CELL THERAPY ON THE RADAR

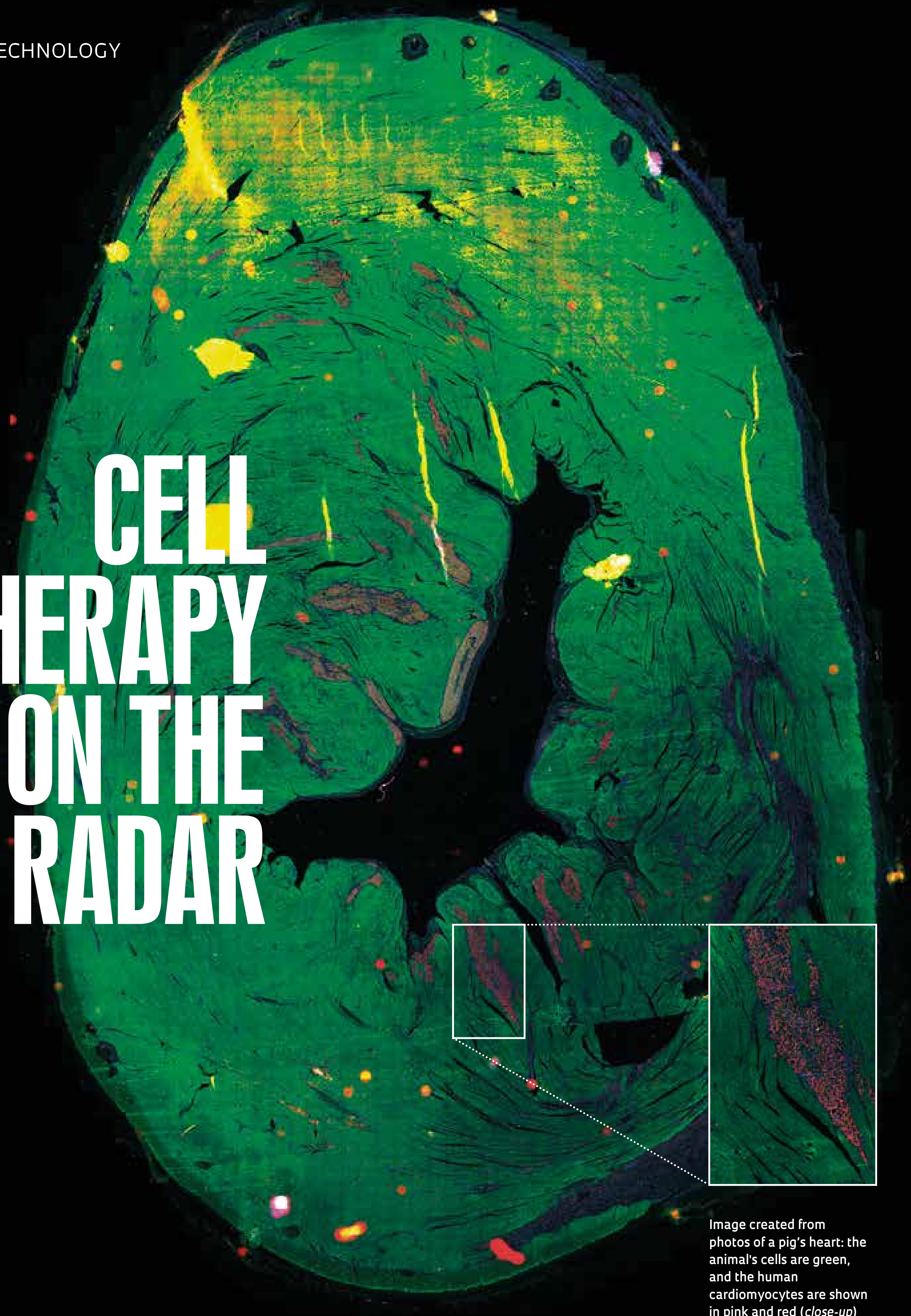

Image created from photos of a pig's heart: the animal's cells are green, and the human cardiomyocytes are shown in pink and red (*close-up*)

Company changes business plans and invests in regenerative medicine in search of cures for diseases that currently have no treatment

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For academic researchers, entrepreneurship requires determination, knowledge of the risks, and a good dose of courage to navigate the countless obstacles that will be encountered along the way. Therefore, imagine the hurdles that one must overcome when leaving a well-known field to invest in a more complex and challenging field. This is what the founders of the São Paulo-based company Pluricell Biotech decided to do. In 2014, less than a year after it was founded, the biotechnology startup obtained its initial funding from FAPESP's Research for Innovation in Small Businesses (RISB, or PIPE in Portuguese) program for a project aiming to produce human cardiomyocytes. At the time, the company's objective was to offer these cells, which are responsible for making the heart muscle pulsate, to academic researchers aiming to conduct in vitro testing of new drugs.

