diabetes

A risky bet against

Experimental treatment with chemo-therapy and stem cells frees 14 patients from insulin injections

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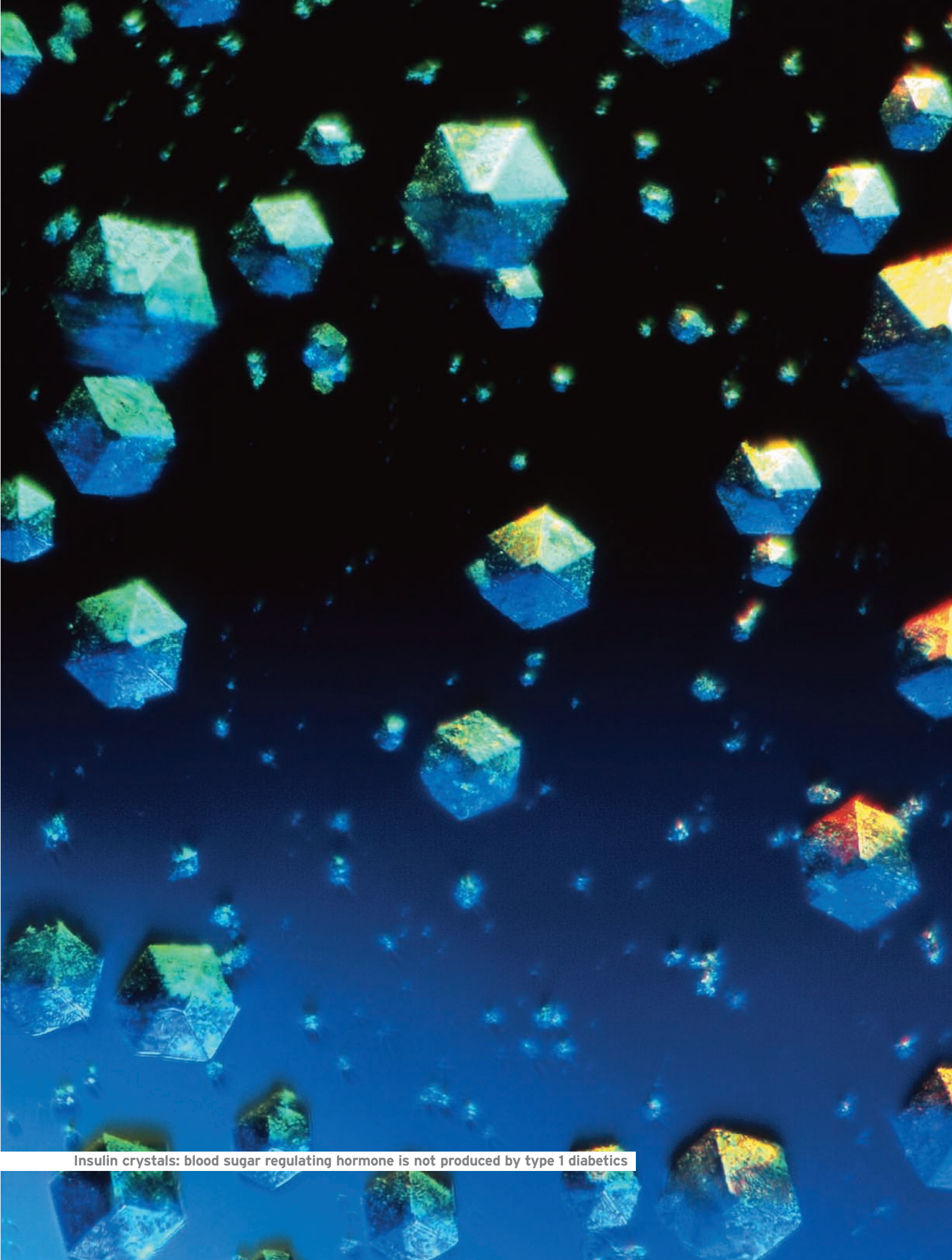
On the 13th of May of last year, dentist Jaider Furlan Abbud, who lives in the São Paulo state town of Pontal, 30 km away from Ribeirão Preto, celebrated his 31st birthday. It was a Saturday and, as is usually the case at these parties, the 'birthday boy' overdid things in terms of food, especially sweets. On Sunday, when he went into the bathroom, he had a surprise: the toilet was surrounded by ants, a classic sign that someone there, probably him, had too much urine sugar. On Monday he went to the doctor and his suspicions were confirmed: he suffered from type 1 diabetes, also called juvenile or insulin-dependent diabetes. Still suspicious of the diagnosis, he consulted one more specialist, whose response was the same as that of the first doctor. In order to control the disease, he would have to take, for his entire life, daily injections of insulin, the hormone responsible for getting glucose out of the blood, which his pancreas had stopped producing due to inflammation that is typical of this type of diabetes. The unpleasant injections routine had to be immediately incorporated into his day-to-day life. "I could hardly believe it", recalls the dentist.

