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Translational strategies and challenges in regenerative medicine

Stefanie Dimmeler[1,6], Sheng Ding[2,6], Thomas A Rando[3,4,6] & Alan Trounson[5,6]

The scientific community is currently witnessing substantial strides in understanding stem cell biology in humans; however, major disappointments in translating this knowledge into medical therapies are flooding the field as well. Despite these setbacks, investigators are determined to better understand the caveats of regeneration, so that major pathways of repair and regrowth can be exploited in treating aged and diseased tissues. Last year, in an effort to contribute to this burgeoning field, *Nature Medicine*, in collaboration with the Volkswagen Foundation, organized a meeting with a panel of experts in regenerative medicine to identify the most pressing challenges, as well as the crucial strategies and stem cell concepts that can best help advance the translational regenerative field. Here some experts who participated in the meeting provide an outlook at some of those key issues and concepts.

Harnessing the potential of endogenous stem cells

It has been almost 40 years since Ray Schofield put forth the idea of a stem cell 'niche', a concept taken from ecological science and the relationship of a species to its habitat[1]. This analogy is certainly relevant in that the function of stem cells cannot be characterized or predicted without a detailed understanding of their relationships to and interactions with their respective niches. And, just as in ecology, the interaction is bidirectional—the niche influences the stem cell, and the stem cell influences the niche. These points are brought into sharp relief in the context of experimental stem cell therapeutics, where the focus is on influencing this dynamic environment, typically in a 'gain-of-function' manner—for example, to enhance stem cell activity for the treatment of injury, disease or age-related dysfunction[2]. Whereas most current studies of stem cell therapies focus on the transplantation of exogenous somatic or differentiated pluripotent stem cells, the primary focus here is on the enhancement of endogenous stem cells for therapeutic purposes and the challenges of that approach.

Targeting the stem cells. Targeted activation of endogenous stem cells to repair the tissues in which they reside requires an understanding of the molecular pathways that normally control stem cell function and how those signals might have changed in the setting of injury or disease, perhaps rendering the stem cells less responsive to extrinsic cues. For example, adult muscle stem cells (MuSCs) require activation of Notch signaling for proliferative amplification to respond to muscle injury, but aged MuSCs show a marked delay in this response, resulting in impaired tissue regeneration, which is reversed upon pharmacological activation of Notch[3]. Similarly, adult neurogenesis, which declines precipitously with age, can be enhanced by exercise in a manner that can be attenuated by blocking insulin-like growth factor-1 (IGF-1) or vascular endothelial growth factor (VEGF)[4–6], and the administration of exogenous IGF-1 can enhance proliferation of neural stem cells (NSCs)[5]. The development of small molecular activators of endogenous stem cells holds great promise as well[7]. A major challenge for the development of targeted therapeutics to enhance endogenous stem cell function is the modeling, *in vitro*, of the environment that sustains the stem cells in a state of reduced responsiveness *in vivo*. Typically, plating stem cells in culture induces them to begin dividing and differentiating, thus altering the cellular state that would be the therapeutic target. The ability to generate multicellular structures *in vitro* that possess many of the features of stem cells and their niches *in vivo*, as is under development for the study of intestinal stem cells and colorectal cancer[8], will markedly accelerate the optimization of therapies directed at the endogenous stem cells themselves.

Targeting the stem cell niche. Although it may be possible to promote the activity of endogenous stem cells to enhance tissue repair, it is becoming increasingly clear that as much attention needs to be paid to the environment in which the cells reside as to the nature of the cells themselves[9]. As noted above, the ability to model the multicomponent nature of a stem cell niche *in vitro*, for example to screen for biologics or small molecules to promote tissue restoration or repair, has remained a major and largely unmet challenge. All of the issues, some of which are discussed below, that relate to the importance of the environment for developing therapeutics to enhance endogenous stem cell function

[1]Institute for Cardiovascular Regeneration, Centre of Molecular Medicine, Goethe University Frankfurt, Frankfurt, Germany. [2]Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, San Francisco, California, USA. [3]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California, USA. [4]Glenn Center for the Biology of Aging at Stanford University, Stanford, California, USA. [5]California Institute for Regenerative Medicine (CIRM), San Francisco, California, USA. [6]These authors contributed equally to this work. Correspondence should be addressed to S. Dimmeler (dimmeler@em.uni-frankfurt.de), S. Ding (sheng.ding@gladstone.ucsf.edu), T.A.R. (rando@stanford.edu) or A.T. (alan.trounson@gmail.com).

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also pertain to the transplantation of exogenous stem cells to replace or augment the endogenous cells.