Almost eight years later, the startup changed course. In April 2021, the company name was changed to LizarBio Therapeutics as it shifted its attention to the international market. It began focusing on the potential use of cardiomyocyte grafts for regenerating injured heart tissue in people who have suffered heart attacks. The biotech company's new goal was to develop cell therapies for diseases for which there is currently no cure—a much more challenging objective than offering cells for research into new compounds. The RISB program was awarded funding for this new scope in 2021.

In the corporate world, this type of change in business direction is known as pivoting. Some people compare it to the pivot of a basketball player who keeps one foot on the ground as a fixed base while rotating his or her body, searching for the best move to make. The base, in this instance, was the subject biologist Diogo Biagi, one of the founders of Pluricell, studied during his PhD studies between 2011 and 2014.

To investigate changes in the heart cells of individuals with heart disease, Biagi used their skin cells to generate induced pluripotent stem cells (iPSCs), which are similar to embryonic stem cells, and then transformed them into cardiomyocytes. His PhD work, carried out at the Heart Institute (InCor) of the Hospital das Clínicas, School of Medicine, University of São Paulo (USP), was funded by a grant from FAPESP.

The technology needed to produce iPSCs was developed and published in 2007 by the Japanese scientist Shinya Yamanaka, who won a Nobel Prize in Medicine five years later. The technology's primary application, according to Biagi, has been in modeling diseases and developing new drugs. Cells are obtained from patients and used as experimental models that allow scientists to observe the behavior of diseased cell tissues and their reactions to new compounds.

When Pluricell was founded by Biagi and two of his colleagues—biologist Marcos Valadares and cardiologist Alexandre Pereira, who is no longer with the company—producing iPSCs for Brazilian laboratories seemed to be a natural direction for the business. Biagi's doctoral supervisor José Eduardo Krieger, director of InCor's Laboratory of Molecular Genetics and Cardiology, was unsure about the decision. "I found the initial business model strange. They wanted to sell cells, but there was no market. The sector they are working in now—regeneration—is more suitable," says the physiologist, who was one of the pioneers of stem-cell studies for cardiac regeneration in Brazil in the 2000s.

The partners of the startup, which today numbers 10 researchers, six of whom hold PhDs, soon reached the same conclusion. "We made the de-

JULLIANA CARVALHO CAMPOS-OLIVEIRA AND ANTONIO FERNANDO RIBEIRO JÚNIOR cision to pivot at the end of 2017, after concluding that the research market in this field is small and dispersed, even worldwide," says Valadares, CEO of LizarBio. "We realized there would be more added value in medicine if we offered the product to patients rather than researchers."

With this change in direction, the startup stopped selling to the medical research market and thus lost its only source of revenue. However, it chose to remain at the forefront of the incipient field of regenerative medicine in Brazil, relying solely on investments from funding agencies and the private sector. In 2019, it received US $1 million from the pharmaceutical company Libbs Farmacêutica to study regenerative cell therapy for cardiovascular diseases, and in 2021, it was given R $2 million by an undisclosed investment firm. It has also obtained approval from FAPESP for eight RISB projects, totaling approximately R $4.5 million.

The startup's decision to move into cell therapy for cardiac regeneration was followed by efforts to internationalize. A partnership was formed with the Brazilian neuroscientist Alysson Muotri of the University of California, San Diego, who uses pluripotent stem cells to study Rett syndrome, a neurological disease caused by a genetic mutation that occurs primarily in girls (*see* Pesquisa FAPESP issue *no. 173*).

"Children who suffer from Rett syndrome begin to show signs of developmental delay in the first few years of life. They usually have motor problems and speech and communication difficulties," says Estela Cruvinel, a biologist and project manager at LizarBio. "It is a spectrum similar to autism."

Muotri uses iPSCs to create cerebral organoids: pea-sized, three-dimensional cell models that mimic the behavior of the human brain. With these mini-brains, he has been studying drugs that could treat Rett syndrome, which currently has no cure or specific medication. "I met the Pluricell team some time ago, and I was impressed. The connection between us was inevitable, and LizarBio was created soon after, incorporating my research on Rett syndrome and other neurological diseases," says the neuroscientist, who is a cofounder of the new company.