On the 29th of July of last year, less than two months after he had been diagnosed, Jaider left the Clinicas Hospital of the Ribeirão Preto Medical School, the University of São Paulo (USP) 13 kg thinner. He was, however, extremely happy: he no longer needed two daily shots of insulin in order to control his disease. He had been submitted to an aggressive and expensive experimental treatment against type 1 diabetes, combining painful chemotherapy sessions with drugs that depress the immune system and an auto-transplant of the bone marrow, and his pancreas had started producing insulin again. Married and childless, the dentist has now been free of the injections for more than nine months and is one of the 15 Brazilians aged 14 to 31 who, from November 2003 to July 2006, tested this therapy, which was entirely developed by a team from the university's Cellular Therapy Center (CTC). All the patients, save one, the first person submitted to the treatment started producing insulin again. "We can't talk about a cure for diabetes. We must still monitor the patients for a long time to see whether the effects are lasting, and also conduct trials on more people", states immunologist Júlio Cesar Voltarelli, the main proponent of this line of research. "But our work will have a great deal of impact on this area." It was this apparent success of the unprecedented therapeutic approach – the adjective apparent applies because it is not yet known whether the benefits will be temporary or lasting – that led a team of researchers from CTC, one of the Centers of Research, Innovation and Dissemination (*CEPIDs - Centros de Pesquisa, Inovação e Difusão*) financed by FAPESP, to publish a nine page scientific paper in the April 11 edition of the *Journal of the American Medical Association* (*JAMA*), one of the world's most prestigious medical journals. The periodical recognizes the pioneering nature of the work and makes the following comments in its editorial: "Voltarelli's study is the first of many cell therapy attempts that will probably be tested to stop the progress of type 1 diabetes", states Jay S. Skyler, from the University of Miami Diabetes Research Institute,



Insulin crystals: blood sugar regulating hormone is not produced by type 1 diabetics

in this *JAMA* editorial. What was also noteworthy was that the experiment was essentially conducted by Brazilians. "It's a national contribution to research into diabetes", comments Marco Antonio Zago, CTC coordinator. Of the 13 authors of the paper publishied in the *JAMA*, 11 are from USP in Ribeirão Preto and only two come from abroad.

There are a lot of unanswered question regarding the experimental treatment tested by USP in Ribeirão Preto, and the researchers themselves do not deny these uncertainties. What causes the combined therapy to apparently work? Did the patients recover insulin production because of chemotherapy or the auto-transplant of their bone marrow? Or was it the synergism between the two procedures? None of this is known yet. And it is precisely because of this that the Brazilians want to continue pursuing their research. "This first study is of an exploratory nature", states Voltarelli. In other words, the treatment is still shrouded in mystery, as is the very origin of type 1 diabetes. Despite the existence of genetic factors favoring its appearance, the disease manifests itself in the organism due to contact with some external element that causes the dysfunction of the immune system. The problem is that nobody, to date, has managed to discover what causes the human body's defense cells to attack the pancreas region, where insulin is produced.

And the worst thing is that there may be more than just a single external element triggering the entire process. There is also speculation, which still lacks proof, that the inflammation may be caused by a virus, free radicals, or cow's milk, among other agents.