What lies within the niche. The physical niche for any somatic stem cell is composed of two basic components: an acellular extracellular matrix (ECM) and local cellular constituents. The molecular components of the matrix have a profound effect on the biology of stem cells in regulating their quiescence, proliferation, symmetric and asymmetric divisions and fate[10]; conversely, stem cells typically produce constituents of their own matrix. The cells found in the niche, such as differentiated cells of the tissue, interstitial mesenchymal cells and cellular components of the vasculature, may influence stem cell functionality by direct contact or by locally secreted paracrine factors. For example, osteoblasts within the niche of hematopoietic stem cells (HSCs) have a crucial role in HSC homeostasis[11]. Finally, stem cells and all components of the niche are influenced by soluble factors from the circulation, allowing for the regulation of stem cell function at a distance. Alterations in any combination of these components may critically limit stem cell function and may therefore be targets to enhance stem cell functionality. The question is how we identify the environmental cues and the resulting signaling pathways that affect stem cell function and promote endogenous regeneration. Studies of diseases, disorders or conditions that alter these cues can provide insight into the normal physiological processes that regulate stem cell function and may be therapeutic targets. This focuses attention on key molecular pathways or cellular constituents whose disruption is associated with stem cell dysfunction.

Modeling and targeting the disrupted niche. A prime example of altered environmental cues occurring in the setting of many degeneration diseases is fibrosis, a process associated with alterations in the composition and extent of the ECM and, invariably, with defects in tissue regeneration. For example, a wide range of diseases and toxic injuries result in fibrosis of epithelial tissues in the skin, lung, liver and kidney[12], and the resulting pathology thwarts the ability of resident stem cells to maintain normal tissue homeostasis. Modeling of such changes in ECM composition has revealed both biochemical and biophysical signals, such as matrix patterning and stiffness[13,14], that are sensed by stem cells and that alter the fate of their progeny, including aberrant differentiation, cell senescence or apoptosis[10]. Thus, numerous antifibrotic drugs and interventions to prevent or reverse tissue fibrosis that are being developed and tested could have the potential to enhance endogenous stem cell function by improving the properties of the niche in a wide variety of degenerative diseases and aging.

An even more dramatic defect occurs in the tissue matrix after traumatic injury, which can completely eliminate the very scaffold in which stem cells reside. Stem cell–mediated regeneration in such a setting is typically aborted at an early stage. For example, blast injuries in combat often result in very limited soft-tissue regeneration because of the absence of a scaffold to guide stem cell–mediated repair. To enhance stem cell–mediated restoration, bioengineering approaches are now exploring strategies to provide appropriate structure in the form of natural and synthetic scaffolds to foster stem cell growth, differentiation and self-renewal[15]. Likewise, full-thickness skin wounds do not repair properly, and new biomaterial systems are being tested for their ability to successfully recapitulate the microenvironments to facilitate stem cell–mediated skin regeneration[16]. In such settings, the development of *ex vivo* approaches to regenerative biology, including three-dimensional (3D) culture systems with multiple cellular components and the use of 3D printing to create defined models of stem cell niches[17], is likely to complement approaches to enhancing endogenous stem cell activity.

Harnessing the vasculature of the niche. In many tissues, such as bone marrow, skeletal muscle and brain, stem cells are intimately associated with the capillary endothelium, which may directly secrete signals to local stem cells[18]. The proliferative progeny of NSCs are found in a vascular niche associated with vascular endothelial cells that are also proliferating, suggesting a co-regulation of neurogenesis and angiogenesis[19]. As such, one potential approach may therefore be to enhance stem cell function by promoting angiogenesis within a tissue, which has shown promise for enhancing neural stem cell function both *in vivo* and *in vitro*[18]. Co-cultures of vascular progenitors with tissue-specific stem cell progenitors have been shown to enhance tissue regeneration[20] and can serve as an *in vitro* model for understanding the role of the vasculature in the stem cell niche and how that aspect of the niche is disrupted in pathologic conditions that either primarily or secondarily affect the local microvasculature.

The tissue vasculature also has a profound influence on stem cell function by virtue of its physiological role in regulating the delivery of oxygen to a tissue. There is evidence that low-oxygen tension is an important factor in maintaining quiescence of HSCs and NSCs, in a manner that is dependent on hypoxia-inducing factor-1α[21]. Although often referred to as hypoxic, this low-oxygen environment can be considered normoxic for stem cells as a physiological adaptation. Different partial pressures of oxygen *in vitro* elicit different behaviors from stem cells, with functional loss occurring at both lower and higher concentrations of oxygen[21]. Therefore, altered wound healing in diseases such as diabetes mellitus, in which there are disruptions in the microvasculature of tissues, may be due, in part, to changes in the local oxygen levels in the stem cell niche. The study of stem cell function *in vitro* by the use of microfluidics is certain to provide insight into the real-time dynamics of stem cell function by mimicking *in vivo* conditions of the delivery of oxygen and nutrients that occurs in vascularized tissues[22].

