sanitaria Gregorio Marañón” (IiSGM) our cost will be approximately:

- Central Unit of Clinical Research and Clinical Trials (UCAICEC) support on elaboration of a complex protocol: 2100€

- Methodology and Biostatistics Unit will prepare a specific budget for our project in which it will provide assistance on data analysis: 200€ approx

- Cell producción for 150 patients will have a cost of approximately: 329.000€, individually for each patient the cost would be 2.193€
- A Clinical Research Association is neede in order to cover results management: 300.000€
- An insurance with a cost of 50€ per patient will be take into account: 7.500€

- Diagnostic tests will have a budged of:
    - · Blood análisis: 70€ each
    - · Ecocardio: 48’33€ each
    - · Cardiac MR with contrast: 347’19€ each
    - · Ecocardio and RM parameters analisis: 72’22€ each, (32’35€ Eco/39’87 MR)

- Publication and dissemination expenses:
    - · Publication in a scientific journal will cost 1.500€ approximately
    - · With the idea of diseminating knowledge, we will go to the next TACTICS Congress, which will cost about 2000€ taking in account travel, inscription, accomodations…

Total costs of publication and dissemination will be 3500€.

EXPENSES	COSTS	
PERSONNEL		
IiSGM personnel	0€	
PROCEDURE AND DATA COLLECTION		
CELL PRODUCTION (CSC)	$35x10^6$ cells (2.193€) x 150	329.000€
BLOOD ANALISYS	70€ x 4 (times each patient) x 150	42.000€
ECOCARDIOGRAM	48’33€ x 4 (times each patient) x 150	28.998€
CARDIAC MR WITH CONTRAST	347’19 x 3 (times each patient) x 150	208.314€
STATISTICAL ANALYSIS		
ECOCARDIOGRAM AND MR ANALYSIS	32’35 x 4 (times each patient) x 150 + 39’87 x 3 (times each patient) x 150	37.351’5€
UCAICEC SUPPORT	2.100€	
METHODOLOGY AND BIOSTATISTICS UNIT	200€	
PUBLICATION AND DISSEMINATION EXPENSES		
PUBLICATION	1.500€	
CONGRESS ASSISTANCE	2.000€	
RESULTS MANAGEMENT AND INSURANCE		
CLINICAL RESEARCH ASSOCIATION	300.000€	
INSURANCE	50€ each patient	7.500€
TOTAL		
955.463’5€		

16. CLINICAL AND HEALTH CARE IMPACT:

Understanding of mechanisms in cardiac regeneration is a safe bet against several pathologies which have, for now so far, devastating prognoses.

For almost twenty years now, advances in this field have consolidated the idea that regeneration, not only of cardiac tissue, but of many other organs is possible and feasible. Many of the cellular mechanisms involved in this complicated process are still unknown and could have a great implication at the time of obtaining good results in different studies, even so, the eagerness of the scientific community to improve every day and to be able to find a definitive solution to these problems is greater every day.

With this essay we try to contribute in the great community of which we are part and to be able to enligth a little more the ins and outs of these pathologies.

The ultimate goal of this project is to be able to offer an effective solution to a specific population of patients who need a treatment that will be able to improve their quality of life.

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18. ANNEXES:

ANNEX 1: PROCUREMENT OF BMMNC WITH DENSITY GARDIENT SEPARATION (FICOLL) (SPANISH)

1. Tratamiento con Ficoll:

- Diluir la MO a ½ aprox.

Ejemplo: 30 ml de MO llevarlo a 60 ml con PBB

- Preparar tantos tubos como sean necesario; añadir 20 ml de Ficoll a cada uno

- A continuación, añadir 30 ml de MO diluida a cada tubo con Ficoll. La medula se debe añadir lentamente para no mezclar las fases.

Centrifugación: Proceder a la centrifugación de los tubos de acuerdo a los siguientes valores.

400 g
30 min
22 º C
Sin freno y sin aceleración

2. Retirar la interfase de cada tubo no más tarde de 10 min desde la finalización del centrifugado.

3. Reunificación de la interfase de dos tubos de tratamiento con ficoll en un nuevo tubo para a continuación llevar a 50 ml con PBS.

