Allogeneic Cell Therapy
A New Paradigm in Therapeutics

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Cell-based therapy is at the forefront of clinical investigation for cardiovascular disease, backed by over a decade of rigorous preclinical study of cell biology, mechanism(s) of action, immunology, and phenotypic efficacy.1 After early proof-of-concept and safety clinical trials,2-5 the field is entering the next phase of clinical evaluation to delineate clinical efficacy. However, several questions still need to be addressed, namely the optimal cell delivery method, cell dosage range, and cell characteristics. Importantly, translating cell therapy into standard clinical practice requires the ability to readily administer a safe and efficacious product at the optimal dosage. An opportunity that greatly enhances the ability to develop such a product is the use of allogeneic therapy, which offers an efficient way to achieve both immediate availability of product and the appropriate number of cells.

The Paradigm of Allogeneic Therapy
Allogeneic therapy is clearly a disruptive concept in biology. Standard immunologic dogma holds that any foreign tissue will elicit an immune reaction.6 This concept is clearly evident in solid organ and hematopoietic transplantation, in which aggressive immunosuppression is the norm to protect allografts from rejection.6 As the field of cell-based therapy evolves, it has become evident that various cell types—mesenchymal stem cells (MSCs) being the prototype—have sufficient ability to evade7 or come evident that various cell types—mesenchymal stem cells (MSCs) being the prototype—have sufficient ability to evade7 or suppress8 the immune system to the extent that they may be used as allografts without requiring concomitant immunosuppression.

Preclinical Studies
More than 10 years ago, it was suggested that MSCs act as immune system modulators.9 Bartholomew et al10 performed a mixed lymphocyte reaction in which baboon MSCs were cocultured with stimulated allogeneic peripheral blood leukocytes and demonstrated that the MSCs suppressed leukocyte proliferation. Extensive studies have subsequently revealed that MSCs evade the immune system via multiple mechanisms, which include having moderate levels of human leukocyte antigen class I expression, lacking expression of human leukocyte antigen class II, B7 and CD40 ligand11 (the underpinning of being immunoprivileged), and secreting paracrine factors12 and exosomes13 (which underlie, among diverse activities, immunosuppressive actions). With regard to the latter, MSCs suppress proliferation of both T helper and cytotoxic T cells,8 decrease the production of the proinflammatory cytokines interferon-γ, tumor necrosis factor-α, and interleukin-214, inhibit the activation of natural killer cells,15 arrest B-cell maturation, and block maturation of dendritic cells, resulting in reduced expression of antigens and costimulatory molecules necessary to activate T cells (Figure 1).16 These data formed the basis for the preclinical and, subsequently, clinical deployment of allogeneic MSC therapy. Indeed, our group demonstrated in porcine models of myocardial infarction17 and chronic ischemic cardiomyopathy18 that delivery of allogeneic MSCs is effective in improving cardiac structure and function, through engraftment, cell-to-cell interactions, and trilineage transdifferentiation, and does not elicit detrimental immune reactions.

It is important to note, however, that some studies are emerging that raise cautionary notes. Huang et al19 in a rodent study reported that allogeneic MSCs may lose their immunoprivileged status during differentiation, but this result has not been confirmed in other studies. As previously mentioned, we have demonstrated persistence of differentiated cells, 3 months after intramyocardial delivery in a model of chronic ischemic cardiomyopathy in the pig.18 Some have argued that MSCs are not fully immunoprivileged but rather immunoevading.7 The difference seems to rely on concentration. In in vitro studies where MSCs were in high concentrations, the immunomodulatory capabilities prevailed.7

Clinical Testing of Allogeneic Stem Cells
Clinical trials have shown that allogeneic bone marrow-derived MSCs may be safely administered to humans without eliciting clinically relevant immune reactions.3,4,20 In the first clinical trial of allogeneic MSC therapy for acute myocardial infarction,4 intravenous infusion of MSCs did not produce an immune reaction and led to improved outcomes with regard to cardiac arrhythmias, pulmonary function, left ventricular function, and symptomatic global assessment. Subsequent to this acute myocardial infarction trial, we performed a randomized clinical trial in patients with chronic ischemic cardiomyopathy, POSEIDON (The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis),3 which had as its main goal the comparison of transendocardial injection of autologous and allogeneic bone marrow-derived MSCs. Although it was not powered to show efficacy as a primary outcome, the trial reported similar safety profiles between the 2 sources. MSC therapy improved indices of physical functional capacity and

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quality of life (6-minute walk test and the Minnesota Living with Heart Failure Questionnaire score, respectively) and reduced scar tissue and left-ventricular sphericity index, markers of ventricular remodeling. Importantly, only 2 patients receiving allogeneic MSCs developed sensitization as measured by the panel reactive antigen (PRA). One exhibited low-level antibodies to antigen specificities not expressed by the donor MSCs and the other showed low-level donor-specific human leukocyte antigen class I antibodies. Flow cytometric cross-match with serum from the second patient to fresh donor T cells showed a weak positive reaction, indicating low titer, de novo allogeneic sensitization with class I donor antigens. Neither incident developed clinical significance. Similarly, in another clinical trial using allogeneic cardiac stem cells has been reported yet. The currently ongoing trial ALLogeneic heart STem cells to Achieve myocardial Regeneration (NCT01458405) is testing the safety and efficacy of cardiospheres without the use of immunosuppression, based on favorable preclinical data. Of note, emerging evidence shows that cardiospheres have many properties of MSCs, as they express classic markers including CD105, CD90, and CD73, and as such may be cardiac-specific stromal or mesenchymal cells. The finding that these cardiac-derived CD105+ cells may be used as an allograft suggests that allogeneic cell therapy may be broadly applicable.