Finally, any discussion of the impact of the vasculature on stem cell function must also consider the role of the circulation in delivering signals, including endocrinological and immunological signals, to tissues to regulate stem cell function in the setting of tissue injury, disease or aging. In particular, heterochronic studies, whether by cell transplantation, parabiosis or transfusion, have highlighted the role of systemic factors that either promote stem cell functionality in young tissues or suppress stem cell functionality in aged tissues[23–25]. Researchers have identified cytokines and growth factors in the blood, such as C-C motif chemokine 11 (CCL11, or eotaxin) and growth differentiation factor 11 (GDF11), that change with age and whose modulation can, in turn, alter endogenous NSC activity[24,26]. Likewise, the concentration of oxytocin declines with age in the blood, and administration of oxytocin to aged mice can enhance MuSC function[27]. The search for such endogenous regulators and for small molecules that mimic their effects to enhance stem cell function will be greatly facilitated by the development of *in vitro* models of these *in vivo* heterochronic studies[23]. Furthermore, extending all of the biological and bioengineering approaches mentioned above to model that aged niche *in vitro* will certainly allow for more high-throughput screening of compounds that may have therapeutic potential to enhance aged tissue repair by promoting endogenous stem cell function.

Endogenous regeneration in the spotlight. Harnessing the potential of endogenous stem cells to repair and restore tissue structure and function holds great promise for the treatment of tissue damage and degeneration. The stem cell environment, both local and systemic, contains a rich and diverse set of cues that impinge constantly on stem cell activity and that may be modulated for therapeutic gain. Of course, major challenges exist. With safety as the first concern for any new therapy, long-term systemic treatment that enhances the function of an endogenous stem cell population will need to be evaluated for the probable, untoward effect of stimulating the division of other cells in the body. Clearly, carcinogenesis is at the top of the list of potential adverse effects. With that in mind, spatially and temporally restricted therapies, such as

for the healing of a wound, are likely to be of lower risk. To the extent that it is possible to find targets, such as a specific receptor isoform, that are specific to the stem cell population of interest, the hope for a higher therapeutic index increases.

All issues and biological and bioengineering approaches mentioned above address the major challenge to model the complexity of stem cell niches *in vitro*, without which the development of meaningful high-throughput screening for compounds to stimulate endogenous stem cells will not be possible. Finally, the ability to monitor the efficacy of endogenous stem cell activity noninvasively over time will greatly facilitate the translation of therapies from laboratory animals to humans. Nevertheless, at the heart of advances in the development of therapies to enhance endogenous stem cell function will be a more detailed understanding of the cells themselves as well as signals within the stem cell niche and from the systemic milieu, and how those signals are interpreted and transduced to realize this goal of regenerative medicine. —*TAR*

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Deciphering therapeutic reprogramming

Recent advances in stem cell biology and engineering, aided by new concepts and discovery approaches in other fields, may make possible new therapeutic developments in regenerative medicine. Our improved ability to generate safe and functional cells for transplantation from various sources to replace lost cells or to promote the repair and regeneration of target tissue through paracrine mechanisms can increase the therapeutic potential of cell-based therapies. Transplantation of cells is not only particularly useful when endogenous repair and regenerative mechanisms are not adequate (as during acute and massive tissue damage from disease or injury), but also may provide superior specificity and efficacy as compared to conventional drugs. This is partly because these cell therapies, which aim to be molecularly and functionally identical to the relevant cells in the targeted tissue, would behave as endogenous cells and can modulate more than one relevant target by, for example, secreting multiple factors.

Particularly exciting are developments in therapeutic reprogramming of diseased or healthy cells—either *ex vivo* or *in vivo*—to restore normal cell function or to license cells' enhanced ability to rescue diseases or tissue or organ injuries. The discovery of induced pluripotent stem cell (iPSC) technology, which allows reprogramming of differentiated somatic cells into cells with the ability to become any cellular component of organs and tissues, has invigorated interest in exploiting cellular plasticity to enhance functions for therapeutic purposes. Continued understanding of *in vivo* cellular behaviors and fates will lead to better cell sources and therapeutic strategies to manipulate them, but much of the current effort is focused on challenges associated with limited cell number, poor initial cell survival and/or long-term engraftment upon transplantation, impaired function due to senescence and imprecision in controlling cell fate.

The *ex vivo* paradigm. Conventional (unmodulated) cell-based therapies, such as HSC therapy, have shown substantial clinical validation in patients with hematologic diseases, but inadequate cell survival, homing, proliferation and differentiation limit their clinical efficacy. Moreover, upon transplantation these cells may have lower therapeutic activity, such as the secretion of specific functional molecules, because of intrinsic properties or cell manipulations that can result in culture-induced cellular senescence and functional impairment such as that caused by freezing and thawing. Reprogramming cells *ex vivo* can potentially overcome these issues and add to the standard clinical practice of cell transplantation by incorporating these modulated cells that are licensed with enhanced abilities. Safety—especially over the long term—would need to be monitored and managed before 'super' cells with enhanced capabilities could be used routinely.

