

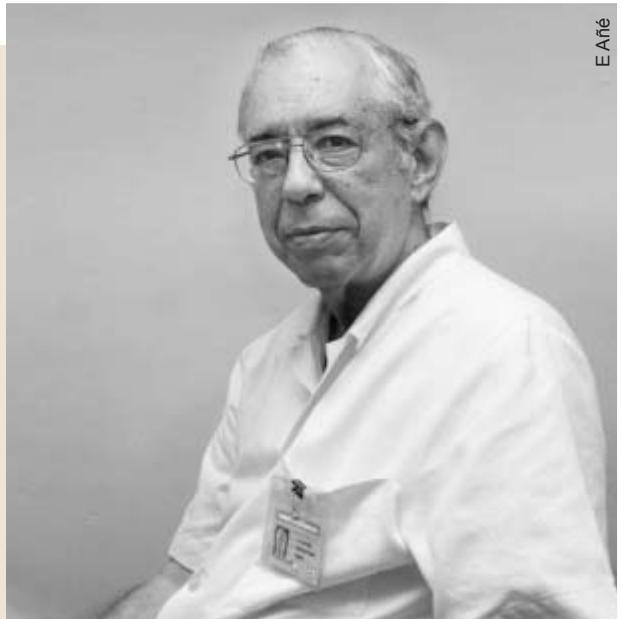
Generating Potential for Regenerative Medicine in Cuba: Porfirio Hernández MD DrSc

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Regenerative medicine has emerged over the last two decades as one of the most rapidly developing fields in medical science, demonstrating unprecedented potential to restore severely damaged or destroyed tissue and even “grow” new tissue in its place. Although the most advanced research, both laboratory and clinical, is taking place in well-financed high-tech settings—including centers in China, India and Brazil—Cuba is one of a handful of other developing countries—along with Argentina, South Africa, Egypt, Iran, and Malaysia—producing regenerative therapies suited to the needs and capacity of their health care systems.[1]

Dr Porfirio Hernández has been at the forefront of the field in Cuba, helping pioneer stem cell research at the Hematology and Immunology Institute in Havana, where he has worked in internal medicine and hematology as clinician and researcher since the 1970s. In this interview with *MEDICC Review*, he discusses Cuba’s regenerative medicine program, particularly development of a simplified method of obtaining adult mononuclear stem cells using existing resources at moderate cost to the national public health system.



MEDICC Review: Regenerative medicine is a new field that isn’t yet widely understood. How is it defined in the Cuban context?

Porfirio Hernández: In general, not just in Cuba, regenerative medicine is understood as the substitution or regeneration of cells, tissues or organs for the purpose of restoring or establishing normal function, based on mechanisms an organism uses to replace damaged cells with healthy ones.

Many of the newer technologies in regenerative medicine have evolved from basic scientific principles demonstrated by technologies and procedures in use for a long time—such as blood transfusions, bone marrow and organ transplantation, and skin and bone grafts—all of which are now considered part of the field. Today, there are four main sub-fields in development: cell therapy, using different types of stem cells; soluble molecules that stimulate tissue generation, including growth factors, cytokines, chemoquines and others; gene transplantation; and tissue engineering.

In Cuba, we have focused primarily on stem cell therapies, which emerged from our experience with conventional bone marrow transplantation to treat blood diseases dating from the 1980s. A bone marrow transplant does, in fact, replace hematopoietic stem cells destroyed by disease or cancer treatment, filling the bone marrow space with new cells that reconstitute the blood-producing tissue.

Our work in hematology and immunology over the next two decades enabled us to begin applying stem cell therapy in 2004, using autologous adult bone marrow stem cells. Since then, we have

treated over 1500 patients in 18 hospitals and research centers around the country. Phase I treatment trials in angiology, orthopedics and periodontology are currently underway in seven provinces, including Havana City. These are all small single center studies, the majority designed to replicate a study initially carried out in 2007 here in Havana at the Enrique Cabrera General Teaching Hospital in coordination with the Hematology and Immunology Institute.

