REVIEW ARTICLE

Stem Cells

Review and Update

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Regenerative medicine and emerging biotechnologies stand to revolutionize the practice of medicine. Advancements in stem cell biology, including embryonic and postnatal somatic stem cells, have made the prospect of tissue regeneration a potential clinical reality. Short of reproductive cloning, these same technologies, properly used, could allow for the creation of replacement tissue for the deficient host. To provide a concise review for surgeons on the current science and biology of stem cells, we surveyed the scientific literature, MEDLINE, and relevant political headlines that illuminate the stem cell discussion; the issues are summarized in this review. Building on this conceptual framework, the related issues of clinical promise and the political debate enveloping this emerging technology are examined. A basic understanding of stem cell biology is paramount to stay informed of this emerging technology and the national debate.

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Stem cell biology is currently one of the most exciting areas of biomedical research, as enthusiasm for the application of this technology toward regenerative medicine continues to expand. The application of cells in a therapeutic fashion may become a natural extension of the presumed potential of these unique cell populations with wide-ranging capabilities. As with many new and exciting technologies, much remains to be tested, proved, and delivered to separate the hope from the hype. In this review, we attempt to deliver the current "state of the art" in stem cell research and to provide a conceptual framework that can be used by surgeons as a basis for critical assessment of this quickly expanding and fascinating field.

The first large mammal cloning experiment, widely publicized in 1997, provided new impetus to the prospect of regenerative medicine through stem cell research.[1] In the case of Dolly, an entire adult ewe was successfully cloned as an exact phenotypic and genetic match of its founder organism.[1] This startling achievement was a reminder that DNA is conserved during the development of complex multicellular organisms. If an entire adult ewe could be recapitulated from a postnatal somatic cell, then clearly the genetic potential should persist to regenerate whole tissue and organ systems. Nuclear transfer, the same technology that created Dolly, could be used to create the raw material to replace defective or senescent tissue as a natural extension of the biology of stem cells.[2-5] The specter of human cloning and nuclear transfer as a means of creating autologous embryonic stem (ES) cells (each individual's identically matched ES cells) has also stirred a parallel political debate.[6,7] The controversy divides the potential hope given to many present-day patients against the requisite and ethically contentious creation of human blastocysts for therapeutic intent.[6]

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ES CELLS: CONCEPTS AND DEFINITIONS

Embryonic stem cells are totipotent cells that can be derived from the inner cell mass of a blastocyst during gastrulation.[8] If separated from the remainder of the blastocyst, with concomitant inherent arrest of