Progress has already been made in preclinical and clinical studies to support this *ex vivo* reprogramming paradigm. For example, cancer immune therapy with T cells armed with either conventional αβ T cell receptors or chimeric antigen receptors has achieved remarkable clinical outcomes in advanced metastatic melanoma and B cell leukemias[28]. Similarly, modification of the HIV entry cell receptor CCR5 on $CD4^+$ T or hematopoietic stem and progenitor cells (HSPCs) using zinc-finger nucleases has resulted in resistance to HIV infection, which may provide a way toward functional eradication of the virus[29]. Other relevant examples include the increase in trafficking of stem and progenitor cells, which has been fertile ground for enhancing stem cell therapy. Particularly, enhancing the chemotactic SDF-1–CXCR4 signaling axis has been used to augment stem cell homing and retention, and *ex vivo* temporal treatment of murine and human HSPCs with a stable analog of prostaglandin E2 has enhanced their engraftment *in vivo* by inducing expression of genes involved in HSPC homing, including CXCR4 (ref. 30). These findings rapidly led to the clinical studies to test whether this *ex vivo* treatment of cells could improve transplantation outcomes in adult patients with hematologic malignancies. Dipeptidylpeptidase IV (DPP4), a membrane-bound extracellular peptidase that cleaves and inactivates SDF-1, negatively regulates engraftment of donor HSPCs upon transplantation; consequently, a DDP4 inhibitor has been found to enhance HSPC homing and increase transplantation efficiency[31].

The *ex vivo* use of growth factors, microRNAs (miRNAs) and cytokines can modulate the function of stem cells, as has been shown for interferon-γ, which substantially enhances the immune suppressive function of mesenchymal stromal cells to suppress autoimmune and alloimmune diseases[32]. Similar treatment of proangiogenic cells, such as endothelial cells, with various miRNAs or antagomirs can also enhance their *in vivo* survival and homing and angiogenic activity or reverse their senescence phenotype[33]. Clearly, a better understanding of stem cell biology with respect to survival, homing, self-renewal, differentiation, senescence and cellular functions in the appropriate contexts will lead to improved strategies to fully harness stem cells' therapeutic potential through such *ex vivo* reprogramming strategies.

The *in vivo* paradigm. An alternative approach to cell-based therapy is to administer chemical, biological or gene therapies directly to patients to specifically modulate cell fates, states or functions by regulating survival, proliferation, differentiation, reprogramming, trafficking, niche interaction, quiescence and polarization. This strategy would ultimately offer less invasive and more convenient 'individualized' precision treatments for individuals with various diseases and needs. Modulation of stem cell trafficking *in vivo* using a small-molecule antagonist of CXCR4, and its combination with granulocyte colony–stimulating factor (G-CSF), have been used clinically to mobilize HSPCs[34]. Conversely, DPP4 inhibitors given directly to mice enhance the recruitment of CXCR4-expressing stem and progenitor cells (including endothelial progenitors) to ischemic heart tissue, which releases high level of SDF-1, leading to improved myocardial function and animal survival through neovascularization, serving as a reparative or regenerative strategy for myocardial infarction[35]. Interestingly, DPP4 has also been shown to have a more general role in regulating hematopoietic cytokines through cleavage of molecules such

as granulocyte-macrophage colony–stimulating factor (GM-CSF), G-CSF and erythropoietin, whose truncated forms act in a dominant-negative fashion to inhibit the activity of their full-length counterparts. The DPP4 inhibitor, sitagliptin, potentiates the activity of hematopoietic cytokines and accelerates hematopoietic recovery after chemotherapy or radiation in mice and is being further evaluated in clinical trials[36].

A more dramatic therapeutic reprogramming *in vivo* would be built on cellular transdifferentiation. This strategy, catalyzed by iPSC reprogramming using multiple cell type–specific transcription factors and miRNAs, induces adult cells such as fibroblasts to transdifferentiate into various cell lineages, including functional neurons, cardiomyocytes, hepatocytes and endothelial cells *in vitro*[7]. Similarly, *in vivo* reprogramming in mice of relevant tissue-resident cell types was found to generate neurons, cardiomyocytes and pancreatic beta cells in specific tissues and organs through ectopic expression of lineage-specific transcription factors *in situ*[7]. A gene therapy delivering the necessary reprogramming genetic factors and/or a pharmacological approach that can activate reprogramming transcription factors could ultimately be developed for such therapeutic reprogramming *in vivo*. Achieving targeted tissue delivery and exquisite reprogramming of selective resident cell type within the targeted tissue remains challenging.

An alternative to expressing or activating cell type–specific transcription factors in the target tissue is to mimic epimorphic regeneration. This regenerative mechanism, which is evident in some lower organisms but absent in mammals, allows cells at the injury site to undergo partial de-differentiation to generate lineage-specific precursor cells that can re-differentiate to replace lost cells[37]. This process inspired the paradigm of cell activation through transient overexpression of transcription factors and/or treatment with small molecules that induce deprogramming along with tissue-patterning cues to transdifferentiate somatic cells into diverse lineage-specific progenitor cell types without entering the pluripotent state. The basis of this strategy is that molecules that induce deprogramming can silence somatic cell-specific gene expression and induce epigenetically plastic states at the initial stages of reprogramming, which can respond to tissue-specific signals to transition toward different lineages[38]. This strategy has been applied to mouse and human cells to generate cardiac, neural, endothelial, pancreatic and hepatic cells[39], and could ultimately be applied *in vivo* to awaken and orchestrate a regeneration for each tissue type within their niche. The therapeutic development of this paradigm may require attention to precise temporal control.