The search for a treatment for type 1 diabetes capable of doing away with the uncomfortable daily insulin injections is understandable. Though they account for 10% of the total population of diabetics, estimated at 200 million individuals worldwide and some 10 million in Brazil, insulin dependent patients are the most severe cases. Among people suffering from type 2 diabetes and pregnancy diabetes, which temporarily affects certain women, the disease can generally be kept under control just with dieting and physical exercise. For juvenile diabetes, on the other hand, which usually appears during childhood or at the beginning of adult life, these measures are not enough. Fighting the pathology necessarily demands external insulin doses. Otherwise, the sick person might die soon. Insulin is essential for life, because it removes glucose from the blood and pushes it into the cells, where it is transformed into energy. The symptoms of the three types of diabetes are the same, though normally they are more acute in type 1 patients: thirst, a constant need to urinate, weight loss even if the person is not dieting, blurred vision, tiredness and pains in the legs.

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Three years with no insulin - The figures that prove the Brazilian experiment's success are impressive. One of the patients treated has had no insulin for 37 months, or more than three years. Another four have done without their needles for at least 23 months and seven have been free of injections for eight months. In two cases, the experimental treatment did not produce immediate results. However, one year after being submitted to the therapy, these diabetics also ceased to be dependent on external doses of the hormone. Of the 14 patients who responded to the therapy, one suffered a relapse, caught a virus and had to go back to taking insulin. The side effects of the new therapeutic approach, even though the latter is aggressive, have shown themselves to be mild so far: one patient had pneumonia and two had endocrinal dysfunctions. However, for the chemotherapy/auto-transplant of stem cells to be able to work, the researchers believe that it is necessary to take great care selecting the patients who will be submitted to the experiment. All the people who in some way benefited from the therapeutic scheme had been formally diagnosed with type 1 diabetes at most six weeks before starting treatment. They were people who had only just become diabetic.

This type of selection has a scientific justification. The researchers believe that in the early stages of the disease, there is still a small number of beta cells in the insulin-producing islets of Langerhans in the pancreas. As the disease progresses, these remaining cells will have the same end as the others: they will be destroyed by the immunological dysfunction that causes type 1 diabetes. The people who took part in the