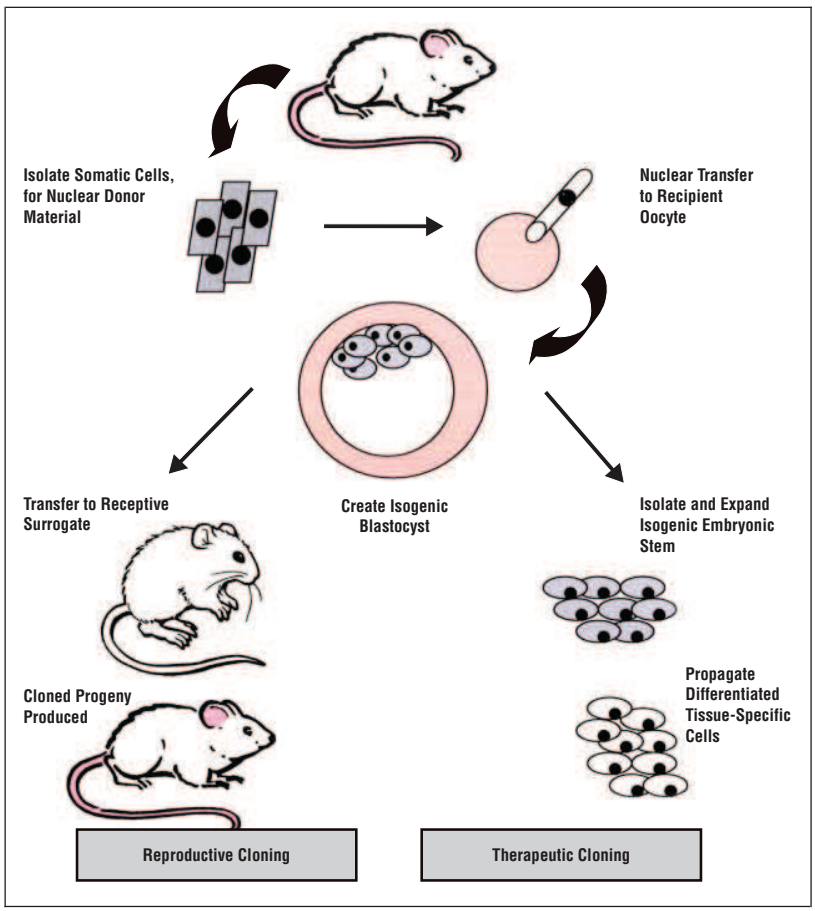

Figure 1. The divergent processes of reproductive and therapeutic cloning. The common steps of somatic cell nuclear isolation and injection into an enucleated oocyte are demonstrated. Once a blastocyst is created, it can be used for either distinct process.

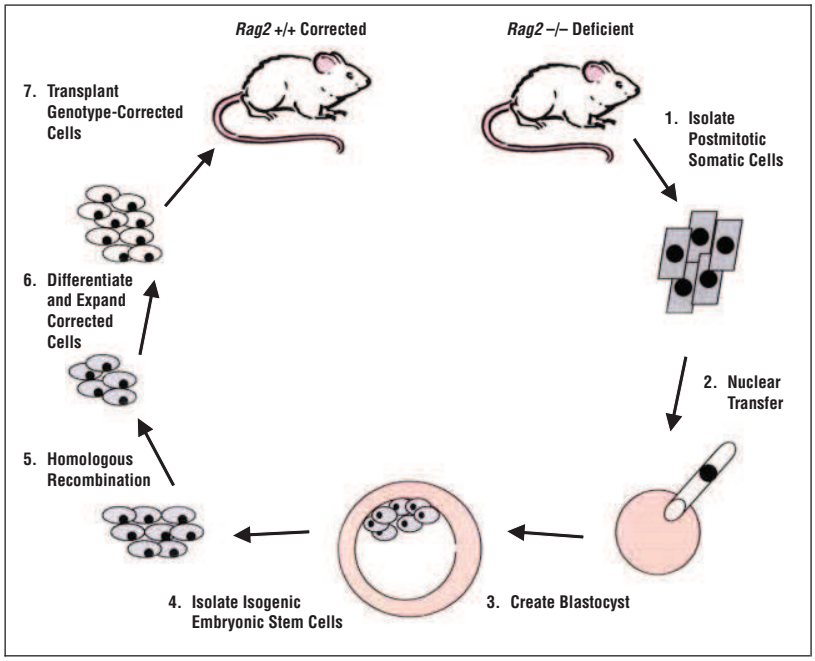

Figure 2. Nuclear transfer, gene therapy, and cell transplantation as a possible clinically applicable paradigm for genetic and subsequent phenotypic correction. This schema was successfully used as proof of principle in a murine model by investigators at the Massachusetts Institute of Technology.[16]

further embryonic development, the inner cell mass can be maintained in a largely undifferentiated state, forming embryoid bodies in which early embryonic cell lineages can develop.[5,8] Embryonic stem cells represent a potential source of cells with practically unlimited self-renewal and differentiation capacity. Able to give rise to all of the somatic and germ line cells of the fully developed organism, these cells are the "uncommitted" progenitors of the subsequent 3 embryonic germ layers: ectoderm, endoderm, and mesoderm.[4,5,8,9] The ES cell is the prototypical stem cell, as defined by its ability to indefinitely expand, self-renew, and give rise to more specialized progeny cells.

Nuclear transfer is a process wherein the nucleus of a postmitotic somatic cell is injected into an unfertilized, enucleated oocyte (**Figure 1**).[1,5,10] Through this nuclear manipulation, a blastocyst can be achieved that may realize one of several alternative fates. If the blastocyst is transferred to a receptive maternal surrogate, fully replicated progeny can be achieved in a process of *reproductive cloning* (Figure 1).[1,11] Alternatively, if the inner cell mass is isolated and separated from the blastocyst, then undifferentiated ES cells can be derived.[10,12] This totipotent ball of cells is capable of reproducing individual cells and, therefore, tissues of the postnatal organism from which it was derived in a process known as *therapeutic cloning* (Figure 1).[5,8,9] Through this process, each individual could potentially create an autologous source of his or her own fully immune-compatible ES cells. It is paramount to distinguish the divergent processes and outcomes of reproductive and therapeutic cloning from the same technique of nuclear transfer (Figure 1). Uncertainty persists as to the true histocompatibility of clonally derived ES cells given the persistence of mitochondrial DNA in the recipient enucleated oocyte.[13,14] Moreover, the biological potential and the competitive characteristics of cloned cells compared with ES cells derived from blastocysts as a result of gamete fusion remain unknown.[11,15]