Research in soluble molecules has also been ongoing for some time, and Cuba currently produces several growth factors with regenerative applications. These include recombinant human erythropoietin (RHuEPO), used to stimulate red blood cell replenishment in patients with certain types of anemia; epidermal growth factor (EGF), which has shown consistently positive results treating diabetic foot ulcers; and the granulocyte colony stimulating factor (G-CSF). This last has been key in developing the procedure we use to obtain adult mononuclear stem cells mobilized into peripheral blood. G-CSF is most frequently used to mobilize blood cells—primarily leukocytes—into peripheral blood in patients with a low white blood cell count, usually as a result of chemotherapy, thereby reinforcing the immune system to prevent infection. Experimental gene transplantation research and *in vivo* tissue engineering studies are also being conducted.

MEDICC Review: What had to happen for you to make the leap from bone marrow transplantation to stem cell therapy?

Porfirio Hernández: We used to think that an adult stem cell could only reproduce one kind of tissue—a skin stem cell could

only make skin tissue; a liver stem cell, only liver; a blood stem cell, only blood, etc. But not long ago, it was discovered that most adult stem cells have the capacity to form different lineages. This was a major breakthrough that overturned one of the dogmas of cell biology.

We were aware of promising preclinical research with bone marrow stem cells, and evidence began appearing in the literature referring to successful treatment of patients with severe limb ischemia using autologous mononuclear bone marrow cells. Based on those reports and our clinical capability, we selected one case to receive treatment in February 2004.

MEDICC Review: Tell us about that first case.

Porfirio Hernández: The patient was a 72-year-old man, hospitalized in the Enrique Cabrera Hospital in Havana with swelling and severe pain in the right leg, even at rest, as well as other classic signs of critical ischemia. There was necrosis in two toes with signs of spreading to the rest of the foot. Arteriography was performed, and the patient was diagnosed with stage IV critical occlusive arteriosclerosis using the Fontaine classification. After 19 days of standard treatment—administration of analgesic, anti-inflammatory, anticoagulant and vasodilator drugs—his condition remained unchanged, and amputation of the limb above the knee was indicated.

Therefore, after obtaining the patient's consent, we provided alternative treatment by intramuscular injection of autologous mononuclear bone marrow cells into the ischemic limb. The results were surprising—much better than we expected. Within 72 hours, severe pain, swelling and other critical signs had subsided, and the patient's condition continued to improve steadily. Only the two toes already affected by gangrene required amputation; the rest of the foot and the leg were saved. Arteriography at four weeks showed increased vascularization, and at 24 weeks the patient was walking without pain and reported normal activity.

We were very encouraged by this, because not all cases progress favorably. If that case had not been such a spectacular success, beyond what standard treatment had ever achieved, we might not have decided to move forward so quickly.

MEDICC Review: What was the next step?

Porfirio Hernández: At that point a team of angiologists from the Enrique Cabrera Hospital and hematologists from the Hematology and Immunology Institute began working with specialists in other fields to treat conditions likely to benefit from therapies using the same type of stem cell. Initially these were cases in cardiology, orthopedics, and neurology, and we obtained good results, comparable, in fact, to those reported in other studies using more complex methods. Furthermore, there were almost no adverse reactions or harmful effects from the treatments. This convinced us that we should continue.

The next step was establishing a Regenerative Medicine Group in Cuba to conduct multidisciplinary research, specifically on therapeutic stem cell applications. This was proposed to the Ministry of Public Health and approved in 2005.

MEDICC Review: Who is involved in the Regenerative Medicine Group and how does it function?

Porfirio Hernández: The Regenerative Medicine Group is multidisciplinary, including representatives from major hospitals and research centers such as the Center for Genetic Engineering and Biotechnology (CIGB), the International Neurological Restoration Center (CIREN) and the Medical-Surgical Research Center (CIMEQ), as well as consultants in medical ethics, drug regulation and clinical trials.

We operate as an advisory group to the Ministry of Public Health. One of our principal functions is helping participating institutions develop a research protocol or adapt an existing one for each clinical trial. To establish eligibility criteria for each study, hematologists and immunologists—who know the most about stem cells—work closely with specialists in other fields, who best understand the conditions recommended for treatment. Treatments are then administered by a multidisciplinary team in consultation with specialists in the Regenerative Medicine Group. The group also coordinates training for physicians and laboratory personnel from different institutions.

Another major function is obtaining and disseminating information essential to keeping abreast of developments in the field and to comparing methods and results of our studies with similar research internationally. We created a website that makes international research more easily accessible to Cuban health professionals and also serves as the main source of information about regenerative medicine in Cuba (see box).