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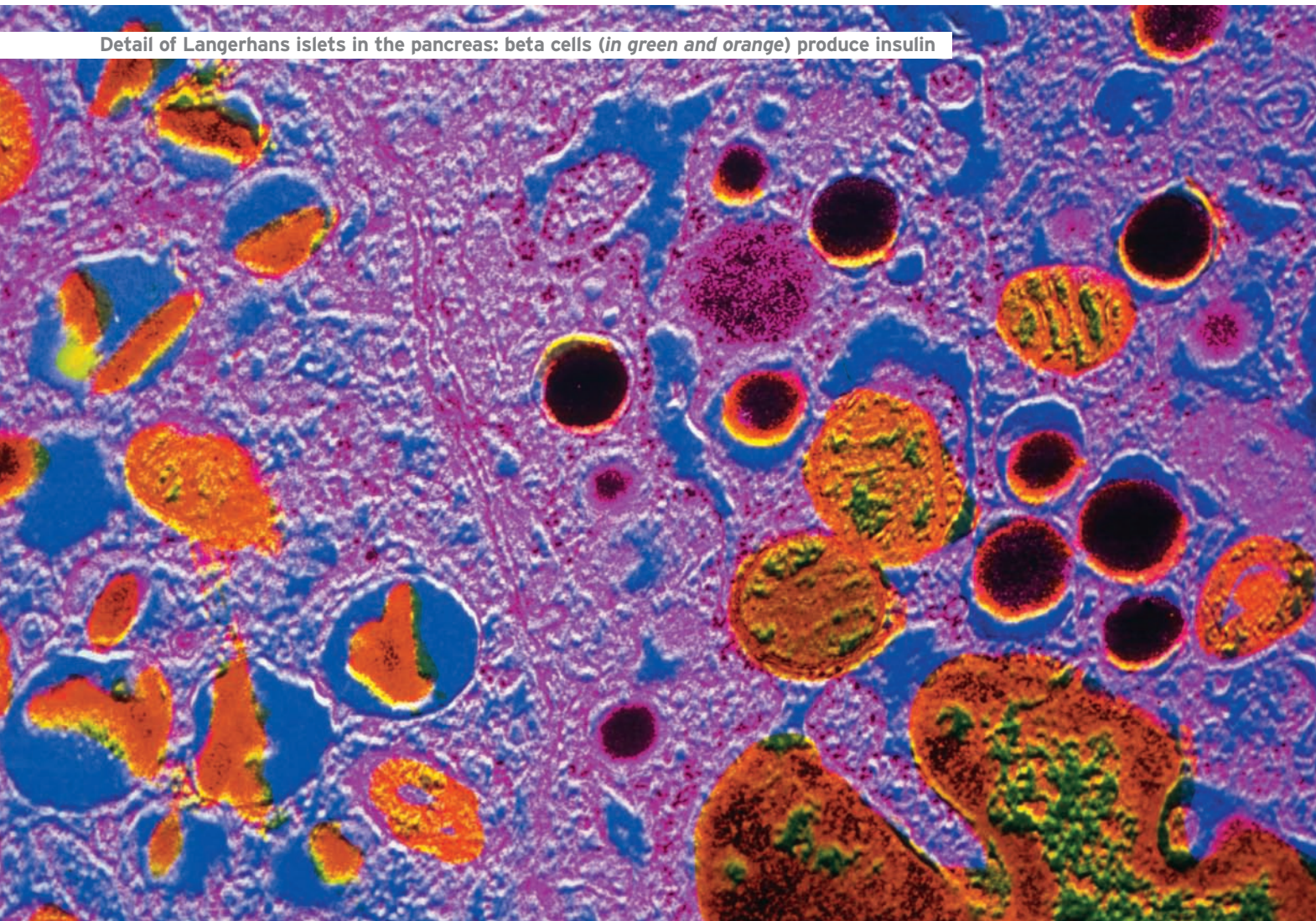
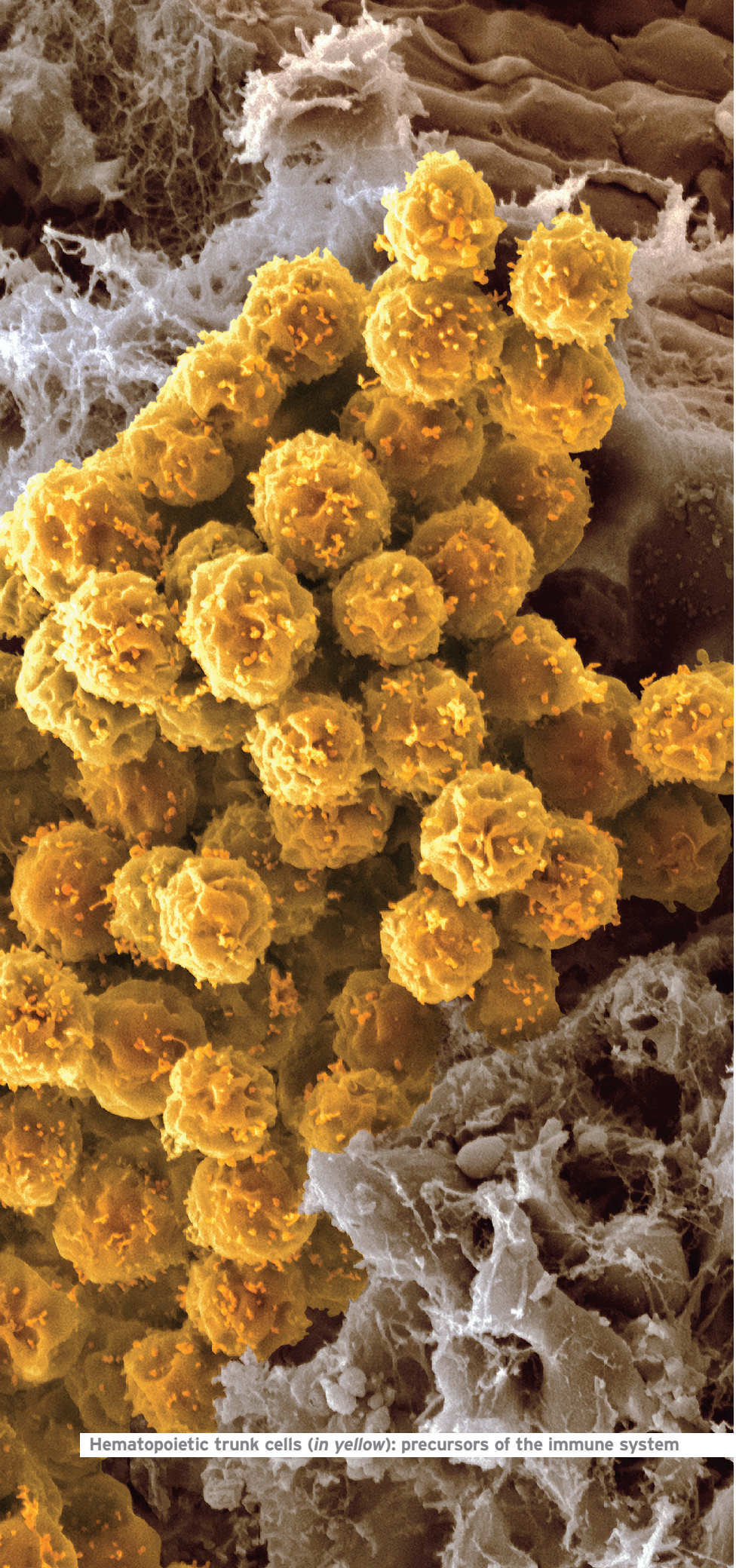
Ribeirão Preto experiment, for instance, still had between 20% and 40% of the beta cells normally found in a healthy body. With this clinical assumption as a starting point, namely, that at the onset of the disease there are still pancreas cells that can be saved from the inflammation attack that is typical of diabetes, the CTC researchers decided to test the treatment in newly-diagnosed diabetic patients only. Thus, they say, the therapy would have a better chance of working. The rationale is simple. If the non-destroyed beta cells are preserved, the body, once it has been freed of the immunological dysfunction that attacks the pancreas, will be able to multiply them and thus resume normal insulin production. This is what may have happened with the patients who responded well to the treatment.