Besides enhancing and regenerating cells *in vivo*, reprogramming strategies could also be applied to eliminate undesired cell populations, as in cancer, where therapies that selectively target 'addicted' metabolic and/or epigenetic mechanisms are bearing fruit. This is exemplified by the use of a chemical inhibitor of mutant isocitrate dehydrogenase, which produces a metabolite structurally similar to α-ketoglutarate that causes epigenetic alterations by inhibiting histone lysine demethylases and the ten-eleven-translocation (TET) family of 5-methylcytosine hydroxylases[40]. The elimination of tissue-specific senescent cells could also serve to promote tissue regeneration, as dysfunctional senescent cells impede regenerative processes, not only by occupying tissue compartments but also by secreting harmful molecules[41]. Targeting senescence may be a unique opportunity for treating aging-related and/or DNA damage-associated diseases, as senescent cells that accumulate in those setting are responsible for many aspects of the underlying pathologies.

The challenges ahead. Although cell-based therapy may have inherent limitations, such as issues for cell production and treatment of patients, there are also classic challenges to *in vivo* therapeutic reprogramming approaches, including achieving the necessary specificity for a given molecule, pharmacokinetic properties of the therapy and mechanism-based safety issues. For example, certain regenerative mechanisms may be harmful in other normal tissues and/or in an imprecisely controlled manner. To mitigate systemic liability that may occur when some of these potential therapies are given orally or intravenously, tissue-specific delivery strategies could be exploited: localized or topical applications to certain tissues (as in the case of the eye, inner ear, lung, knee or skin); cell type–specific delivery through, for example, antibody-drug-conjugates; prodrugs (such as those that use specific enzymatic activity of certain cell types to release the active drug piece) or soft-drug strategies (such as those with a short range or time of activity via inactivation) or even drugs with cell type–specific enrichment (for example, those that use a cell type–specific drug transporter). Another attractive strategy is to exploit synergistic activation of a specific pathway and/or mechanism involved in tissue regeneration, as has been shown for the small molecule QS11, which synergistically activates the Wnt–β-catenin signaling pathway only in the presence of Wnt ligands[42]. This strategy would be particularly useful in cases where an endogenous regenerative mechanism exists but has inadequate activity, as it could drastically amplify the mechanism in the target tissue but not in other tissues.

With these and other strategies being explored for better controlling cells, therapeutic reprogramming *ex vivo* or *in vivo* has a bright future with continued research and translation in stem cell biology and regenerative medicine. The *ex vivo* approach may be closer to reaching translational settings, as it would be built largely on existing clinical proof-of-concept studies and would address current particular limitations in a similar clinical setting. Knowledge gained from the *ex vivo* approach may well inform how such therapeutic molecules can be applied in a specific *in vivo* context. Furthermore, decades of practice and experience in the development of conventional drugs would also strengthen the *in vivo* reprogramming approach, which may become a more convenient and cost-effective form of regenerative medicine. —*S. Ding*

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Disease challenges cell integration and function

The goal of long-term engraftment. The limited functional integration of transplanted stem cells into the target tissue is a problem common to most, if not all, autologous and allogeneic stem cell therapies. Even with established clinical procedures, such as the transplantation of HSCs, the number of integrating cells is rather low and the therapy, in this case, is effective only because the locally generated cells can systemically and easily circulate throughout the body. After tissue injury, such as that occurring in myocardial infarction or stroke, a large number of new cells must replace billions of dead cells to rebuild organ function; however, the number of transplanted stem cells that acutely home and survive in these organs is generally quite low. For example, cell-tracing experiments have shown very limited long-term incorporation of diverse cells (2–10% in the first few days after transplantation and almost none after 3 months), including skeletal muscle cells, bone marrow–derived cells, cardiac stem cells and cardiomyocytes derived from pluripotent stem cells (PSCs) that

were transplanted in infarcted hearts of mice[43,44]. Engraftment of transplanted cells to the brain after stroke is also quite low, with <1% of infused bone marrow–derived cells detected 24 h after infusion in recent clinical studies[45]. Only undifferentiated ESCs, which formed tumors after transplantation in the heart, have been detected after longer-term follow-up[43].

It is not surprising that cells die soon after transplantation into ischemic or inflammatory environments, as they are vulnerable to lack of oxygen and the presence of inflammatory cytokines and reactive oxygen species after reoxygenation. Preconditioning of these cells with antiapoptotic and prosurvival factors such as the kinase Pim-1 can increase acute survival of transplanted cells in infarcted hearts[46], although this only delays—not prevents—the subsequent elimination of the cells, which are not detectable in the organ after 8 weeks[46]. Two important standing questions are: what causes the elimination of these cells in the long term when they are functionally incorporated during the first days after transplantation, and why they survive only in healthy tissue when forming tumors. Unfortunately, the reasons for this failure of long-term engraftment and integration are unclear. Functional integration in tissues is best during tissue and organ development; in mature tissue, where the united cell structure is formed, injected cells cannot be easily integrated. Consequently, integration in the adult tissue is very limited, and most, if not all, transplanted cells are eventually removed by the host immune system. After injury, the tissue seems to be more accessible to cell integration, and increased homing has been observed to the heart after infarction and to injured regions of the brain, probably because of the increase in cytokines and chemokines, such as SDF-1, which can promote the migration of these cells to the target tissue[47] It might be interesting to study whether a re-expression of embryonic genes, such as occurs after the induction of cardiac hypertrophy, which induces a gene-expression pattern normally associated with fetal heart development[48], may facilitate the functional integration of applied stem or progenitor cells. One may envision that the reset to a fetal environment might provide more plasticity of the tissue for the integration of new cells.