4. Centrifugación: Proceder a la centrifugación de los tubos de acuerdo a los siguientes valores:

300 g
10 min
22º C

5. Si fuese necesario realizar el choque osmótico:
   - Añadir 1 ml de agua inyectable al pellet y mezclar golpeando suavemente el tubo durante 1 minuto
   - Bloquear el choque añadiendo medio de cultivo al 10 % de suero hasta completar el volumen del tubo. Centrifugar a:

300 g
10 min
22º C

6. Decantare el sobrenadante y añadir 10 ml de medio de cultivo al 10 % para realizar el contaje con la cámara de neubauer.

7. La CMNs se plantan a una concentración de 160.000 – 200.000 cel/cm^2

TRIPSINIZACIÓN (4 pases)

Cuando el cultivo ha llegado a una confluencia del 85-90 %, procedemos a la tripsinización:

1. Retirar el medio y hacer un lavado con PBS.
2. Poner TryPLE Select (2 ml F.25 cm2, 5 ml F.75cm2 o 10ml F.175 cm2)
3. Poner en agitación a 37ºC durante unos minutos hasta que las células se despeguen del flask (ir comprobando en el microscopio)
4. Una vez estén despegadas las células bloquear la acción del TryPLE Select con la misma cantidad de medio.
5. Recoger todo el contenido del flask y ponerlo en un tubo para centrifugar.
6. Centrifugar 1200 r.p.m. 10 minutos
7. Decantar y resuspender el pellet en un volumen de medio para realizar un contaje.
8. Contar en la cámara de Nuebauer para hacer el cálculo de los flask necesarios para seguir con el cultivo.

Nº células x 10^4 x dilución x Volumen

En la primera Tripsinización una parte del cultivo va destinada para la diferenciación celular (Adipogenica, Osteogénica y Condrogénica)

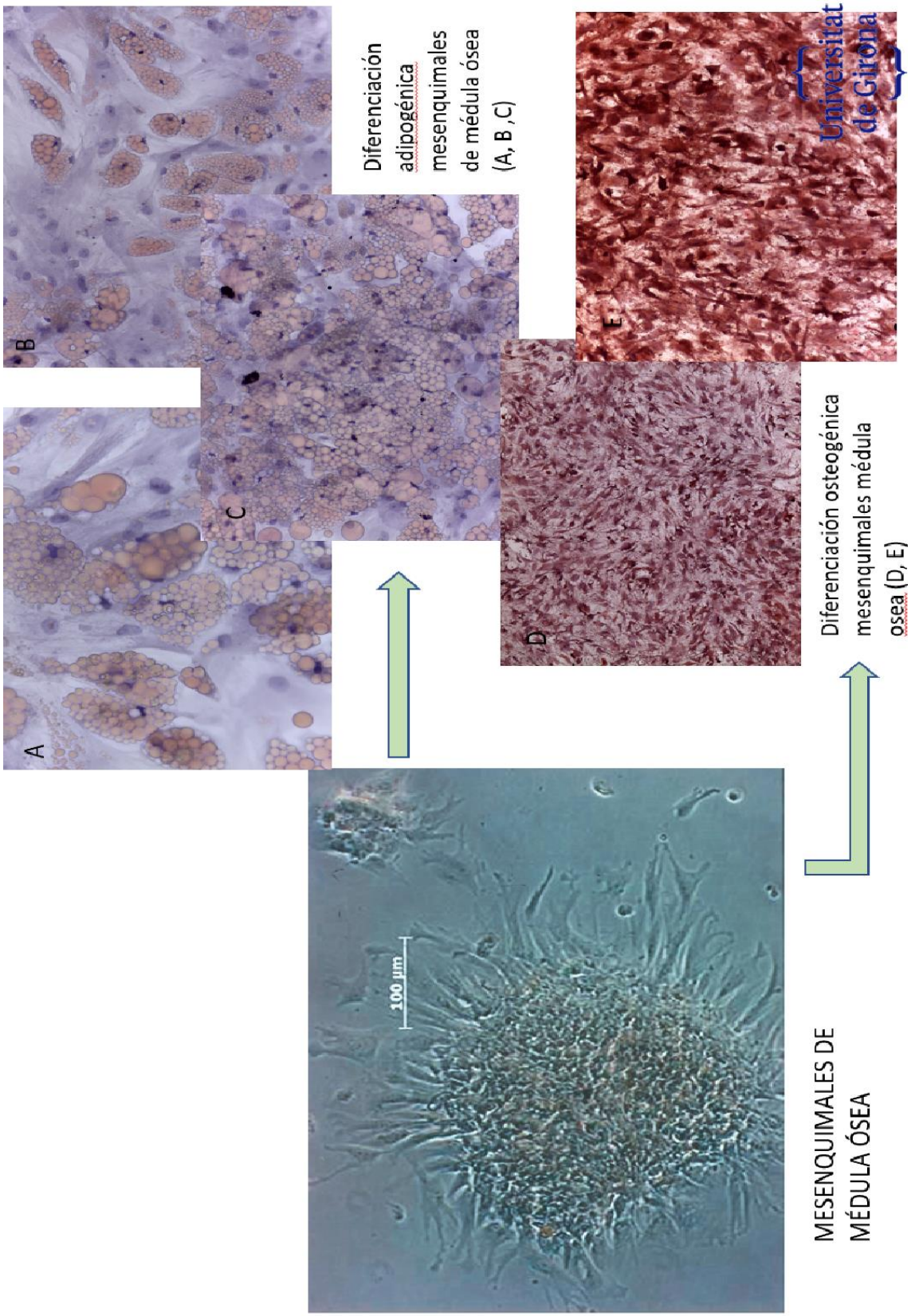
El resto de la muestra lo ponemos a cultivar a 5.000 cel/ cm^2. Aproximadamente