MEDICC Review: In addition to severe lower limb ischemia, what diseases or conditions have been treated with stem cell therapy in Cuba?

Porfirio Hernández: Of 1478 patients receiving stem cell therapy in Cuba between February 2004 and May 2010, 74% were angiology cases and 20% were in orthopedics and traumatology. The rest were mainly in periodontology and neurology, and a few in cardiology.

The majority of angiology cases were like the first—critical lower limb ischemia from arteriosclerosis with a stage III or IV Fontaine classification. Stage III means persistent resting pain. At Stage IV, patients have gangrene or open sores in their toes or foot that do not respond to standard treatment, and they are usually facing major amputation. In about 80% of those cases limb amputation was averted after stem cell treatment.

At first, we were seeing only the most critical cases—those facing inevitable lower limb amputation—but we have since broadened the inclusion criteria to consider patients with severe intermittent claudication; that is, those who can't walk more than 150 meters without stopping because of intense pain. After treatment, most of these patients no longer had claudication or they could walk much longer than before without any pain. Needless to say, this greatly improved their quality of life.

MEDICC Review: What about applications in the other specialties you mentioned?

Porfirio Hernández: Results in those fields are also very promising, but given the small number of cases and the short time since

Resources on Regenerative Medicine in Cuba

About Regenerative Medicine in Cuba (Spanish):
<http://www.sld.cu/sitios/medregenerativa/>

Case study of the first patient receiving stem cell treatment in Cuba (Spanish):

Hernández P, Dorticós E, Hernández C, Cortina L, Marsán V, Macías A, et al. Transplante de células madre autólogas en el miembro inferior isquémico de un paciente con arterioesclerosis obliterante crítica. *Rev Cubana Hematol Inmunol Hemoter.* 2005;21(1). Available from: <http://scielo.sld.cu/pdf/hih/v21n1/hih07105.pdf>

Recent published reports of stem cell studies in Cuba

English

Alberti E, Los M, García R, Fraga JL, Serrano T, Hernández E, et al. Prolonged survival and expression of neural markers by bone marrow-derived stem cells transplanted into brain lesions. *Med Sci Monit.* 2009 Feb;15(2):BR47–54.

Hernández P, Cortina L, Artaza H, Pol N, Lam RM, Dorticós E, et al. Autologous bone-marrow mononuclear cell implantation in patients with severe lower limb ischaemia: A comparison of using blood cell separator and Ficoll density gradient centrifugation. *Atherosclerosis.* 2007;(194):e52–6.

Suárez-Monteagudo C, Hernández-Ramírez P, Alvarez-González L, García-Maeso I, de la Cuétara-Bernal K, Castillo-Díaz L, et al. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study. *Restor Neurol Neurosci.* 2009;27(3):151–61.

Spanish

Cortina L, Hernández P, López M del R, Artaza, H, Dorticós E, Macías C, et al. Aislamiento de células mononucleares de sangre periférica para trasplante de células madre. Método simplificado. *Rev Cubana Hematol Inmunol Hemoter.* 2008 Sep–Dec;24(3). Available from: http://scielo.sld.cu/scielo.php?pid=S0864-02892008000300004&script=sci_arttext

Oral presentation summaries. *Hematology. Revista Cubana de Hematología, Inmunología y Medicina Transfusional.* 2009;25(Suppl):27–40. Available from: http://bvs.sld.cu/revistas/hih/vol25_04_09/hih02409.pdf

research began, it is too soon to draw definitive conclusions. In orthopedics, stem cell therapy is now being applied to seven specific conditions, including intervertebral disc degeneration, bone cysts, pseudarthrosis, avascular necrosis of the femoral head, and some chronic spinal cord injuries. Over 300 orthopedic patients have been treated to date.

In 2008, we began treating a few cases of periodontitis at the dental clinic in the William Soler Pediatric Teaching Hospital in Havana. These were adult patients with bone loss from severe gum disease. Standard treatment, involving gum surgery, tooth scraping and root planing, is traumatic, and the results are often unsatisfactory. There is also a laser treatment, but we don't have the equipment it requires. However, we are finding that stem cell therapy is simple, low-cost, safe and effective.