Recently, investigators[16] at the Massachusetts Institute of Technology demonstrated proof of principle for therapeutic cloning by correcting the gene defect in *Rag2* immunodeficient mice (complete lack of B and T cells) (**Figure 2**). Through a combination of

nuclear transfer to create major histocompatibility complex–compatible ES cells, homologous recombination for genetic correction, in vitro differentiation to hematopoietic stem cells (HSCs), and final transplantation to *Rag2* recipients, mice were successfully genetically and phenotypically corrected (Figure 2).[16] Whereas this set of experiments demonstrates the utility of nuclear transfer for therapeutic cloning, equally numerous studies now demonstrate the frailty of nuclear transfer for reproductive cloning. Reproductive cloning has been found to be mostly a highly inefficient process, moreover producing abnormal progeny in what is becoming known as the "large offspring syndrome."[15] In whole-organism cloning, there seems to be epigenetic derangements, resulting in fetal overgrowth, placental defects, and a myriad of at least common skeletal abnormalities.[15,17,18]

Despite the unquestioned totipotency of ES cells, there are numerous unanswered biological questions as to the regulation of their growth and differentiation. The safety profile of unselected ES cells for transplantation has early on demonstrated dysregulated cell growth with transplantation to the immunocompromised host, resulting in teratoma formation.[19] This example speaks to the need to explore strategies for ES cell predifferentiation or selection for lineage specification before attempts at in vivo use. Early studies[20,21] in murine ES cells have developed cell trapping mechanisms based on lineage-specific gene activation. Strategies currently being investigated seek to predifferentiate ES cells in vitro before functional in vivo testing.[22-24] Many of the master transcription factors, such as stem cell ligand, first identified as controlling differentiation of postmitotic HSCs are now being exploited to control differentiation of ES cells.[25,26] Furthermore, gene profiling of many stem cell lines for master transcription factors, such as the *Oct4* gene, are under way in an effort to understand the signals that control cell proliferation and differentiation in ES cell and postnatal stem cell sources (ie, HSCs).[25-27]

Substantial roadblocks must be overcome before attempting clinical application. Foremost is the need for bulk cultures of ES cells for studies addressing basic biological questions. Given the wide variety of genetic variability and epigenetic changes that occur in ES cells, large numbers of cell lines in addition to those with current federal government approval for study are needed to successfully study these complex biological processes.[17,18,27] Beyond the use of ES cells for replacement biology is their practical utility for the study of the genetic basis of human disease. The current US government moratorium on federal funding for human ES cell studies stands to considerably impede the pace at which this critical work can proceed.[28,29]

Even with governmental support, practical and biological barriers to wide applicability can be foreseen. For example, current culture techniques for human ES cells require a xenoculture feeder cell system that would meet with considerable Food and Drug Administration restrictions.[10] In the murine system, ES cell expansion can be supported by the growth factor leukemia inhibitory factor, a supplement that does not work with human ES cells.[23] Since the first description of successful human ES cell isolation, subsequent work has struggled to define an efficient system for human cell growth and expansion.[10] These examples substantiate concerns that the mouse and human systems are sufficiently diverse in that there cannot be a reasonable expectation that lessons learned from work with murine ES cell systems will find direct translation to the human systems. From a practical standpoint, even long-term batch cultures of allogeneic cells would be beset by histocompatibility barriers to effective and widespread ES cell transplantation strategies. This issue further speaks to a current shortcoming of strategies to "scale up" ES cell production for clinical use unless autologous ES cells are created on an individual need basis via nuclear transfer, for example. An alternative strategy would be to create large-scale banks of ES cell lines, most likely derived from in vitro fertilization blastocysts, for HLA typing and cell matching to potential recipients.