Cada 3-4 días observar el flask y cambiar el medio.

DESCONGELACION MSC

Introducir los viales de la muestra, en el baño termostático a 37ºC hasta que estén completamente descongelados.

- Preparar un tubo de 15 o 50 ml con medio completo y atemperar a 37ºC
- Introducir el vial a descongelar en baño de 37 ºC y agitar con suavidad
- Una vez descongeladas las muestras proceder a resuspender inmediatamente en cantidad suficiente de medio de cultivo completo atemperado, añadiendo la muestra gota a gota al medio y agitando el tubo para homogeneizar la mezcla en los crioviales.
- Centrifugar a:
    - 300 G
    - 10 min
    - 22º C
- Resuspender el pellet celular en el medio completo y contar en cámara de neubauer



Procurement and differentiation of bone marrow derived mesenchymal cells into adipogenic and osteogenic cells. My experience on Gregorio Marañón Laboratory.

ANNEX 2: PROCUREMENT OF SVF FROM ADIPOSE TISSUE (SPANISH)

PREPARACIÓN DE MUESTRAS VIABLES. OBTENCIÓN DE SVF

- Pasar la muestra de grasa a tubos de 50 ml, a razón de 25 ml de grasa por tubo (para lipoaspirado
- Añadir PBS hasta completar el volumen de los tubos mezclando suavemente por inversión del tubo.
- Centrifugar
  300 g
  10 min
  22 º C
- Eliminar la fracción soluble (inferior) con una pipeta.
- Repetir el proceso de lavado detallado en los tres puntos anteriores.

DIGESTIÓN CON COLAGENASA I. OBTENCIÓN DE SVF

- Añadir 10 ml de colagenasa I al (previamente activada) a cada tubo de 50 ml o 2 ml al tubo de 15 ml.
  (Preparar la colagenasa a una concentración de 0,74 mg/ml. Por ejemplo; resuspender 37 mg de colagenasa en 50 ml de HBSS o PBS según instrucciones del fabricante. Filtrar la solución con filtro de jeringa de 0,22 µm.)
- Incubar en el agitador orbital a 37 º C, durante 45-60 minutos a 120 rpm.
- Bloquear la digestión añadiendo un volumen equivalente al de la colagenasa de Medio de cultivo al 10 % de suero, a cada uno de los tubos.
- Centrifugar los tubos de acuerdo a los siguientes valores:
  300 g
  10 min
  22 º C
- Retirar con pipeta la fracción superior dejando el pellet celular.
- Añadir 25 ml de PBS a cada tubo de 50 ml. Reunificar el contendido de cada dos tubos en uno, reduciendo a la mitad el nº de tubos a manipular. Llenar el tubo de 15 ml de PBS.
- Centrifugar a:
  300 g
  10 min
  22 º C
- Filtrado de la muestra. Filtrar la muestra utilizando filtros de cazuela de 70 µm
- Centrifugar la muestra filtrada
  300 g
  10 min
  10 º C
- Verificar que el aspecto de los pellets es blanco marfil a ligeramente rosáceo. En caso contrario proceder al choque osmótico