Achieving functional integration in diseased and aged tissues. Even when there is physical engraftment of transplanted cells in injured tissues, functional integration, such as coupling of cardiomyocytes to the host tissue to allow a synchronized beating of the heart, is uncommon. It has been discussed that transplantation of more immature, not fully differentiated cells in a progenitor state might enable better *in vivo* functional incorporation and maturation. But, although the higher plasticity of more immature cells may allow them to more easily integrate and mature *in vivo*, transplantation of more immature cells can pose the risk of tumor formation[49]. That risk is lower if ESCs or iPSCs are differentiated before transplantation, but the extent to which intermediate progenitor cell states pose a safety concern in humans is unknown, particularly in patients receiving immunosuppressive therapy to prevent cell rejection.

The problem of functional long-term integration is aggravated when cells are transplanted into chronically diseased tissues. Several of the diseases with the highest medical need, such as heart failure or neurodegenerative disease, progress over many years and cause major changes in the organ that may affect cell integration and function. For example, declining connexin expression and altered gap-junction organization develop during the progression of heart failure[50]. As translocation of calcium from myocytes to transplanted stem cells via connexins is important for functional integration and affects the acquisition of a myocyte phenotype of progenitor cells, the downregulation of connexins during heart failure poses an additional challenge for the functional integration of injected cells[50].

Chronic diseases such as heart failure do not only affect cardiomyocyte function; they are also associated with tissue fibrosis and changes in the immune-related cytokine profile, thus generating an entirely different environment for the transplanted cells. In this case, the exposure of transplanted cells to a diseased niche may have a negative impact on cell functionality. Niche cells, such as mural, endothelial or glial cells, are functionally modulated in chronic disease and aging. For example, the uncontrolled microglial activation and neuroinflammation that occurs during the progression of neurodegenerative diseases may contribute to neuronal damage and influence grafted stem cells[51], and the profound upregulation of inflammation in the failing heart certainly does not make exceptions for newly transplanted cells. Furthermore, endothelial function is impaired by aging, cardiovascular, neurodegenerative and other chronic inflammatory diseases, which underscores the importance of vascularization of newly regenerated tissue for long-term survival and function and suggests that an impaired microcirculation might not adequately support the transplanted cells. In light of recent reports suggesting that the vascular niche provides a paracrine environment for regeneration[52,53], one could also speculate that endothelial dysfunction may not only interfere with oxygen delivery but also negatively affect the paracrine signals provided by the endothelium to control endogenous or stem cell–induced organ regeneration. General changes in the secretome have been reported in the context of aging, as the induction of senescence affects the secretion of diverse paracrine signals that control endogenous regeneration of solid organs[54,55].

Looking ahead. To cope with these challenges, integrated therapeutic approaches that will improve the niche and/or make the transplanted cells resistant to the disease-induced alterations of the environment might be necessary. For example, rejuvenation of the vasculature could activate endogenous regeneration, as has been recently shown[55], and support the survival and function of transplanted stem cells. In addition, transplanting stem cells together with cytokines, biomatrices or mural cells could transiently create a healthy paracrine environment, providing additional support to promote functionality of transplanted cells[56]. Alternatively, given the recent advances in gene editing, these tools could be exploited to modulate signaling pathways in the transplanted stem cells to make them resistant to the disturbed signals in the chronic disease state and able to maintain long-term function.

The impairment of stem cell functions and niches in chronic disease is even more relevant for autologous cell therapy, in which stem cells are isolated from patients, many of whom are aged and have chronic conditions. Aging, and diseases commonly associated with it, such as diabetes, impair stem cell function and cellular processes by affecting stem cells directly or by affecting the niche[57]. Consequently, progenitor cells derived from these patients show substantially less therapeutic benefit in animal models of disease compared to cells from healthy, young donors[57]. It might be possible to overcome the dysfunction of autologous cells by implementing cell-isolation procedures to select and enrich stem cells that will show healthy, functional phenotypes or through *ex vivo* correction of cellular dysfunction using small molecules, miRNAs or genes[43,53]. For example, inhibition of the age-induced miR-34a enhances the cardioprotective activity of bone marrow cells[58].

