The intrinsic regenerative capacities of the adult mammalian heart are believed to be limited and insufficient to compensate for major cell loss upon injury. Transplantation of cells bearing regenerative potential emerged as a possible therapeutic strategy more than a decade ago; however, investigators continue searching for the ideal cell and implantation strategy.1 Mesenchymal stromal cells (MSC) are among the most widely used cell types for regeneration studies.2 MSC are multipotent, but rigorous in vivo proof of their stem cell identity is still lacking. Residing in the perivascular space of nearly all tissues, MSC are most commonly retrieved from bone marrow or adipose tissue aspirates. MSC are immune evasive and exhibit immunomodulatory properties,3 essentially making them suitable for possible allogeneic use. MSC are identified by a defined panel of surface markers in the absence of hematopoietic markers and by their in vitro trilineage differentiation potential into osteogenic, adipogenic, and chondrogenic lineages.4 Irrespective of these criteria, MSC represent a heterogeneous population, and their properties depend on a variety of factors, which include donor and tissue of origin as well as isolation and culturing techniques. In particular, the choice of culturing media, supplements, and oxygen supply may greatly influence their characteristics, which can be exploited to modify their regenerative potential.

“Fortune favors the prepared mind” (Louis Pasteur, 1854), as biology favors the prepared cell. Besides molecular or pharmacological manipulation and genetic engineering, culturing of MSC under hypoxic conditions before transplantation, also known as hypoxic preconditioning, is one of the strategies to enhance their viability and engraftment and to modify their secretome, thus promoting anti-apoptotic, anti-inflammatory, and proregenerative properties.5 Hypoxic preconditioning has the advantage that it is broadly available, easily applicable, and free of safety concerns inherent to pharmacological or genetic manipulation. Nevertheless, because hypoxic preconditioning profoundly alters cell properties and their interaction with the microenvironment, it may also affect their safety profile.

In the present issue, Hu et al undertook the great effort to investigate whether transplantation of allogeneic hypoxia–preconditioned MSC is safe and efficacious compared with MSC cultured under ambient conditions (N-MSC) in a randomized trial in monkeys subject to acute myocardial infarction.6 MSC were isolated from the bone marrow, labeled with green-fluorescent protein by lentiviral transfection and cultured either under ambient conditions (21% O2) or under hypoxic conditions (0.5% O2, HP-MSC) for 24 hours. Cells (1x10^7 per heart) were injected intramyocardially at 5 different sites into the infarct border zone 30 minutes after ligation of the left anterior descending coronary artery. Applying a thought-through study design using magnetic resonance imaging and positron emission tomography to determine cardiac function and metabolism as well as telemetry and electrophysiology to monitor rhythm and assess proarrhythmogenic potential, the investigators report improved viability, function, and remodeling of HP-MSC-injected as compared with N-MSC- or vehicle-injected monkey hearts. These beneficial effects occurred in the absence of arrhythmogenic complications and were associated with enhanced cell engraftment, cardiomyocyte proliferation, increased vascularization, and less apoptosis of intrinsic cardiac cells. Hu et al did not, however, observe neocardiomogenesis or long-term persistence of MSC.

Given that the benefits of hypoxic preconditioning of MSC have previously been explored in various disease models with positive outcome,7 how does this study expand current knowledge? Large animal studies have proven informative regarding safety and outcome of cardiac cell therapy in clinical trials,8 but primate studies are rare. Not only are they expensive and time-consuming, but also they require special expertise and are under tight regulation. With respect to hypoxic preconditioning in MSC, only a single study exists in chronic ischemic heart failure in pigs (cell transplantation 30 days after infarction). This prior work reported improvements in left-ventricular function in animals receiving allogeneic HP-MSC as compared with N-MSC.9 The study by Hu et al is the first study in primates and the first large animal study in acute myocardial infarction. Cynomolgus monkeys (macaca fascicularis), which belong to the Macaques family, are frequently used for toxicology and preclinical safety studies, and their anatomy and physiology share great similarities with humans.
They are also well suited for cardiac imaging\textsuperscript{10} and telemetry testing,\textsuperscript{11} which was used by the investigators who chose a clinically relevant study design to monitor arrhythmogenic complications and cardiac metabolism and function in vivo in a similar fashion to a clinical trial in humans. The improvements in myocardial viability and function in the absence of significant arrhythmias indeed raise hope that HP-MSC therapy may be safe and efficient for potential use in humans. An additional strength of large animal studies is the possibility of long-term follow-up. This is particularly relevant in view of clinical studies in humans, in whom long-term outcome is of utmost concern and importance. Hu et al opted for long-term follow-up, and data are reported for \( \leq 270 \) days. Although the number of animals is too small to draw definitive conclusions, the effect of HP-MSC therapy appeared to diminish over time from an increase of roughly 10\% in left ventricular ejection fraction day 90 versus day 3 to 2.8\% day 270 versus day 3 post infarct and treatment. The authors have stated that follow-up is still ongoing, and long-term outcomes will be of great interest in view of future clinical trials using hypoxia-preconditioned MSC in patients.