CHOQUE OSMÓTICO

Realizar las siguientes actividades:

- Añadir 1 ml de agua inyectable estéril a cada tubo, mezclar golpeando suavemente el tubo durante un minuto.
- Bloquear el choque añadiendo medio al 10% de suero hasta completar el volumen del tubo.
- Centrifugar a:
  300 g
  10 min
  22º C
- Retirar el sobrenadante

CULTIVO DE LA FRACCIÓN DEL ESTROMA VASCULAR DE LA GRASA

- Añadir 10 ml de medio de cultivo al 10 % de medio de cultivo para resuspender el pellet y contar.
- Calcular el número de flasks necesarios para sembrar de acuerdo al siguiente cálculo y registrar el número de los mismos para cada superficie de flask.

  nº de células totales / 100.000 = A (nº de cm2)

ANNEX 3: CSC PROCUREMENT AND EXPANSION FROM CARDIAC BIOPSES (SPANISH):

Para la obtención y expansión de las células progenitoras cardíacas, las muestras se procesaron siguiendo dos protocolos distintos: el protocolo de Marbán [5] .

3.1.2.1. Obtención de CPCs mediante el protocolo de Marbán

PROTOCOLO DE MARBÁN

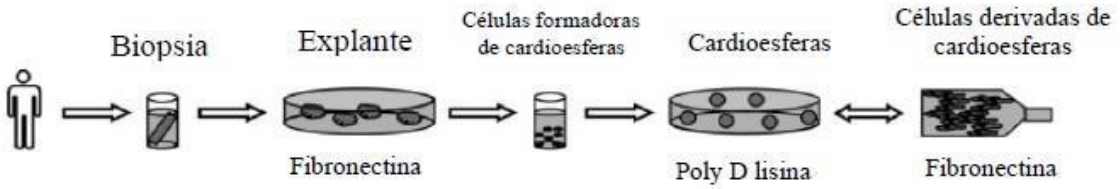


Imagen 1. Esquema del protocolo de Marbán

Las biopsias se transportaron del quirófano al laboratorio en una solución de suero fisiológico al 1% de Penicilina-Estreptomicina-Anfotericina (PSA), solución ideal para lavar la muestra y minimizar la posible contaminación por microorganismos que ésta pueda contener.

En el laboratorio, se trocearon las muestras en pequeños fragmentos los cuales fueron posteriormente digeridos parcialmente con colagenasa IV (1mg/ml). El objetivo de este paso del procedimiento era promover la hidrolisis y eliminación del tejido conectivo existente en la muestra inicial y facilitar la posterior salida de las células del explante.

Los fragmentos resultantes de la digestión se sembraron en una placa de 35 mm de diámetro previamente tratada con fibronectina, polímero glicoproteico que facilita la adhesión de las células al plástico. A continuación, se añadió una pequeña gota de medio específico para el crecimiento de los explantes (Tabla 2A) sobre cada uno de éstos y se incubó la placa a 37ºC durante 3-4 horas. Pasado este tiempo, se añadió un volumen adecuado del mismo medio. El pretratamiento de la placa junto con la digestión parcial de los explantes permite reducir considerablemente el tiempo necesario para que las células salgan, colonicen la placa y lleguen a confluencia.

Siguiendo las indicaciones del protocolo base, cuando el cultivo celular llegó al 80-90% de confluencia se descartaron los restos existentes de los explantes y se levantaron las células mediante el tratamiento enzimático con TrypLE™ Select (Gibco), enzima recombinante que promueve la hidrólisis de las conexiones proteicas entre las células y la superficie de la placa. A diferencia de la tripsina, el TrypLE™ Select permite que el proceso de trispinización sea más suave y asegura una mayor estabilidad de las células [17]. A continuación, las células fueron plantadas a una concentración de 30.000 células/cm^2 en placas de 60mm de diámetro previamente tratadas con poli-D-lisina.

