# Stem Cells

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## Abstract

Stem cells offer the potential of new therapies for previously untreatable diseases. This session focuses on different aspects of stem cells from embryonic stem cells to adult stem cells and the biology and therapeutic impact of cancer stem cells.

### Keywords

Stem cells; ES cells; regenerative medicine; cancer

## INTRODUCTION

Stem cell research offers opportunities for developing new medical therapies for debilitating diseases and a new way to explore fundamental questions of biology. Stem cells (SCs) are defined as cells that can self-renew and differentiate into mature functional cells. If we can determine the mechanisms that control the proliferation and differentiation of SCs, it may be possible to engineer cells for therapeutic benefit. Therefore, it is important to explore all sources of SCs: embryonic (ES), adult, and induced pluripotent stem cells (iPS). Each population offers different advantages and disadvantages. Some of these properties will be presented in the presentations that follow, but it is unlikely that a single SC source will be applicable for all diseases. In contrast, each disease presents its own set of problems and complexities, and subsets of patients within diseases present differing challenges. Most likely patient-specific cellular products (eg, products generated from iPS cells) will be limited because of cost and time for manufacture. Allogeneic cellular products will be limited by HLA barriers and potential rejection or graft-versus-host disease (GVHD). A number of clinical trials are evaluating the use of autologous cellular products and other complexities will limit access to these products because of factors such as time for production. Therefore, carefully designed clinical trials are needed to evaluate the potential of cellular products from various SC sources to define the limitations in production, delivery, and clinical benefit.

This session on SCs consists of 3 presentations that will focus on the biology and translational aspects of human embryonic stem cells (Jane Lebkowski), cancer stem cells (Richard Jones), and adult stem cells (Ian McNiece).

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## HUMAN EMBRYONIC STEM CELLS (hESCs) JANE LEBKOWSKI

Many investigators have been evaluating the potential of hESCs to generate cells for clinical applications. Embryonic SC-derived neural cells have been used to treat nervous system disorders in animal models. In the case of spinal cord injuries, neural cells derived from animal ESCs and injected into the spinal cord injury site produced significant recovery of the animal's ability to move and bear weight. To apply those observations to humans, Geron has derived oligodendrocyte progenitor cells (GRNOPC1) from hESCs. Oligodendrocytes are naturally occurring cells in the nervous system that have several functions. Oligodendrocytes produce myelin (insulating layers of cell membrane), which wraps around the axons of neurons to enable them to conduct electrical impulses. Myelin enables efficient conduction of nerve impulses in the same manner as insulation prevents short circuits in an electrical wire. Without myelin, many of the nerves in the brain and spinal cord cannot function properly. Oligodendrocytes also produce neurotrophic factors (biologics that enhance neuronal survival and function) to support the maintenance of nerve cells. Oligodendrocytes are lost in spinal cord injury, resulting in myelin and neuronal loss that cause paralysis in many patients with spinal cord injuries.

In our collaboration with researchers at the University of California, Irvine, we have shown in animal models that GRNOPC1 can improve functional locomotive behavior after implantation in the injury site 7 days after injury. Histologic analysis also provided evidence for the engraftment and function of these cells. These data were first published in May 2005, in the *Journal of Neuroscience* [1]. In additional studies, the lesion site of animals 9 months after injury and subsequent injection of GRNOPC1 was observed to be essentially filled with GRNOPC1 and myelinated rat axons crossing the lesion. These animal observations serve as the rationale for the use of GRNOPC1 in treating spinal cord injuries in humans.

We have developed a functional cryopreserved formulation of GRNOPC1 for use in clinical trials, and have initiated current good manufacturing practices (cGMP) production of GRNOPC1 in our qualified manufacturing facilities.

After completion of extensive animal toxicology testing, which included 24 separate studies in rats and mice that required more than 5 billion GRNOPC1 cells, we filed a 21,000 page investigational new drug (IND) with the U.S. Food and Drug Administration (FDA) containing data from the animal and in vitro testing of the cells to ensure the highest possible degree of safety of the product before initiating human clinical trials.

In January 2009, we received clearance from the FDA to begin the world's first human clinical trial of an ESC-based therapy using GRNOPC1 for acute spinal cord injury. The IND is currently on clinical hold by the FDA pending the agency's review of new non-clinical animal study data submitted by the company.