It is now clear that the functional engraftment of transplanted stem cells is a prerequisite for achieving efficient regeneration. Even if cell therapy is used to provide paracrine factors or exosomes to support tissue repair or activate endogenous regeneration, initial engraftment of the transplanted cells to the target organ is necessary, unless transplanted cells act in a systemic manner. So far, engraftment is

limited in most cases, and strategies to overcome low engraftment are needed to support the therapeutic benefits of cell therapy. Such attempts may include pretreating the cells *ex vivo* to augment survival and functional integration or treating the target tissue to make it more supportive of integration and survival of transplanted cells. It might be interesting to systematically study the extent to which restoration of a 'healthy' secretome in target tissue during aging or disease can improve the therapeutic benefits of stem cell therapy. —*S. Dimmeler*

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Roadblocks to translation of stem cell therapies

Translating stem cell discoveries takes strong motivation on the part of academic researchers and considerable financial resources from both the nonprofit and for-profit sectors. In many respects, it is best achieved by merging academic and medical researchers with industry resources[59], as this enables soundly based scientific discoveries to transit from the laboratory to the clinic with deep-enough research resources and infrastructure. Key academic researchers who make initial discoveries can ideally revise details associated with the therapeutic product, as unexpected outcomes can arise during preclinical translation. The combination of academic and industry partners enables deeper research as well as the experience to obtain the demanding regulatory data on pharmacology, bioactivity, cell distribution and survival, potency and animal safety required for trials. The lack of public funding for academics to work effectively in translation and the scarcity of venture capital finance for these relatively expensive studies have been, and will continue to be, major barriers to progress, unless public investment increases by recognition of the role of academics and private capital returns to support life-sciences opportunities. In California, large public and growing private investment has been a beacon for the field of regenerative medicine; other state and national funds and infrastructure are now emerging as confidence rises with the flow of potential cell products and newly discovered treatments. Although a number of large pharmaceutical and biotech companies are entering the regenerative medicine field, at the same time that many teams with major academic involvement are getting involved in clinical trials, sufficient public-private funding is still essential to support the expected large raft of therapies that are now in the pipeline[60].

Unknown mechanisms of action of cell therapies and disease pathways. Frequently, the mode of action of stem cell products remains obscured because the cells are inefficiently integrated into the tissue and so disappear rapidly, although clinical trials have shown some evidence for their therapeutic benefit. Bone marrow cells, mesenchymal stem cells (MSCs) from bone marrow and other sources and expanded cardiac cells remain in the body only briefly, but they seem to improve heart function and reduce infarct lesions. However, in the case of autologous bone marrow cells, it is doubtful that these improvements are evidence of any real therapeutic benefit. In an analysis of clinical trial reports of autologous bone marrow transplants[61], studies with no discrepancies showed no effects of cell administration, whereas higher numbers of discrepancies correlated closely to larger effect size. This calls into question the clinical data and the interpretations of clinical benefit, irrespective of method of administration and selection of cells for transplantation. Any regenerative response of transiently surviving transplanted cells may therefore be a trophic consequence of their cytokine secretory properties on endogenous stem cells or tissue vascularization and/or their influence on dampening inflammation through direct immune suppressive influences[62], rather than the result of the donor cell repopulation of targeted tissues. MSCs[63], NSCs—in some applications, and expanded cardiac cells all seem to have these transitory properties *in vivo*; however, replicating such functions and the clinical benefits or errors observed will be challenging, as it is unclear how they induce these effects. The actual mechanisms of disease perturbation also remain major limiting factors in cell-based therapies, as many of them are still uncertain, particularly for neurodegenerative diseases. Cellular therapies involving bone marrow transplants or any other cell type have not yet had any substantial impact in multiple sclerosis or in Alzheimer's, Parkinson's or Huntington's diseases. Although cell therapy–based treatments may be implemented and eventually prove efficacious in some patients, as has been the case for fetal brain cell grafting in Parkinson's disease, the cause of neurological diseases remains largely obscure, which complicates identifying the appropriate target[64].

Present and future translational techniques. There is much optimism that modeling disease *in vitro* using iPSCs will enable elucidation of genetic pathways involved in pathogenesis. Although preclinical studies may help elucidate aberrant genetic pathways in complex diseases, finding new and effective drugs and more personalized approaches to responsive drug therapies remain aspirations for now. Recently, studies have shown that gene editing tools such as transcription activator–like effector nucleases (TALENs), zinc-finger nucleases (ZFNs) or clustered regularly interspaced short palindromic repeats (CRISPR) could be used to determine cell function through analyses on identical genomic backgrounds involving knockouts or knock-ins to verify gene pathway influences on the expression of pathological phenotypes[65]. This removes the background 'noise' of genomic variance between individuals, particularly in complex diseases.

There are some views holding that the preclinical breakthroughs necessary to find effective therapies in complex diseases will require a more industrial-scale approach that brings numerous platform technologies to bear on disease-focused research programs. California recently established a stem cell genomics center of excellence that could contribute to this approach by enabling genomics, gene editing, protein and RNA interactome studies and epigenetics to be merged with cell imaging and studies of cellular interactions with other cells, the matrix and other microenvironmental cues and metabolite signaling, among others. Such initiatives will probably advance understanding of the cause of disease and the fidelity of different approaches to correcting disease phenotypes. Large databases for these studies will require major bioinformatics and systems biology resources to be applied to genomics, cell biology and pathology and patient data to enable the confident identification of interactive mechanisms that predict therapeutic targets. The sharing of this approach by California and large institutions elsewhere will facilitate robust therapeutic models to evolve with a sound scientific basis and accelerate effective therapies in regenerative medicine.