The treatment consists of infusing a concentration of adult mobilized peripheral blood mononuclear cells in periodontal pockets and areas of bone loss. In some quadrants this was done in conjunction with gum flap surgery. Evaluation of the first 18 patients showed reduced inflammation and normal gum color at seven days and healthy gums at six months in all patients. X-rays showed new bone formation at three months and increased bone density in all patients as well. Results of stem cell infusions combined with flap surgery were similar to those obtained using infusions only, which is a simpler procedure and much less traumatic for the patient. This study continues with new patients enrolled.

Maxillofacial specialists at Abel Santamaría Provincial Hospital in Pinar del Río are conducting another study using stem cell implants to treat maxillary cysts and other conditions. And in Havana, the International Neurological Restoration Center (CIREN) completed a study applying bone marrow stem cells in stroke patients suffering neurological damage.

MEDICC Review: Does tissue regeneration resulting from these treatments usually last? Can stem cell therapy permanently reverse arterial occlusion, for example?

Porfirio Hernández: Usually, but it depends on the patient. The vast majority of patients with lower limb ischemia from occlusive arterial disease are heavy smokers. If they don't stop smoking, the problem can recur or may present in the other limb. The long-term outcome can also be affected by secondary factors, such as an injury or another disease—diabetes, for example.

We follow patients' evolution for at least two years after treatment to determine if initial results are sustained, to see which patients suffer relapses and why, and to detect tumors or other delayed adverse effects that could be attributed to the treatment.

MEDICC Review: Do you foresee introducing any of these therapies in standard care anytime soon?

Porfirio Hernández: Not very soon. All the current trials are small pilot prospective studies. Before we can think about standardizing any of the procedures, we need to conduct larger randomized trials to confirm the efficacy and safety of the treatments in the context of the Cuban health system. This is a long, gradual process of accumulating experience and innovating with available resources. For example, we can't extend the Phase I studies to more sites as fast as we would like now because of the time it takes to train staff who already have a full workload.

MEDICC Review: What are the primary benefits of the regenerative therapies developed in Cuba so far?

Porfirio Hernández: The positive results we've obtained treating diseases and conditions that do not respond, or have a limited response, to standard therapies is one main benefit. Since the treatments we are using are autologous—that is, using the patient's own stem cells—there is no risk of rejection or transmission of anything harmful that could occur using cells from a donor or manipulated *in vitro*.

Another main benefit is developing techniques that are affordable and applicable in low-resource settings. Extracting a sufficient quantity of a specific type of stem cell from bone marrow or pe-

ipheral blood is a complex process requiring expensive equipment and accessories. A cell separator machine, for example, costs about US\$50,000, and the accessories needed for each use, such as disposable collection bags, cost about US\$200. The three institutions that perform bone marrow transplants in Cuba (all in Havana) have separator machines, but we must be careful to use them as efficiently as possible due to cost. If we had to depend on a separator to obtain the stem cells we are using now, we could not even think about extending their application beyond those institutions. So, in most cases, we are using a simpler method that is more practical and more economical, and that can be applied in provincial hospitals with affiliated blood banks.

This method uses G-CSF produced in Cuba to mobilize mononuclear bone marrow stem cells into peripheral blood. Then, the blood is extracted the same way it is for donation, under the same conditions, using the same type of collection bag. The patient does not need to be hospitalized, does not need surgery, or even anesthesia.

Stem cells are then obtained manually using Ficoll density separation or centrifuge and precipitation techniques that can be done with standard blood bank equipment. This method doesn't cost much more than the cost of collecting donated blood and preparing it for autologous transfusion.

MEDICC Review: To what extent has availability of Cuban-produced G-CSF influenced development of the method?

Porfirio Hernández: If G-CSF were not readily available, we would not be able to obtain sufficient concentrations of stem cells from peripheral blood, and we would be limited to extracting stem cells directly from bone marrow, which is more complex, more costly, and more invasive and uncomfortable for the patient.

Use of G-CSF would also be limited if we had to rely on imported products. Two Cuban G-CSF products—LeukoCIM and HeberVITAL—are distributed through the national health system at much lower cost. Comparing results using Cuban and imported products, we found no difference. This gave us confidence to continue using Cuban-produced G-CSF, enabling us to extend the method to more institutions, particularly outside the capital.