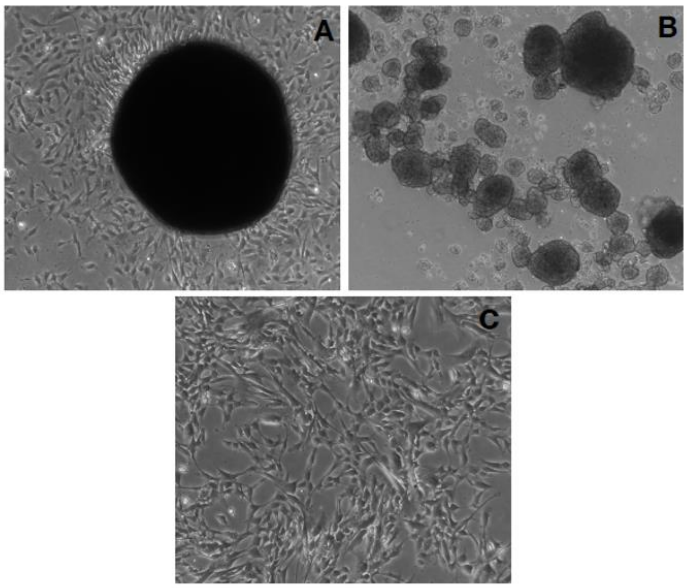
Este polímero juntamente con el medio específico para la expansión de células progenitoras cardíacas (Tabla 2B), favorece que las células fibroblásticas se adhieran a la superficie de la placa y que, por lo contrario, aquellas que tengan potencialidad de célula madre, se levanten y formen cúmulos brillantes de células en suspensión, a los que se denomina como cardiosferas [14]. El tiempo de incubación con poli-D-lisina es limitante ya que las células con potencialidad de célula madre requieren su tiempo para desprenderse poco a poco de la placa y agruparse progresivamente en suspensión.

Finalmente, estos cúmulos celulares (cardiosferas) fueron recogidos y plantados en una p35 previamente tratada con fibronectina. A partir de este punto, se siguió expandiendo el cultivo

hasta obtener la densidad celular deseada para la realización de las pruebas de caracterización.

Tabla 2. Composición de los medios de cultivo utilizados en el Metodo I (protocolo de Marbán)

A) Medio de tratamiento de explantes	**B) Medio de expansión de CPCs**
Iscoves's modified Dulbecco médium 20% fetal bovine serum (FBS) 1% PSA 1% L-glutamina 0,1 Mm 2-βmercaptoetanol	65% DMEM + F12 (1:1) 35% Iscoves's modified Dulbecco medium 10% fetal bovine serum (FBS) 1% PSA 1% L-glutamina 0,1 Mm 2-βmercaptoetanol



A: Explant derived cells
B: Cardiosferas
C: CSCs morfology

Procurement and culture of Cardiac Stem Cells. My experience on Gregorio Marañón Laboratory.

ANNEX 4: INFORMATION SHEET AND INFORMED CONSENT

INFORMATION SHEET AND CERTIFICATE OF CONSENT

This informed consent is adressed to patients aged ≥18 years old who meet trial inclusion criteria and who agree to participate in it, either at Gregorio Marañón Hospital or at any cooperating hospital.

- **Principal Investigator Identification:**
- **Main Responsible Entities:**

All patients will be given a copy of the full Informed Consent Form. In this document and in accordance with the 2013 updated regulations of the Helsinki Declaration on the ethical principles for medical research in human beings, the following information is presented:

This informed consent has two parts:

· Information Sheet with all protocol information.

· Certificate of Consent for signatures if you agree on protocol procedures.

1. Information Sheet

Introdution

As the researcher in charge working in Hospital Gregorio Marañón i will inform you about all protocol information and procedures. This is a clinical trial with stem cell therapy which aim is to find a safe and effective treatment for chronic patients with ischaemic heart infarction.

We are going to give you all information and invite you to participate in this trial. You do not have to decide today whether or not you agree to participate. Before you decide, you can talk to anyone you feel comfortable with.

If there are parts of the information sheet that you do not understand, please ask anyone on the research team as you go througth all information and they will take time to explain. If you have questions later, you can ask them the study doctor or anyone on the staff.

Protocol Nature and Objectives

The main objective of this trial is to obatin totally anonymous medical information of all patients undergoing the procedure.

The aim is to use this data in order to achieve more comprehension about heart regeneration and to aply this data on patient future management.