Mouse and *in vitro* models of human disease represent only part of the human disease condition and have major limitations in designing effective human therapies. The ability to design human 'diseases in a dish' using iPSCs has enabled the modeling of human heterogeneity in cardiac and neurodegenerative disorders and the identification of

distinct and common aberrant gene pathways (mutations that affect the central nervous system, for example, may also have an impact on heart function). This approach can provide clues about the origins of complex disease and related phenotypes that are new starting points for human therapeutic targets[66].

Surmounting immune rejection. The lack of an effective method for inducing immune tolerance of allogeneic cell transplants remains a serious roadblock to cell therapies. Patients receiving allogeneic cells need long-term immune suppression to avoid rejection of grafted cells, and the alternative—autologous cells or the patient's own specific iPSCs—is unlikely to be cost-effective for large-scale clinical demands or to be clinically sustainable. The field of regenerative medicine is concentrating on the use of allogeneic donor cells that disappear quickly from the body and generally do not elicit an immune rejection response. As scientists are now pursuing donor cell–based therapies to repopulate, integrate with and renew these tissues[67], they also need to avoid rejection. Although iPSC derivatives would be tolerated in the donor patient, they would not be a large-scale or affordable solution, or an option for patients not eligible for such therapies. Haplotyping iPSC lines may enable a finite number of banked cells compatible with major histocompatibility antigens[68]. Alternatively, constitutive expression of programmed death ligand-1 and a fusion of cytotoxic T-lymphocyte antigen 4 and immunoglobulin, which can reduce T cell activity, may also remove immunoreactivity from transplanted cells[69]. However, these genetically engineered cells may also escape immune surveillance even if infected or cancerous, which is a risk that must be weighed and would leave few or no options other than elimination of all of them—for example, through the use of a suicide gene.

Optimizing differentiation of transplanted cells. Cell therapies aimed at long-term repopulation of damaged or diseased tissues requires understanding of the cell type and maturity needed for the transplanted cells. PSCs can usually be grown only up to immature fetal-type cell stages in culture, but these progenitor cell types can be transplanted into people, where they will mature into targeted tissue cells. This maturation *in vivo* is commonly observed, as with pancreatic beta islet cells derived from pancreatic progenitors[70]; however, this will challenge the search for useful functional biomarkers of manufactured progenitor cells before transplantation.

The heterogeneity of the stem cell population could also pose a problem, which may be exacerbated if cell manipulation and growth in culture introduce additional genomic variants, which may affect normal functioning. Culture systems can add heterogeneity to the phenotype and genotype, thus complicating selection criteria for transplantation[71]. Incorrect or incomplete differentiation can also be a concern, as in the case of improperly differentiated endothelium from transplanted PSCs, which can lead to maladapted fibrosis and affect organ function[72]. Although these potential risks can be partly investigated and addressed by transplantation of cells into suitable immune-suppressed animals, these models may only approximate the human disease and often do not account for the intact human immune system.

Clearly, the lack of standardization of the materials for potential use in patients raises concerns for regulators and regarding therapeutic approval. In addition, some tissues, including blood, skin, gut and liver, have a very high turnover rate of mature cells and high stem cell activity, whereas others, such as the heart, pancreas, muscle and bone, have very little turnover with few active stem cells. Regenerative therapies in the eye are developing rapidly because relatively few cells are needed, local immune suppression is possible and the regenerating cells can be tracked[73]. In contrast, the repair of damaged heart tissue may require innovative approaches such as patch technology, in which cardiomyocytes are embedded in a scaffold that is attached to the ventricular wall over the damaged tissue[74], or direct reprogramming of heart fibroblasts to cardiomyocytes to ensure repopulation of ischemic ventricular tissue[75].

Finally, the age of the tissue can have an impact on disease phenotype and gene-expression profiles of transplanted cells, yet it is rarely considered in translational studies[76]. Because of the acquisition of mutations, tissue damage and dysfunction and the accumulation of senescent cells and protein aggregations that can occur in tissues from aged people[77,78], transplanted cells will probably have very different outcomes during the regenerative process from those predicted in animal studies, which are often done with young animals. Aged animals that mimic the human patient should therefore be included in preclinical studies to better predict therapeutic outcomes.

In the workshop on stem cell therapies held by the US National Academies' Institute of Medicine, the National Academy of Science and the International Society of Stem Cell Research in 2014, it was concluded that of one of the most important problems for clinical trials in regenerative medicine is that "compelling evidence of therapeutic effectiveness has generally not been delivered by the field." This has led to some serious concerns about the readiness of the industry for major investment, and these were amplified by a disappointing meta-analysis showing a high degree of clinical data error in studies of autologous bone marrow cells for the treatment of heart disease[61]. To increase the chances of success in future clinical trials, preclinical studies should show sufficient scientific evidence, the lack of which still remains the main impediment to moving the field forward. —*AT*

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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