Unprecedented worldwide, the therapeutic approach used on the 15 patients relies on high doses of chemotherapic and immunotherapic drugs (cyclophosphamide and antithymocyte globulin), followed by a transplant of hematopoietic stem cells, capable of differentiating themselves and generating other types of cells, such as red blood cells, platelets and the white blood cells of the body's defense system, which had previously been removed from the bone marrow of the same patient and conserved in liquid nitrogen. This second procedure is know as an autologous bone marrow transplant (or auto-transplant) and is free of rejection risks. Therefore, the experimental treatment attacked diabetes on two fronts, using a scheme that is similar to what is used to fight certain types of cancer, such as certain kinds of leukemia. First, the chemotherapy practically destroys the patient's entire immune system, which is the source of the inflammatory problem that attacks and kills the pancreas's beta cells. Then, an intravenous injection of the hematopoietic stem cells is meant to speed up the reconstruction of the patient's immune system. Or rather, of a new immune system that, for reasons as yet unknown, seems to be free of the inflammatory dysfunction that attacks beta cells. "It's as if we brought the body's defenses down to zero and the patient went back to having a child's immune system", states Voltarelli, who also tests stem cell therapies on other autoimmune diseases, such as lupus and systemic sclerosis. Therefore, those who submitted to this treatment, besides losing their hair, vomiting and undergoing other forms of discomfort, must also take vaccines all over again. After all, the immune system's memory has apparently been erased or made dormant.