Despite their initial promise, ES cells have met with mixed enthusiasm for their use and investigation given the considerable moral objections surrounding their derivation and procurement.[6,7] In 2001, the executive and legislative branches of the US government introduced the Human Cloning Prohibition Act (the Brownback Bill), which called for a ban on nuclear transfer for the production of therapeutic stem cells and on human cloning altogether.[6] Similarly, in 2001, US President Bush[28] limited "allowable" federally funded research on human ES cells to the "more than 60 genetically diverse stem cell lines that already exist." White House policy also established eligibility criteria for additional lines in development to qualify for federal funding.[29] Most researchers dispute the claims by President Bush and argue that more reasonable estimates place the number of usable human ES cell lines at a much smaller number.

In August 2002, the US Senate defeated the Brownback Bill, effectively preventing a promised presidential signing into law. The US Congress will continue to revisit these earlier decisions just as more lenient regulations and governmental investment in the United Kingdom, Australia, and Singapore are facilitating a broadening interest in human ES cell research.[30-32] Many researchers argue that US restrictions will stymie stem cell research in the United States and result in, at minimum, a flight of intellect to countries with more liberal regulations.[33] Other investigators see the United States falling behind in a critical technology that, if ignored, will be developed elsewhere only to be imported or altogether unattainable in the United States because of the political atmosphere.[6] For now, there will be lobbying on both sides of the argument in the United States that should be influenced by a broad national dialogue on the societal, ethical, and scientific issues raised by this technology. Although few individuals outside the theological fringes would argue the merits of human reproductive cloning, the scientific community, via the National Academy of Sciences, has argued strongly for (1) a continuation of human ES cell research with allowable nuclear transfer for ES cell derivation for therapeutic intent and (2) a continued ban on human reproductive cloning.[34-36]

POSTNATAL SOMATIC STEM CELLS

In response to these political and biological roadblocks, investigators have sought other possible sources of pluri-

potent cells.[2,9,37,38] Tissue-specific stem cells have long been recognized to exist in postnatal and adult animals.[9] In their tissue of residence, these cells function as lineage-committed progenitors to cells capable of more highly specialized tasks. Many examples exist in such disparate locations as the gut crypt cell, the skin bulge cell, and the hepatic oval cell.[9] Perhaps the best characterized and most widely understood are the HSCs. These cells, by their known biology and clinical success, have provided for a classic paradigm of sorts to which all other candidate postnatal stem cell populations are held. The HSCs fulfill the requirement of asymmetrical cell mitoses, whereby each cell division is capable of producing another pluripotent progenitor and a more specialized daughter cell.[39,40] Furthermore, the true HSC is capable of indefinite self-renewal, and until recently it had a restricted ontogeny that included all the cells of the myeloid and lymphoid systems.[39]

The concept of adult tissue-specific stem cells has been a fundamental premise and has served as the model of renewal for postnatal tissue. Developmental biology has long held that lineage determination is an irreversible commitment to a particular tissue type fixed by trilaminar embryonic differentiation. A series of recent startling findings[41-45] have challenged this dogma, as cells that originate in the bone marrow (BM) have taken on new tissue-specific phenotypes as broad ranging as neurons, hepatocytes, myocardium, and skeletal muscle. In an experiment of nature, multiple postmortem examinations of hepatic, cardiac, and brain tissue in patients who had received cross-sex BM transplants have revealed some startling findings.[46-49] The findings of *Y* chromosone–positive hepatocytes, cardiomyocytes, and neurons in female recipients of male BM transplants have caused long-standing biological dogmas to be questioned. These observations have led many researchers to question the stochastic or possibly physiologic relevance of these seeming cell fate switches.[50]

In one of the more robust experimental demonstrations of cellular plasticity, Lagasse and colleagues[43] used whole BM enriched for HSCs to rescue a murine model of fatal hereditary tyrosinemia by regenerating deficient hepatic mass. Similar previous descriptions of cell fate switches have almost uniformly been described for the HSC. These observations have disarmed long-held beliefs in the lineage-restricted stem cell with finite specialization repertoire. These lineage changes have been termed *cellular transdifferentiation* and have served to renew interest in the HSC for more widely applied clinical intent other than BM reconstitution.[51-54]