Pursuing a potential future treatment for Rett syndrome, the researchers are differentiating iPSCs into glial cells—a varied range of cells found in the brain and other organs of the central nervous system affected by the disease. "Tests using pluripotent stem cells in mice have been started in Alysson's lab. The first results should be released in the coming months," says Cruvinel.

BOTTLENECKS AND CHALLENGES

Since it was founded, LizarBio has been based at USP's Center for Innovation, Entrepreneurship, and Technology (CIETEC), but it plans to move to its own headquarters soon. "The aim of the next round of investments is to move into a more adequate space," says Valadares. The new funds will also be used to study how cardiomyocytes can alleviate heart failure, an area of research at a more advanced stage than nerve tissue cell research.

According to Biagi, the RISB phase 2 funding includes the first stage of a study assessing the potential for grafting cardiomyocytes into cardiac tissue, which involves producing and multiplying the cells on a large scale. "The next step, which is an assessment of cardiomyocytes in pigs, still requires further investment, which we hope to obtain soon." The company has already conducted initial tests to validate the methodology that it will use in preclinical trials involving eight pigs, half of which will receive the stem cells while the other half serve as controls.

Tests on rats have yielded promising results, which are described in an article published in the *Journal of Personalized Medicine* in April 2021. "The injected cells improved cardiac function, although it is impossible to say how many of the cardiomyocytes were retained in the heart," says Biagi. These are the results he hopes to obtain from the studies on pigs, whose heart muscles are of a similar size to those of humans.

According to Krieger, this is one of the largest bottlenecks of the research on cardiac regeneration. "We have successfully developed cardiomyocytes in the lab. The problem is now how to deliver these cells to the heart so that they are incorporated into the organ and function properly." The physiologist explains that cardiomyocytes made from stem cells are still immature cells. While they are good at proliferating—something that is necessary for cardiac tissue regeneration—there is also a great risk that they will be rejected or cause arrhythmias. "Cardiac function is rhythmic; the cells need to work together," explains Krieger.

Another challenge is the number of cells that need to be produced for the experimental treatment. While the group injected approximately 10 million cardiomyocytes into rats, the pig trial will require approximately 1 billion cells per animal. This number is similar to the number of cells a human loses when he or she suffers a heart attack. The optimal number of cells to graft into heart tissue is still being investigated at LizarBio.

Lab work: a researcher holds up pig heart histological slides (*right*) and another looks at human heart cells produced from iPSCs

To increase its production capacity, the company entered into a partnership with the São Paulo State Institute for Technological Research (IPT) via USP's Interunit Graduate Program in Biotechnology. Under the supervision of IPT researcher Patrícia Leo, LizarBio's Sirlene Rodrigues is studying the cultivation, multiplication, and differentiation of iPSCs into cardiomyocytes as part of her master's degree, with funding from FAPESP. The goal of this partnership with the IPT is to master mass production, which would reduce the cost of producing cardiomyocytes.

Another obstacle to regenerative medicine is the current regulations on the use of stem cells; however, as Valadares highlights, Brazil has made an important advance in recent years in this regard. "Until 2018, there was no formal regulatory framework allowing for the registration of advanced therapeutic products," he says. "In February 2020, Brazil's Health Regulatory Agency [ANVISA] published RDC [Collegiate Board Resolution] 338, which governs the registration of these products."

In Japan, the government authorized clinical trials using iPSCs in 2013. Several research groups from other countries have continued to look for an effective cell therapy. "Encouraging results are attracting more funding from companies. When the risk decreases, private capital input increases," says Krieger.

There is still no guarantee that regenerative therapy will be available in the short term, says Marimelia Aparecida Porcionatto, a professor of molecular biology at the Federal University of São Paulo (UNIFESP) and researcher at the National Institute of Science and Technology for Regenerative Medicine (INCT-Regenera), a research network composed of 28 associated laboratories from different institutions. "The entire development process can take 10 to 20 years—it's hard to predict," she says. "There has been a lot of progress since the 2000s, when labs began more actively studying mesenchymal stem cells isolated from adult tissue. It was thought that they would fix all evils, but they didn't."

Mesenchymal stem cells have not proven effective for cardiac regeneration, for example. "Today, we know that they play a more immunomodulatory role, reducing local inflammation," explains Porcionatto. However, they could still play an important role in repairing diseased hearts. Krieger agrees: "It is a new technique with great potential for improvements, but it is not a miracle cure."

With much hope now placed on induced pluripotent stem cells, the researchers at LizarBio know that in addition to overcoming technical, economic, and legal challenges, they have to deal with high expectations. "We know how long things can take and we are carefully planning each step," concludes Valadares. ■

The research projects and scientific articles consulted for this feature are listed in the online version.

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