Distribution of Stem Cell* Treatments in Cuba, by Province



◆ Cumulative number of cases treated, May 2010
 *Autologous adult stem cells
 N = 1478: Angiology 1093 (74%); Orthopedics 301 (20%); Other 84 (6%)

MEDICC Review: You mentioned the need to scale up professional training; what does the training involve?

Porfirio Hernández: The Hematology and Immunology Institute gives hands-on workshops for doctors and laboratory scientists. Sometimes a team from one institution comes and works side-by-side with their colleagues here. They study the research protocol criteria and select two or three cases for treatment. The scientists work with our blood bank staff to obtain the stem cells, and the doctors—those with experience and those in training—apply the treatments as a team. Other times, we send an experienced team to another institution and conduct the training there.

Trained blood bank personnel are essential. The periodontal treatment, for example, could easily be applied in dental clinics around the country, but most are not directly affiliated with a blood bank equipped to prepare stem cells. Setting up a system to coordinate this in each province will take time.

In the meantime, we get several calls a day inquiring about stem cell treatments, and we have to explain that these are clinical trials, and we can only accept patients who meet the research protocol eligibility criteria—and that means exclusion as well as inclusion criteria. For example, patients with poorly controlled diabetes mellitus are excluded.

MEDICC Review: Is Cuba the only country using the “simple” method you describe for obtaining adult mononuclear stem cells mobilized into peripheral blood?

Porfirio Hernández: Mobilization of stem cells using G-CSF and other mobilizing factors is well known. The difference is in the process used to separate the stem cells. Most studies in other contexts report using an automated method, requiring a separator machine. There are very few reports in the literature describing the manual method we are using.

MEDICC Review: Are there plans to explore therapies using other types of stem cells?

Porfirio Hernández: As long as we are getting good results using adult mononuclear stem cells, either from bone marrow or mobilized into peripheral blood, we will continue to concentrate on therapies using those. At the same time, we're following the scientific literature and looking ahead to the possibility of obtaining stem cells from fat tissue, amniotic fluid, the amniotic membrane, and umbilical cord blood, all of which are rich in different types of adult stem cells.

Mesenchymal stem cells, for example, are found predominantly in fat tissue and are reportedly very potent, capable of greater and more varied regeneration than hematopoietic stem cells. But the procedure for extracting and cultivating them is complex and costly. Adult bone marrow stem cells, however, contain not just hematopoietic stem cells but also mesenchymal stem cells and a variety of other stem cell types with regenerative potential. Therefore, instead of separating hematopoietic stem cells to use alone, we are

Interview

taking advantage of this “cocktail” to stimulate regeneration of different types of tissue. And we have obtained results comparable to what other researchers report using only mesenchymal cells.

MEDICC Review: Is any research being done in Cuba using embryonic stem cells?

Porfirio Hernández: No, we are not investigating embryonic stem cell therapies and currently have no plans to do so. There is a lot of interest in embryonic stem cells because they have the greatest potential for differentiation and generation of any type of tissue, but they have many disadvantages. First of all, they have to be extracted from a human embryo, which is ethically and sometimes legally problematic. Secondly, cultivating them in sufficient quantities is complex and requires special laboratory conditions. Thirdly, they carry a risk of overgeneration resulting in production of tumors.

Advanced stem cell research is looking at ways to minimize that risk and also ways to reprogram adult stem cells to become like embryonic stem cells with almost the same differentiation potential but without the risks or the same ethical constraints. This has created a lot of expectation, and we are following these developments very closely. Right now we don't have the capac-

ity to do that level of research here, but we are in contact with laboratories in other countries about training opportunities that will enable us to do more advanced research and improve our technical know-how.

The media and the scientific community are focusing attention on those stem cells and applications that appear to have maximum potential but that also require maximum resources. We, on the other hand, are focused on obtaining maximum potential using minimum resources. If we wait until a better or more effective treatment becomes available within our means, a lot of patients who might benefit from the method we are using now would be left out.

We face a lot of difficulties, but if we dwell on them, we'll think what we're trying to do can't be done. Our results are showing this method can achieve positive outcomes for patients. I take great satisfaction from seeing remarkable improvement in patients who generally have chronic conditions with few therapeutic alternatives. 

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