## CANCER STEM CELLS RICHARD JONES

### Background

Only a minority of cells from most hematologic malignancies and solid tumors are clonogenic in vitro and in vivo. This low clonogenic potential could represent proliferative capacity exclusively restricted to a small subset of cancer cells, or alternatively, all the cells within a cancer retaining the capacity to proliferate but only at a low rate. Which of these 2 possibilities explains the low clonogenicity of most cancers has been debated for years. Fialkow and his colleagues [2] first suggested that chronic myelogenous leukemia (CML) arose from rare transformed hematopoietic stem cells (HSCs) nearly 40 years ago, when they showed that both granulocytes and red blood cells from CML patients were derived

from a common cell. The first modern use of the term cancer or tumor SCs is attributed to Park et al. [3], who found that only a minority of mouse multiple myeloma cells were capable of clonogenic growth. The SC origin of CML was confirmed more than 15 years ago when several groups utilized phenotypic characteristics of HSC to identify and isolate CML cells capable of expansion ex vivo [4]. Dick and colleagues [5,6] extended these observations, showing that phenotypic primitive HSCs purified from patients with both acute myelogenous leukemia (AML) and CML would generate leukemia in vivo when injected into nonobese diabetes/severe combined immunodeficiency (NOD/SCID) mice.

Such cells have more recently been described in many other malignancies [7,8]. A recent consensus conference defined Cancer Stem Cells (CSC) cardiac stem cells (CSCs) as tumor cells possessing the capacity for self-renewal and generating the cells that comprise the tumor bulk [8]. Although CSCs have been hypothesized to arise from transformed normal cells with SC properties, it is very possible that the transformation process itself may be able to induce self-renewal capacity. Hence, these cells have also been designated "tumor-initiating" or "tumorigenic" cells, although the most commonly used label remains CSCs [8]. The current gold standard for identification of CSCs requires that they possess the ability to engraft immunodeficient mice [5,9]. However, this is an artificial culture system that could either under- or overestimate the true frequency of CSCs.

### Clinical Relevance

Therapeutic advances over the past 3 decades now allow most cancer patients to achieve major clinical responses. Although clinical responses can clearly decrease side effects and improve quality of life, most cancer patients still eventually relapse and die of their disease. Putative CSCs have been reported to be relatively resistant to standard anticancer therapies [10-12], at least in part by co-opting normal SCs' intrinsic defense mechanisms such as quiescence, efflux pumps, and detoxifying enzymes [11]. The CSC concept proposes that initial responses represent therapeutic effectiveness against the cancer cells making up the bulk of the tumor, whereas CSCs are responsible for most relapses, and must be eliminated to realize cures [7,13]. However, currently there are no definitive data that CSCs from any malignancy are, in fact, responsible for disease relapse or resistance. Thus, the data on CSCs remain largely laboratory curiosities, leading many investigators to question the biologic and clinical relevance of these cells [14].

However, emerging data suggest for the first time a clinical relevance for CSCs. Multiple myeloma (MM) has long been considered a malignancy of plasma cells, because they form the bulk of the tumor. However, several groups have recently shown that the myeloma plasma cells actually arise from a minute population of less differentiated CSCs, which resemble memory B cells and have the ability to self-renew, differentiate, and generate the disease in vitro [15,16] and in NOD/SCID mice [11]. A report using rituximab to target myeloma CSCs (myeloma plasma cells usually do not express CD20) found a strong and significant association between myeloma CSC numbers in patients after treatment with and progression-free survival (PFS) [17]. Moreover, rituximab could be detected on the surface of circulating myeloma CSCs from patients who progressed, suggesting that the therapeutic concept was valid, but that rituximab was unable to kill the myeloma CSCs. Residual breast tumor cell populations persisting after conventional treatment have recently been shown to be enriched for phenotypic breast CSCs [18].

### Summary

Initial responses in cancer represent therapeutic effectiveness against the cancer cells making up the bulk of the tumor; emerging data suggest that resistant CSCs are often responsible for relapse. Because many currently active treatments have been developed to

target the cancer cell bulk, they may have little activity against biologically distinct CSCs. Moreover, traditional response criteria measure tumor bulk and may not reflect changes populations of rare CSCs [7]. Standard response parameters may not only potentially overestimate the effect of therapy on the minute population of stem cells, but may also underestimate it. Therapy selectively directed at CSCs will not immediately eliminate the differentiated tumor cells; such therapy, therefore, might be prematurely abandoned if clinical activity is judged solely by standard response criteria that reflect the effects of treatment on the bulk of the cancer. Thus, it is likely that improving the results of cancer therapy requires identification and better understanding the biology of CSCs, as well as reexamining both our preclinical and clinical drug development paradigms to include the CSC concept.