### Meta-Analysis of Allogeneic Cell Therapy

As there are early accumulating data about allogeneic cell therapy, there is significant value in performing meta-analyses of preclinical data. In this issue of *Circulation Research*, Jansen of Lorkeers et al present a comprehensive meta-analysis of large animal studies, investigating the effects of stem cell therapy in ischemic heart disease. After the application of

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**Figure 1.** Mesenchymal stem cells are considered immunoprivileged because of their lack of expression of major compatibility complex class (MHC) II proteins and costimulatory molecules B7 and CD40 ligand and exert immunosuppressive actions through inhibition of innate and adaptive immunity. IFN indicates interferon; IL, interleukin; and TNF, tumor necrosis factor.

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**Figure 2.** Comparison of autologous and allogeneic stem cell therapy. Allogeneic stem cell therapy offers advantages during the autologous counterpart. The stem cells are derived from young healthy donors, eliminating any comorbidities associated with disease states. Allogeneic cells grown and kept in stem cell banks so that they are available for immediate delivery.
strict selection criteria, 82 of 595 publications were included in the meta-analysis. Importantly and expectedly, MSCs were the cell type used in the large majority of the analyzed studies. Three important messages from this meta-analysis are (1) autologous and allogeneic cell therapy exhibit similar effects, (2) cell therapy provides an overall significant improvement in left ventricular ejection fraction and decrease in end diastolic volume, and importantly (3) cell therapy seems to be safe. This study makes a critical statement supporting the findings of the 2 clinical trials in which allogeneic MSCs or mesenchymal precursor cells have been used, and furthermore continues to support the fact that large animal studies are highly representative of translation into humans.

As the authors aptly point out, the major issue for the success of allogeneic cell therapy is the lack of an immune response by the recipient to the transplanted cells. With the exception of 1 study, the MSC studies included in the meta-analysis did not use immunosuppression. Nevertheless, no serious immune reactions were reported in any of the studies and there was no impact on the safety and efficacy profile. In fact, in noncardiac studies, the effect of immunosuppression with cyclosporine or methylprednisolone has been mixed, ranging from a positive effect in the treatment of stroke to a negative effect on efficacy of allogeneic MSCs in spinal cord injury.31

The Application of Allogeneic Cell Therapy

Regarding cell preparation and timely delivery, allogeneity solves logistical issues of a successful stem cell therapy. Allogeneic MSCs are subject to multiple quality controls (Figure 2), from the screening of young healthy donors to pre- and postcryopreservation control of viability and sterility. When an autologous source is used, the cell product derives from a patient that is usually older and with multiple comorbidities and the cell product is manufacturing dependent. The allogeneic product can be expanded in quantities that may be unattainable for the autologous source, undergo cryopreservation, and be readily available for delivery. In comparison, the manufacture of the autologous product may require ≤6 weeks to reach sufficient numbers for administration, and the ability of the patient’s cells to expand is unpredictable.

In noncardiac clinical trials, allogeneic sources of stem cells and more specifically MSCs have been widely tested.32 Le Blanc et al33 reporting on the outcomes for 55 patients receiving intravenous infusions of fresh or cryopreserved allogeneic MSCs for steroid-resistant graft versus host disease, noted no acute side effects. In fact, a growing number of published phase I and phase II clinical studies34,35 of allogeneic MSCs administered for diverse medical conditions have consistently described a lack of acute complications from doses as high as 5x10⁶ cells/kg. On the basis of this background, numerous trials for diverse indications are emerging.

Future Issues for Consideration

Although our immunologic understanding, animal data, and empirical clinical trial data support the use of allogeneic therapy, several issues need to be considered as the field evolves. For instance, tolerance to repeat MSC dosing has not been convincingly demonstrated. And if that is the case, could a different donor be used to avoid anamnestic reaction? Should there be any prescreening of candidates and appropriate immunophenotypic matching as is the norm with blood banks? The preclinical evidence and empirical safety from clinical trials thus far do not support immunomatching, although strict immunologic monitoring in early stage trials is highly recommended to further define whether immune reactions to allogeneic cell therapy are meaningful and clinically relevant.

In light of these highly encouraging results and intriguing pending questions, larger randomized clinical trials are warranted to investigate whether the immunoprivilege of and immune modulation by MSCs/mesenchymal precursor cells observed in preclinical trials in large animals and phase I clinical trials translates to larger patient populations. Rigorous and long-term monitoring of the immunologic profiles of the enrolled patients will provide the evidence needed for stem cell therapy to be transformed into an off-the-shelf treatment option for heart failure and many other diseases.

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