Cell-Based Gene Therapy in Pulmonary Arterial Hypertension
Journeys in Translational Medicine
Vikram Gurtu, Evangelos Michelakis

“As you set out for Ithaka, hope the voyage is a long one, full of adventure, full of discovery. Keep Ithaka always in your mind. Arriving there is what you are destined for... But do not hurry the journey at all. Better if it lasts for years, so you are old by the time you reach the island, wealthy with all you have gained on the way, not expecting Ithaka to make you rich... And if you find her poor, Ithaka won’t have fooled you. Wise as you will have become, so full of experience, you will have understood by then what these Ithakas mean.”

from Ithaka, by C.P. Cavafy

Pulmonary arterial hypertension (PAH) is a relatively rare but fatal disease. However, with the advances in diagnostic tools and our organized approach (most tertiary-care centers now have PAH programs), we now know that PAH is more common than we thought even 15 years ago, although precise estimates of global incidence and prevalence are lacking. PAH is projected to become a 5-billion-dollar industry by the end of this decade, as most of the approved 3 classes of therapies (phosphodiesterase type 5 inhibitors/cGMP modulators, endothelin antagonists, and prostacyclin analogues) are expensive. The vast majority of clinical trials with these drugs have been short (≤6 months) and have not included mortality as a primary end point, and there is no prospective evidence that these therapies can prolong life or reverse the progression of the disease. On the other hand, it seems that they can improve symptoms or decrease hospitalizations. The ultimate treatment remains lung transplantation, and there are currently no therapies to treat the major complication of the disease, that is, right ventricular failure. Yet, there are myriads of promising therapies at the preclinical stage. Like in many other diseases, in the PAH field, there is a tremendous difficulty in translating promising therapies into clinical research. Perhaps the most challenging stage of this translation is its first step, that is, the phase-I trials. Many reasons have been proposed to explain this lack of translation, but the bottom line is that phase-I trials in sick patients are extremely challenging to conduct. One of the most effective ways to approach this is to assemble teams that include the scientists who conceived and conducted the preclinical work, willing (and at times bold) clinicians, and often industry partners. In this issue of Circulation Research, such a team extended 15 years of preclinical research and presents a phase-I trial of the first cell therapy of endothelial progenitor cells (EPCs) overexpressing an endothelial nitric oxide (NO) synthase (eNOS) gene in patients with advanced PAH: the Pulmonary Hypertension And Cell Therapy (PHACeT) trial.

The PHACeT trial was designed to determine the feasibility and safety of genetically engineered EPC therapy in a dose-escalating manner. The authors studied 7 patients with PAH and World Health Organization functional class III–IV, optimally treated with approved PAH therapies. EPCs were isolated from the patients, then transfected to enhance gene expression of eNOS, and subsequently reintroduced to the patients via central access to the right atrium, allowing for direct delivery to the pulmonary microvessels. EPCs were given to each patient on 3 consecutive days to total of 7, 23, or 50 million cells, whereas patients were instrumented with a central catheter for continuous hemodynamic monitoring in an intensive care unit. Patients were followed up for at least 6 months after intervention.

The adverse effects were classified as minor, and although inflammatory markers were generally higher by day 4 post initiation of cell therapy, they normalized by the 6-month follow-up period. However, one of the 7 patients died after a sudden collapse at home, soon after completing the 3-day protocol. Even after an autopsy, the cause of death was not revealed but pulmonary embolism was ruled out. Within the study cohort, this patient had the worst cardiac index at baseline and the highest increase in inflammatory markers (interleukin-6 and C-reactive protein) during the 3 days of cell administration.

Although the trial was not designed to assess efficacy, there was a trend toward a reduction in the total pulmonary resistance during the 3 days of treatment (P=0.06), however, without sustained improvement at the 3-month follow-up reassessment. Despite no change in hemodynamics during the follow-up period, the cohort had an overall improvement in 6-minute walk distance, and half of the patients who completed follow-up had improved functional class by the end of the study. However, because the validity of the 6-minute walk is questionable as an end point, particularly in open-label studies, it is difficult to conclude that there was a true improvement in these patients.
This study has limitations. An important one is the lack of a measurement of the biological activity of the treatment. In this case, this could have been the measurement of NO products (either in the serum or exhaled air) as it was the production of NO that the authors hypothesized would potentially lead to pulmonary vasodilation or reversal of vascular remodeling. Thus, in the absence of a significant hemodynamic response, we cannot assess the dose–response (number of cells) tested. Because of the small number of patients studied, due to the difficulty in recruiting patients (an understandable and expected challenge in this disease), the authors studied the highest dose (50 million cells) only on 1 patient, further limiting the assessment of the dose escalation.

The study started recruitment 8 years ago (registered in ClinicalTrials.gov in 2007) underlying the difficulties in recruiting patients in this field. Although the complexity (and potential dangers) of this particular protocol undoubtedly affected the willingness of patients to enroll, this is a challenge that is not unique to this study. In our view, it is unfortunate that although we know that the 3 classes of approved therapies do not reverse PAH, there are still many ongoing early and late-phase studies on medications within these classes or their combinations, which obviously directly compete with the enrollment in studies with novel and potentially more effective therapies, although they do not advance the field. There are currently (July 2015) 20 phase-I clinical trials studying therapies in patients with PAH which are registered on ClinicalTrials.gov as active or completed within the past 3 years (Table). Of those, 25% have been either withdrawn or terminated. Only 8 trials (completed or ongoing) study novel therapies. Thus, to design, complete, and report an early phase trial in PAH assessing a novel mechanism is a major accomplishment.

