

## Deciphering stem cell therapy for the interventional cardiologist

“...this editorial will discuss the historical perspective of stem cell research in the USA, the pitfalls and barriers to success and the types of studies that are being conducted...”

**Keywords:** angiogenesis • endocardial delivery • NOGA mapping • post-STEMI remodeling • refractory angina • refractory CHF • stem cells • stop flow intracoronary delivery

Despite over 20 years of interest in cardiac applications for angiogenesis and cellular regeneration, no single gene, stem cell or clinical indication has evolved into mainstream practice. There are many reasons for this but it is certainly not for a lack of effort. Time and space do not permit a meaningful review of the large body of excellent work in the field conducted outside of the USA [1–3]; therefore, this editorial will discuss the historical perspective of stem cell research in the USA, the pitfalls and barriers to success and the types of studies that are being conducted with a special emphasis on study design, cell delivery techniques and patient/end point selection. Finally, a call to arms will be offered with a plea to industry, investigators and regulatory bodies to try and standardize study designs in order to make it easier to execute and complete studies, harmonize outcomes and accelerate approval, which is sorely needed.

### Background

Before cardiac applications of stem cell research gained much traction, Isner *et al.* laid the early groundwork for both pre-clinical and clinical applications of gene therapy for angiogenesis in the refractory angina patient [4,5]. Despite very encouraging Phase I and Phase II studies, using both epicardial and transvalvular endocardial injections of VEGF I and VEGF II, a large pivotal Phase III trial was terminated due to ten cases of pericardial effusion and three [3] cases of tamponade [SPONSOR PROVIDED, UNPUBLISHED DATA]. A strategic decision to use a

nonmapping catheter instead of the proven NOGA mapping system (Biologic Delivery Systems, CA, USA) led to the high number of perforations. Interim analysis of the first 300 patients showed failure to reach the primary end point of an improvement in the 3-month exercise stress test and the trial was abruptly abandoned. Despite these disappointing and unexpected results, much was learned from the many errors that were made in conducting this study from patient selection, study design, catheter design and data analysis which ultimately helped in the design and execution of future stem cell trials. As with most negative trials, it did not fail because of a lack of an effect of the gene product, rather the placebo group performed surprisingly well. This was a valuable lesson in trial design, which reminds the investigator to account for a sometimes robust placebo effect. With the failure of this study, interest in gene therapy quickly faded for the indication for refractory angina and was redirected toward cell-based therapy.

### Stem cell lines

There are two basic stem cell lines of interest for cardiac applications, hematopoietic and mesenchymal. The hematopoietic cell, the most common of which is the CD34<sup>+</sup> cell, is unique in its ability to promote capillary budding, hence angiogenesis, in response to an ischemic signal [6]. The typical patient in need of this cell would be one with good left ventricular function and poor coronary blood flow as a result of failed intervention (either bypass surgery or percutaneous coro-



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nary intervention) with persistent angina, unresponsive to medical therapy. Although all patients have intrinsic CD34<sup>+</sup> cells, both in the bone marrow and circulating, there are typically not enough of them to create the collateral flow required to render the patient asymptomatic; therefore, very high concentrations of CD34 cells injected directly into the ischemic zone can recruit growth factors, such as VEGF, PDGF, HGF and SDF1, and amplify the local angiogenic signals to promote capillary growth and thus hopefully improve blood flow.

The second type of cell commonly studied is the mesenchymal stem cell, which is predominantly encoded to promote new cell growth or regeneration of damaged myocytes [7]. The typical patient targeted for mesenchymal cells would have refractory symptoms of dyspnea despite maximum medical therapy, cardiac rhythm therapy (CRT), prior intervention and poor left ventricular function either of ischemic or non-ischemic origin. It is hoped that these stem cells will populate then repair existing tissue or differentiate into functioning/contracting myocytes, thus, improving systolic and diastolic function.

### Clinical indications

There are three clinical indications for stem cell investigations currently in the USA summarized as follows.

#### Refractory angina

Due to safety fears in the early days of investigation only the most refractory, no option patient was considered an appropriate patient for clinical investigation of stem cells. Inclusion criteria were very strict and included persistent angina (class II–IV) despite exhaustion of all approved therapeutics including coronary artery bypass surgery, percutaneous coronary intervention and maximum medical therapy, but with objective evidence of ischemia by noninvasive testing (single-photon emission computed tomography, PET and exercise echocardiography, among others). The only large randomized trials for refractory angina in the USA were Phase I and Phase II trials using autologous CD34<sup>+</sup> cells [8,9]. The cells were uniquely derived from autologous bone marrow stimulation using 4–5 days of subcutaneous granulocyte colony stimulating factor followed by apheresis collection of cells then separation of the CD34<sup>+</sup> cells using the Isolex system (Baxter, IL, USA). These cells were then processed, cleared for threshold cell count, purity, viability and infection, then delivered to the investigator within two weeks for endocardial injection. Using a sophisticated left ventricular mapping system (NOGA, Biologic Delivery Systems) areas of ischemia were confirmed

by normal voltage and reduced linear shortening after which multiple intramyocardial injections were done in the target area. A Phase I study showed that the procedure was safe and following the Phase II study showed clear efficacy with a significant increase in treadmill time at 6 and 12 months compared with placebo along with a significant reduction in angina. Interestingly, the ‘lower dose’ of cells consistently showed more efficacy than the ‘higher dose’ which remains unexplained. These encouraging results prompted a pivotal Phase III clinical trial which unfortunately was abruptly terminated by the sponsor after the first 125 patients were enrolled. At this time, there are no stem cell trials in the USA for refractory angina. It is hoped that analysis of these few patients enrolled in the Phase III trial over time will yield valuable information and might promote further interest in this important clinical subset of patients.