There have been numerous equally varied and compelling results[55-58] on the identity of the cell source present in BM that is capable of lineage transdifferentiation with adoption of heterotopic cellular phenotypes. Whole BM has been fractionated based on specific cell surface antigens that have traditionally identified hematopoietic progenitors, with deletions of lineage-committed cells by the same cell-sorting mechanism of surface epitopes. A variety of cells with similarities to HSCs found in BM have been isolated from such disparate tissue as skeletal muscle and adipose.[52,59] Other isolation methods have sought to define a functional aspect of cell populations based on gene expression under tight control during cell differentiation. One example of these critical proteins are the adenosine triphosphate–binding cassettes (ABC transporters similar to the *BCRP* gene), marking cells as primitive *side population* cells when isolated by flow cytometry.[52,60] Despite these efforts, there are no clear answers as to the identity of the cell type capable of observed phenotypic plasticity. Other researchers argue that cell transdifferentiations may represent a "function" or capability of various cell types rather than a capability limited to a specific subset of cells.[50] Stated differently, perhaps various primitive cell types that persist in the postnatal animal may maintain enough genome plasticity to be capable of reprogramming toward alternative phenotypes. Still other evidence[61,62] has demonstrated that certain cell types are capable of fusing with alternative lineage cells and of subsequently adopting the form and function of the originator cells. Whichever is the true mechanism or cell identity that accounts for postnatal cell plasticity, it seems reminiscent of the lessons learned once again from the cloned sheep Dolly about DNA conservation and reactivation in the postnatal organism.

If mesenchymal cells from BM can in fact become, for example, endodermal hepatocytes, it would be advantageous to prospectively isolate the cell populations capable of this feat. Despite all that is known about the multipotent HSCs residing in BM, there exists a second population of unique progenitor cells in BM, mesenchymal stem cells (MSCs).[3,37,63,64] Originally believed to represent the stromal or supportive cell substrate for the HSC, the MSC has recently been rediscovered of sorts, as has its far-reaching capacity to become multiple mesenchymal lineages.[65] Much work has been done in an effort to isolate and prospectively define the cell type that derives from BM and gives rise in vitro to adipocytes, chondrocytes, and osteoblasts.[65-78] A true MSC, if it exists, would be a highly beneficial biological tool with potential clinical applications for the regeneration of connective tissue. Being that connective tissue is, relatively speaking, metabolically quiescent, in contradistinction to liver, for example, it is little surprise that structural tissue engineering applications for MSCs have garnered such early attention. If MSCs demonstrate sufficient cellular phenotypic plasticity, their potential for use as raw material for tissue engineering would seem a logical extension of this biology.

Despite an apparent capacity to be reprogrammed from what was previously thought to be a terminally differentiated cell type, transgermal plasticity to endoderm and ectoderm has not been observed for postnatal cells until recently. Jiang et al,[79] at the University of Minnesota, recently showed that perhaps BM does contain a pluripotent cell type with capabilities similar to those of the more volatile ES cell. These investigators chose to term their specialized cells "multipotent adult progenitor cells" (MAPCs) to distinguish them as unique from what has been previously identified as MSCs. Unlike MSCs, MAPCs have demonstrated the long-sought transgermal plasticity of more primitive ES cells.[79,80] These same MAPCs could be isolated from numerous tissue compartments, including BM, muscle, and brain.[80] In addition, they were capable of adopting varied functional phenotypes of neu-

rons (ectoderm), hepatocytes (endoderm), and multiple mesenchymal lineages of stromal and hematopoietic lineages.[79] The origin of these cells and whether they exist in various tissue compartments or are created as a by-product of the culture process remain unknown. Once again, a broader theme of biological redundancy and genome plasticity seems to be represented by these findings. Not all investigators agree, and the skeptics are many. Perhaps to avoid some of the controversy altogether, the Minnesota group chooses not to refer to their cells as *stem cells* at all, choosing instead the more generic term *multipotent progenitors*. These descriptions of MSCs and MAPCs have served to further fuel the debate over what constitutes a true stem cell.