The journey for the translation of EPC-eNOS in humans started for the authors 15 years ago, much longer than the 10 years it took Odysseus to reach Ithaka. At that time, it was clear that a major challenge for the treatment of PAH was the selectivity of therapies to the pulmonary vasculature because the systemic vessels are typically not affected in PAH. This can be addressed by either discovering targets that are unique to the diseased pulmonary arteries or by selectively delivering therapies into the pulmonary circulation. Serious complications of adenoviral gene therapy in the 1990s directed researchers to consider using the host cells as vehicles for the desired therapy to reduce the inflammatory/immune response. Although it was hoped that delivery of stem or precursor cells would allow them to differentiate in situ and reverse the tissue remodeling, it was soon realized that the value of delivered cells in general may be their stability as sources of secreted molecules, like the potent pulmonary vasodilator NO, the deficiency of which was confirmed by many studies in PAH.

For the lungs, the idea was that the cells would be trapped in the resistance pulmonary circulation, the heart of PAH's pathology. Dr Stewart’s group was instrumental in the preclinical advances of this concept. In 1999, his group showed that when pulmonary artery smooth muscle cells were harvested from donor rats, transfected ex vivo, and redelivered via the jugular vein, over half of

<table>
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A indicates academic/federal; C, completed; eNOS, endothelial nitric oxide synthase; I, industry; IL-1, interleukin-1; N, National Institutes of Health; PA, pulmonary artery; PDE-5, phosphodiesterase-5; O, ongoing; R, recruiting; sGC, soluble guanylate cyclase; T, terminated; U, unknown; and W, withdrawn.
these cells could be seen in the lungs by 15 minutes, and ≈15% would remain there ≤2 weeks later. When they transfected them with eNOS, delivery of pulmonary artery smooth muscle cells prevented rat PAH. They subsequently found that a transgene of vascular endothelial growth factor in pulmonary artery smooth muscle cells prevented and improved established rat PAH. They then investigated a more readily accessible cell type, skin fibroblasts, and compared the delivery of these cells transfected with eNOS or vascular endothelial growth factor directly as treatment after the establishment of rat PAH. Eventually, they used a similar strategy using bone marrow–derived EPCs. Ex vivo transfection of these cells with eNOS significantly reduced pulmonary artery pressure in established rat PAH, improving lung perfusion and rat survival. Shortly thereafter, a Chinese group reported the first trial of autologous EPCs in patients with PAH, with encouraging results at 12 weeks of follow-up. However, it was becoming clear in the field of cell therapy that any of its modest effects were mediated by paracrine effects of the delivered cells, rather than by cell differentiation. Thus, the logical next step for human translation was to engineer these cells so that they produce larger amounts of a beneficial mediator, in this case NO. Dr Stewart’s team partnered with industry and leading clinical investigators from 2 Canadian centers and designed the PHACeT trial. What did this long journey teach us?

1. The procedure is feasible and opens the door for the study of different mediators using the same cells as vehicle.
2. The procedure may be safe when the right precautions are followed. It may be that the sickest of patients with PAH have to be excluded. In fact, if the delivered gene is that of a vasodilator, the advanced vascular remodeling in the late stages of the disease makes this group of patients less attractive for enrollment. On the other hand, delivery of a gene that has shown promise in its ability to reverse vascular remodeling remains attractive.
3. Monitoring inflammatory indices, although the therapy is not a virus or allogeneic cells, may be important early biomarker for adverse effects.
4. Sustainability of the delivered cell phenotype is a major challenge. Although it seems that the sustainability/visibility of the cells delivered in the PHACeT trial was limited, there have been many advances in the genetic engineering of the delivered cells for cell therapy since the launch of this trial 8 years ago, allowing optimism. Considering the field 8 years ago, the authors of this trial deserve our compliments. Since then, the Food and Drug Administration has streamlined procedures and has developed an active role in facilitating investigators in this challenging field. The Food and Drug Administration has now published documents with advice and checklists for teams planning the conduction of both preclinical and early phase clinical studies in cell/gene therapy.

For many of us, reversal of PAH in humans is our Ithaka. As Cavafy suggested, we need to keep Ithaka always in our mind. The design and execution of our preclinical studies needs to take into consideration and mirror the design of the future early phase trials, to increase the efficiency of translation. This is important because time is of the essence; our patients continue to die. It took this team 15 years to achieve this. We are certain that this translation would have taken much longer if it was not for the same team driving the whole process. We also cannot help not to symbolically note that the preclinical trial that provided the foundation for the PHACeT trial was also published in Circulation Research allowing the readership of a Basic Research journal to witness the conclusion of a beautiful translational journey. We hope that this important trial, despite its challenges and limitations, will inspire young clinician investigators to set out for their own Ithaka.

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References

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