After the initial procedure clinical, analytical, and imaging parameters will be collected in a 12 months follow-up that all patients will have to keep.

Purpose

Ischaemic heart disease is still a huge problem worldwide. It's morbility and mortality is so high and keeps increasing with illness time. Need of an effective treatment is evident.

The purpose of this research is to asses safety and effectiveness of a new therapy that could lead to a definitive solution for this illness. Results on this trial can help us in the future in improving this patient population Life Quality and will also write down clinical and analytical data in order to improve acknowledgement of heart regeneration mechanisms.

So, this Consent Certificate has the purpose of requesting your permission on collecting and analysing all information that we are able to obtain.

Type of Research Intervention

This study has been designed double-blind randomized clinical trial, this means that the study will have a group of patients who will undergo the process with stem cells and a group of control patients who will be infused with placebo.
This separation into different study groups will be completely random and without any type of preference.
In any case, at the end of the trial, if the results of the study are positive and the treatment is expected to be effective, patients of the control group will be given the option to undergo main treatment infusion with stem cells.

Participant Selection

All patients who meet the inclusion criteria of the study will be offered the opportunity to participate in this study.

With this being said, we are inviting you to take part in this trial beacuse it's important to know all mechanisms of this condition in order to achieve an optimal treatment.

Voluntary Participation

Your decisión to participate in this trial is entirely voluntary. It is your choice wether to participate or not. If you choose not to participate all services that you are reeceiving in this hospital will continue and standard procedure for the illness will be applied. You may also choose to change your mind later and stop participating, even if you agreed earlier, and the services you receive at the hospital will continue the same way.

Description of the Process

This process has two different parts: Intervention itself, were cardiac stem cells will be infused and an 12 months follow-up.

The intervention will be a intramiocardial administration with catheters that will infuse cells directly on the myocardium. Although catheterization is an invasive test, current advances make its risks extremely low. Moreover, the possibility of a cardiac arrest is absolutely exceptional.

The main problems derived from catheterization are usually vascular, that is, possibly a bruise in the puncture site. It is an absolutely common technique for several years, and we think possible benefits are far ahead possible complications.

Cell infusion has also proves total safety in other trials and for now no rejection side effects have been noticed.

The second part will be an 18 months follow-up with different clinical, analytical and imaging tests.

Duration

This trial will have a duration of approximately 1’5-2 years per patient and for 12 months will be from taking information about patient condition after the procedure. During that time we will test the parameters taken and you will be informed of any medical condition that we found.

Side Effects

This intervention have proved to have minimal side effects, but if something happens, you can come to the hospital at any time and healt proffesionals will perform better treatment.

No injert rejection side effects have been seen until now, so we asume the procedure is safe and feasible.

Benefits

If you participate in this trial, you will have the following benefits: if this treatments is effective and you are on the treated group your quality of life, mobidity and mortality rates will improve. You will also offer a valuable source of anonymous data that can be used to improve the management of patients with chronic myocardial infarction.

Confidentiality

Patient's information will be kept confidential and anonymous, in the custody of the responsable person for the registration.

No patient identifier data will be recorded and then all data will be anonymized again using a random number code.

In any case, the parameters established by the *Organic Law 15/1999 of December 13, Personal Data Protection* will be fulfilled.

Results Sharing

All knowledge that we get from this study will be shared with you before its made widely available to the public. Confidential information will not be shared.

After a meeting with all participant patients we will publish results in order that other interested groups may learn our research.

Contact

If you have any questions you may contact your cardiology department via e-mail or with your hospital contact numbrer or came to the hospital, and they will put you in contact with a team researcher.

Interest Conflicts

There is no interest conflicto by the register responsable.

2. Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any question I have asked has been anwered to my satisfaction. I consent voluntarily to participate in this study.

Name of Participant _____, with DNI _____ and as a participant on this trial I understand information exposed on this Consent.

For record signature on ____, of _____ 20___

Patient Signature

Responsible Declaration

As the responsable of this trial I certify that I have explained to the patient Nature and Objectives of this study and that the participant completely understands their participation, possible risks and benefits and all the procedure specifications.

All questions have been formulated and answered appropiately and I have read all formulary parts and I'm aware of my patient data custody responsabilities.

Here is my signature on ____/_____/_____

Dr./Dra. _____ signature

ANNEX 5: SCACIHI FLOW CHART