DEFINING THE STEM CELL

As the number of studies claiming cell transdifferentiation have flourished, so have the objections of the most outspoken critics of stem cell biology.[51,81,82] The same scientists with some of the more distinguished records of investigation in stem cell biology have vocally expressed concerns over the rigor of the science and the justifiability of the claims being published.[81] Strict criteria have been called for in order for claims of transdifferentiation to be considered plausible.[83,84] Cells need to be prospectively isolated, purified to homogeneity, and well characterized before in vivo testing.[81,83,84] The analysis of cell fate or function in animal models that place the population of cells in a stressed environment or provide for unique cellular environments with their associated signals has been the proving ground for cell populations of interest. Once localized to a particular tissue, the candidate pluripotent cell must demonstrate tissue-specific function.[81] Furthermore, the cell must contribute substantially to the function of the host tissue.[51,81] The essential characteristics that a cell must demonstrate before being considered a "stem cell" in the classic sense have also been called for. The candidate cell must be capable of asymmetrical cell division, producing an exact multipotent replica cell and an additional progeny cell that can perform a more specialized function.[39,40,85] In this way, cells for tissue specialization are achieved without loss of the full potential of the founder cell population. Despite these calls for a uniformity of approach and lexicon, the relevant literature persists with a certain ambiguity of claims and terms.

PROSPECTIVE CLINICAL APPLICATIONS OF CELLULAR THERAPY

Irrespective of the true "stemness" of these unique cell populations, they may have significant utility in a variety of clinical applications.[2,86-88] Several therapeutic strategies are immediately apparent that may exploit the unique "stemlike" activity of the various cell populations under study. Given their capacity for self-renewal, proliferation, differentiation, and wide distribution, it would be appealing to adopt a gene transfer strategy into HSCs or MSCs.[86] This same strategy has been successfully demonstrated in murine systems as proof of principle (see the *Rag2* example in the "ES Cells: Concepts and Definitions" section and Figure 2).[16] Numerous heritable gene defects and other acquired diseases would be seemingly amenable to this approach.[86,87] Postnatal somatic stem or progenitor cells could also be used in a form of cellular therapy for local tissue repair and regeneration.[2,89,90] Numerous examples of this approach have been initiated. For example, MSCs could be implanted locally to promote or augment repair or regeneration of a fractured or osteoporotic bone.[2,91,92]

In a series of experiments, Pereira et al[74,93] infused donor wild-type MSCs into a transgenic mouse model of osteogenesis imperfecta. In this syngeneic transplant model, transplanted cells populated the BM and replaced defective osteoblasts in weakened bone and cartilage as a result of defective type I collagen synthesis.[74,93] This work resulted in an abbreviated clinical trial[94] for the treatment of collagen defects in children with osteogenesis imperfecta, which demonstrated a decreased fracture rate and increased bone density in children in whom transplantation was successful. This early work and seemingly simple clinical success has provided the requisite proof of principle that a deficient cell population could be replaced by an exogenous source of cells. These experiments exploited several stemlike abilities of MSCs. The transplanted MSCs were able to serve as precursors to the more specialized osteoblasts and were successfully engrafted in sites of deficient collagen synthesis. For the therapeutic cell fraction to engraft, myeloablation was performed to tolerize to the therapeutic cellular fraction.[94] This necessity once again demonstrated one of the more prohibitive biological hurdles to the widespread clinical applicability of a cellular replacement strategy, that is, cell transplant rejection.

If cellular therapy is to become a reality, many of the same hurdles of graft tolerance that face solid-organ transplantation will have to be addressed. Most experiments that have been performed with HSCs as a cellular source have thus far involved a marrow ablative regimen such that hematopoietic chimera are created.[47,95,96] This strategy has provided the parallel benefit of tolerance to the intended therapeutic cellular transplant. If a cell source other than HSCs is intended to be used, this same marrow replacement strategy quickly becomes ineffective. Now that other potential sources of multipotent cells have been identified, alternative strategies for their use in an allogeneic setting still need to be explored. Autologous ES cells derived from therapeutic cloning may address many of the immunologic concerns. However, even autologous ES cells would require ex vivo manipulation to be used for replacement of deficient cells or their gene products.[86] These cells have thus far shown themselves to be somewhat resistant to efficient gene transduction.[86]

SUMMARY

As the political debate about stem cell research continues, the scientific discoveries and substantiation of earlier claims will proceed. Obvious potential clinical benefits may result from much of this work, but in a larger sense the rethinking of long-held biological paradigms may prove to be ultimately as valuable. The concerns

voiced by others to proceed with caution and await the rigors demanded by good science should be the precedent. The alternative is to allow the hype to embolden claims and hopes that may not be deliverable if a "stem cell bubble" goes unchecked. A great deal of basic research is needed to further explore the current candidate cell populations before potential clinical benefits of stem cell research can begin to be realized.

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