## ADULT STEM CELLS IAN MCNIECE

The maintenance of homeostasis requires constant cell production to replace dead and damaged cells. Adult SCs reside in the tissues and differentiate to functional mature cells. The control of proliferation and differentiation of a number of types of SCs occurs in the microenvironmental niche or the SC niche. HSCs have been studied in detail and shown to reside in the BM in association with stromal cells that make up the hematopoietic microenvironment [19]. The stroma consists of several cell populations including mesenchymal stromal cells (MSCs), fibroblasts, and adventicular reticulocytes [20]. HSCs exist in a quiescent state in close relationship with the stromal cells in the BM. These stromal cells produce a number of cytokines and growth factors that are either secreted or expressed as membrane bound proteins, and these cytokines and growth factors control the differentiation and proliferation of the HSCs. In vitro, MSCs have been shown to support the proliferation and differentiation of HSCs, generating committed hematopoietic progenitor cells over a 6-week period [21]. If the microenvironment is compromised, such as in patients who receive multiple rounds of high-dose chemotherapy regimens, normal homeostasis is disrupted and deficiencies in blood cells occur.

### Stromal Cells in Cardiac Tissue

The extracellular matrix (ECM) of cardiac tissue, which is composed of a number of cells including cardiac fibroblasts, mesenchymal cells, fibronectin, and other matrix proteins [22-24], provides elasticity and mechanical strength. We have isolated several stromal cell populations from human fetal heart that are positive for CD105, CD90, and CD73, but negative for CD34 and CD45, which is consistent with the phenotype of BM-derived MSCs. Given the homeostatic role of MSCs in regulation of HSCs, it is highly likely that cardiac stromal cells play a regulatory role in the control of proliferation and differentiation of CSCs and cardiac progenitor cells (CarSC and CPC) (CPCs). This role could be performed through the secretion of a range of growth factors and cytokines.

Myocardial infarction (MI) results in ischemic damage, which results in cell death of not only cardiomyocytes, but also fibroblasts and most likely stromal cells. Even with migration of viable CarSCs and CPCs to the ischemic tissue, the lack of stromal elements would result in the failure of the CarSCs and CPCs to proliferate and differentiate, hence failure of remodeling. Along with the recent identification of cardiac SCs in heart tissue, this offers insights into the biology of ischemic heart damage. Patients with an MI have ischemic tissue that fails to regenerate, and we propose that this is in part because of destruction of cardiac stromal cells.

MSC derived from BM cells have been evaluated for cardiac regenerative therapy [25] and offer advantages over other sources of stem cells because of their availability, immunologic properties, and record of safety and efficacy. Studies of MSC engraftment in rodent and

swine models of MI demonstrate: (1) functional benefit in post-MI recovery with administration, (2) evidence of neoangiogenesis at the site of the infarct, (3) decrease in collagen deposition in the region of the scar, and (4) some evidence of cells expressing contractile and sarcomeric proteins, but lacking true sarcomeric functional organization. Administration of autologous or allogeneic human MSCs to cardiovascular patients has been performed in several clinical studies to date, all in the post-MI setting. The MSCs have been administered via the intracoronary (i.c.) route, via peripheral intravenous (i.v.) injection or direct injection into the cardiac tissue with surgery. Preliminary data suggest improved cardiac function in patients receiving MSC; however, these results need to be studied in larger randomized trials [26].

Based upon data from animal studies and preliminary clinical data we propose that optimal repair of ischemic tissue requires regeneration of both stromal elements and cardiomyocytes. Delivery of MSC to the ischemic tissue can regenerate the stroma and delivery of CSCs/CPCs can regenerate cardiomyocytes. We further propose that the combination cellular therapy is necessary for optimal repair as delivery of CSCs/CPCs will result in minimal repair because of the lack of a niche and the absence of appropriate growth factors and cytokines for these cells to proliferate and differentiate.

## Acknowledgments

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