Large animal models although important in the development of novel therapeutics are not traditionally the first model systems used for deriving mechanistic insights and to develop new biological concepts. Nevertheless, Hu et al provide sufficient data to mechanistically support the beneficial effects of HP-MSC on cardiac remodeling and function seen in the primate heart. Although their findings are consistent with prior findings in MSC, there are several points worth considering in greater detail: MSC have been shown to exhibit cardiomyogenic and vasculogenic differentiation potential in vitro and in vivo, and along with gain in cardiac function, true regeneration of lost cardiac muscle remains among the primary goals of cell transplantation studies, particularly for sustained long-term cardiac improvement. However, bona fide differentiation of MSC is low, and their beneficial effects are mostly attributed to their paracrine activity.\textsuperscript{12} The lack of neomuscularization observed in the study by Hu et al, therefore, is not of great surprise. In light of recently reported neomuscularization of large areas of injured macaque hearts based on transplantation of human embryonic stem cell–derived cardiomyocytes,\textsuperscript{13} though, the role of MSC must be critically evaluated. Efficient differentiation of MSC to meaningfully contribute to neomuscularization of the infarcted heart—if possible at all—may come with important downsides. MSC may not be immunoprivileged after all, but rather immune evasive,\textsuperscript{3} and differentiation markedly alters their immunogenic properties. The increased expression of the immunogenic major histocompatibility complex Ia and II and the reduced expression of the immunosuppressive major histocompatibility complex Ib observed in MSC undergoing differentiation into any of the 3 major cardiac lineages (endothelial, myogenic, or smooth muscle cell)\textsuperscript{14} will ultimately jeopardize this immune evasiveness, making immunosuppression mandatory. In addition, the large bulk of cells needed to engraft bears a much higher risk of electric destabilization than the \(< 1\% \) of \( 1 \times 10^7 \) injected cells engrafted in the study by Hu et al, irrespective of cell type. Indeed, all animals experienced arrhythmias for \( \leq 4 \) weeks.
after transplantation in the study by Chong et al,13 but no significant arrhythmias were observed in association with HP-MSC therapy.6

Rather than neomuscularization, the observed beneficial effects reported by Hu et al likely arise from the paracrine properties of MSC, which were augmented by hypoxic preconditioning. HP-MSC expressed higher levels of hypoxia-inducible factor 1α (HIF1α), erythropoietin, and angiopoietin-1, exhibited enhanced apoptosis resistance in vitro, and more efficiently induced tube formation of endothelial cells than N-MSC. Erythropoietin and angiopoietin-1 are known constituents of the MSC-secreted proteome and have previously been implicated in angiogenesis, fibrosis inhibition, and apoptosis protection.5 HIF1α is the oxygen-sensitive subunit of the transcription factor HIF1, a master regulator of the hypoxia response. Activity of HIF1 is induced under hypoxic conditions through changes in HIF1α mRNA and protein expression, subsequently enhancing transcription of a variety of growth factors and cytokines, many of which can be found in the MSC secretome, including erythropoietin and angiopoietin-1.15

As in previous studies using MSC, the beneficial effects of HP-MSC observed by Hu et al outlasted the traceability of engrafted cells in the heart, a phenomenon also referred to as hit and run-mechanism. How precisely the MSC effects are maintained long after the cells disappear is poorly understood. In addition to proteins, the MSC secretome contains nucleic acids including miRNAs and miRNAs, and lipids, packaged in exosomes or microvesicles. Recent work demonstrates that such microvesicles are capable of transferring mRNA or proteins into recipient host cells thereby altering protein expression16 or epigenetic programming,17 particularly the latter offering a possible explanation for some of the long-lasting effects of MSC. Let alone that the MSC secretome is far from being identified, much more research is needed to define whether and under what circumstances comparable mechanisms may occur in the myocardium. In addition, hypoxic preconditioning could theoretically alter each of the secretome’s components. Collectively, the cardiovascular field will be required to dig deeper into the molecular mechanisms underlying the cardiac benefits of transplanted cells if cell therapy is to become a real treatment option in the future.

In the interim, the findings of Hu et al remain an important step forward (Figure). MSC may indeed be ideal carriers of a beneficial cocktail to induce cardiac repair. Identification of the critical components of this cocktail, as well as the biological mechanisms underlying their effects, will emerge from continued studies such as Hu et al. Biomedicine has long taught that there are no shortcuts when it comes to emerging therapies, and continued studies with long-term outcomes data and mechanistic studies are highly anticipated.

Sources of Funding
The authors are supported by grants from the Swiss National Science Foundation No. 310030_156953 (to G.M. Kuster) and the National Institutes of Health (NIH) HL088533 and HL128135 (to R. Liao).

Disclosures
None.

References


Key Words: Editorial ▪ acute myocardial infarction ▪ hypoxia preconditioning ▪ mesenchymal stromal cells ▪ paracrine effects ▪ primates
Fortune Favors the Prepared: Safety and Efficacy of Allogeneic Hypoxia Preconditioned Mesenchymal Stromal Cells in Primates
Gabriela M. Kuster and Ronglih Liao

Circ Res. 2016;118:908-910
doi: 10.1161/CIRCRESAHA.116.308410

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/6/908

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/