#### Refractory congestive heart failure

Contrary to the seemingly lack of interest in refractory angina patients as a clinical indication, there is robust interest in patients with refractory congestive heart failure (CHF) both of ischemic and nonischemic origin for stem cell therapy. The CHF trials underway in the USA share some similar general designs, but they also have substantial differences regarding inclusion and exclusion criteria. The similarities include the refractory nature of the no-option indication with persistent New York Heart Association class II–IV symptoms but they differ with regard to ejection fraction cutoff, presence or absence of ischemia, recent hospitalizations and presence or absence of an automatic implantable cardiac defibrillator (AICD). Differences include primary end points which vary from repeat hospitalization, VO<sub>2</sub> max, composite major adverse clinical events, ejection fraction, and so on. These are, for the most part, Phase I and II trials with only one Phase III trial currently underway (DREAM trial, TEVA, Petack Tikva, Israel). The cell types used are mesenchymal in origin and are autologous cells from bone marrow harvest (Vericell, MA, USA), adipose tissue from liposuction (Cytori, CA, USA) or allogenic cells from donors (TEVA, Petack Tikva, Israel).

The narrow scope of this paper does not allow an extensive review of all these data from the trials; however, suffice it to say that available Phase I and Phase II data indicate that all of the cell types are safe with no deleterious effects noted compared with controls including allogenic cells. Past fears of mutagenicity and rejection have been muted by these early results. Efficacy data continue to look encouraging, which has prompted investment and pursuit of Phase III trials.

### Postmyocardial infarction remodeling

The post-ST elevation myocardial infarction (STEMI) patient represents a unique indication for stem cell therapy much different from the refractory heart failure and angina patient. Unlike those patients who are in desperate need of some intervention for refractory lifestyle-limiting symptoms and high morbidity and mortality, the postmyocardial infarction patient may be entirely asymptomatic thus the difference in the targeted strategy. In this case, the goal of therapy is to somehow influence the adverse remodeling that occurs in a small percentage of patients post-STEMI despite successful intervention [10]. These patients can deteriorate much later in time and spiral quickly despite the best medical, surgical and interventional efforts to reverse it. There are two randomized trials for post-STEMI remodeling being conducted in the USA at this time. Both target the same type of patient (post successful stenting of a left anterior descending STEMI) but from a very different perspective. The Neostem (NY, USA) trial targets very early intervention (within 2 weeks) compared with a longer one month to one year horizon for Capricor (CA, USA). Neostem uses autologous bone marrow derived CD34<sup>+</sup> cells which are processed then returned to the investigator for intracoronary injection within 2 weeks of the index STEMI event. It has been shown that there is a small window of opportunity during these two weeks where levels of the chemokine SDF-1 are very high in the injured myocardium which amplifies and potentiates the regenerative effects of CD34<sup>+</sup> cells. Early analysis of these data are encouraging and show a clear dose effect with higher doses of cells resulting in improved ejection fraction compared with placebo

[SPONSOR PROVIDED, UNPUBLISHED DATA].

Using a different cell type and strategy, Capricor uses allogenic donor mesenchymal cells (cardiospheres) anytime from one month to one year post-STEMI. The cells are given intra-coronary using a stop flow technique similar to Neostem. The end point is a very objective measurement of scar versus viable tissue by late gadolinium enhancement MRI. The Phase I results (CADEUCEUS trial), were very encouraging and showed safety and a measurable decrease in scar with an increase in viable myocardium compared with placebo [11]. Phase II data are pending.

### Study design

It is apparent from the above review that there is no consensus for study design. While they are all randomized and double blinded, some use 2:1 versus 1:1 randomization; some have US FDA mandated double placebo 'standard of care' arms, others do not; some allow crossover, others do not; some allow

intramyocardial injection of placebo, others use a 'sham' procedure. This can create much confusion for both the operator and the patient in trying to determine eligibility for entry. Patients are surprisingly well informed from perusing the internet when they arrive for consultation for participation in these trials. They come prepared to discuss randomization, placebo risks, crossover and whether they can drop out if they receive placebo. We cannot forget how desperate these patients are with such a poor prognosis and an equally unacceptable lifestyle. Thus, a study design to allow crossover and 2:1 randomization to cell therapy would go a long way to encourage entry and discourage disenrollment. The double placebo arm of the Phase III ACT34 trial (standard-of-care arm) for refractory angina definitely discouraged recruitment as patients were reluctant to enroll with such a low chance getting cell therapy without crossover. Furthermore, patients who were randomized to the standard of care arm quickly disenrolled and looked elsewhere for treatment. This predictable slow enrollment probably influenced the sponsor's termination of the study.

### End point selection

Even more confusing is the wide disparity in primary end point selection for these trials with no two studies having the same primary end point. While the fate of the sponsor may rise or fall on the wise selection of a solid end point that not only gets a 'p-value' and FDA approval at some point but also make sense to the clinician who must ultimately decide whether he believes the data and is willing to expose his patient to the risk and cost of this new product. Our history is full of devices and drugs that received FDA approval as 'safe and effective' only to fail in the marketplace when the clinician ultimately decided that it was just not so. In general, the more objective the end point the better, as subjective patient assessments are too variable for both CHF and angina assessment. Due to cost and statistical power, it is difficult to design and fund a study around the most objective and meaningful end point of survival; so we are left with surrogates and composites to add power and reduce the size and cost of trials.

For angina trials, objective performance on a physician-monitored stress test may be the best single end point even though it still has limitations. Single-photon emission computed tomography or PET may not have the sensitivity and reproducibility to serve as a single end point. Time to angina or ST depression may work well for a study of class I angina patients and a new antianginal drug but not for class III patients who are heavily medicated with  $\beta$ -blockers and nitrates.

For the CHF patient, there are several objective end points to consider: ejection fraction (EF),  $MVO_2$ , pressure volume loops, repeat hospitalizations, AICD events, and so on. EF is very appealing if it goes up but what does one do with a patient who has a negligible increase in EF but improves symptomatically due to a favorable shift of his pressure volume curve from favorable remodeling post treatment? Composite end points which have the power of multiple interactions may be more valuable in this case.

For the post-STEMI patient, late gadolinium enhancement MRI is a superb objective tool to assess the biologic effect of stem cells but we must be careful not to overinterpret the significance of a p-value for scar reduction when considering the larger picture of ultimate clinical benefit. A smaller infarct size is a good surrogate for the more important end point of survival, so it is hoped that this will be confirmed over the long term in a Phase III trial [12].

### Delivery

How to deliver stem cells to the patient is also controversial. There are very good animal and human data now to declare that intramyocardial delivery of cells is superior to intracoronary delivery if one looks merely at cell retention (12 vs 3%) [13,14]. Within hours of delivery with either technique almost 50% of the cells migrate to the lungs then to the liver and spleen over time. Whether 3% retention is enough to establish a biologic footprint is unknown at this time nor if the clinical response is linear with regard to retention. Therefore, we must be prudent in study design to maximize the chance of getting a positive clinical response. Not enough is known right now to declare if one technique is superior to the other across all cell lines and patient populations. The intracoronary injection technique seems easier than mapping as it is a familiar technique to the interventionalist and does not require learning a new skill set as with NOGA mapping. However, it is still fraught with the hazards of balloon occlusion induced ischemia, damage to the recently placed stent, new dissections, sludging or embolization of cell products which may clump and the usual risks of wire access to coronaries. NOGA mapping and injection require dedicated training and experience, take 2–3 h to perform and carry a 1–2% risk of perforation and serious ventricular arrhythmias.

### Mapping versus nonmapping

The high cost and risks noted above of NOGA mapping make endocardial delivery a challenge for mainstream acceptance which has encouraged development of nonmapping biologic delivery catheters. NOGA

mapping has the unique ability to provide 3D awareness to the operator which allows him to avoid thin-walled scar and overlapping injections which is not possible with nonmapping catheters. However, the ease of use and low cost of nonmapping catheters are very appealing when there is no scar to avoid, wall thickness is uniform and global delivery is desired such as in the patient with nonischemic myopathy. All injection catheters are considered experimental at this time and are not approved by the FDA.

### Dosing

The ‘correct’ dose of stem cells is unknown at this time as each cell is very specific and cannot be compared with each other. Even the entire concept of ‘dosing’ with biologics may not be same as with pharmacologics and begs the question of how to establish the right dose. Too many cells may be deleterious as noted in the ACT34 Phase II trial while too few may offer nothing more than a placebo effect. Until more Phase II trials are completed, proper dosing remains unknown.

### Future perspective

It has been nearly 20 years since earnest investigation of stem cells for cardiac applications was begun, yet there is still no end in sight for approval of any cell product. Although data are very encouraging, there are no Phase III data to say with confidence that ‘work’ for patients with refractory angina or CHF. As a result of these delays, many rogue stem cell clinics have appeared worldwide treating desperate patients at great cost, without the transparency of oversight and largely with unproven techniques. With so much at stake and such a dire population of patients already declared refractory, and with no other options, it is imperative to streamline the process for approval. This can only be helped by trying to standardize study designs, protocols, patient selection, delivery and removal of some of the confusion from regulatory agencies that have inhibited patient enrollment. Only then might we be able to fulfill the promise of a cell-based therapy for patients with refractory heart disease in the reasonable near future.

### Financial & competing interests disclosure

The author has a relationship with BDS (Biologic Delivery Systems), which includes a consulting agreement and a licensing agreement. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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