These encouraging results of the experimental treatment of type 1 diabetes, though still preliminary, became hot news all over the world. For better or for worse. Feature articles and more feature articles about the study were produced both in Brazil and abroad. Some of them resorted to a tone that bordered on sensationalism, as if the USP researchers had announced a cure for the disease, which they had not done. Just to men-



Detail of Langerhans islets in the pancreas: beta cells (*in green and orange*) produce insulin



Hematopoietic trunk cells (*in yellow*): precursors of the immune system

STEVE GSCHMEISSNER/SCIENCE PHOTO LIBRARY/LATIN STOCK

tion some of the international media examples, French newspapers such as *Le Monde*, the British *Financial Times* and the North American *The Wall Street Journal* reported the study. In these articles, certain questions arose regarding the results achieved by the Ribeirão Preto team. Perhaps the most critical article was published in the April 21 edition of the *New Scientist*, a highly respected British science magazine . Using a heading a full octave higher than what the CTC team had described, and using the expression "cure for diabetes using stem cells", the publication's text voiced the doubts of foreign researchers regarding technical and even ethical aspects of the Brazilian experiment.

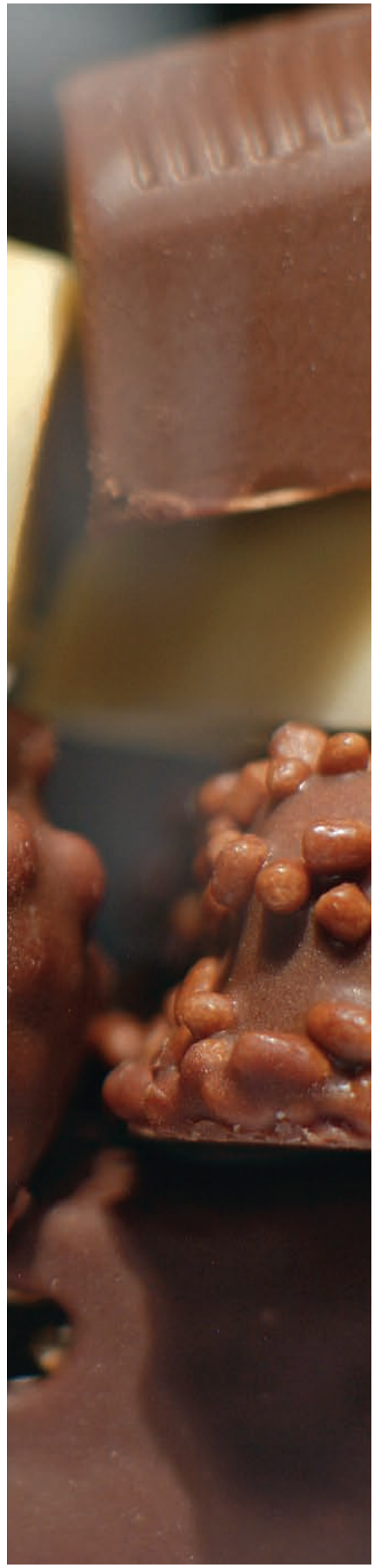
Honeymoon effect - In general terms, the article, which also allowed room for Voltarelli to defend the work, questions whether the experimental treatment really did benefit the patients. It also insinuates that it is easier to test new, high-risk stem cell therapies in Asia and Latin America, where there are allegedly fewer legal controls than in Europe and the USA. Kevan Harold, from Yale University (USA), one the researchers interviewed by the English magazine , states that type 1 diabetes patients can go through a so-called honeymoon stage in which they temporarily resume insulin production. According to this line of thought, the Brazilian team might be ascribing the recovery of hormone production in the pancreas to the effects of the treatment, while everything might be no more than a passing and natural bodily reaction. The CTC research team disagree with this type of argument. "There is no honeymoon period capable of explaining the fact that 14 of our 15 patients have gone back to producing insulin, some of them for years", counter-argues endocrinologist Carlos Eduardo Couri, another author of the paper in the *JAMA*. "It would be too much of a coincidence."

One of the opinions against the Brazilian experiment was collected by the *New Scientist* from Lainie Ross Friedman, a medical ethics expert from the University of Chicago, who also talked to *Pesquisa FAPESP*. Lainie's sharpest observation is about including children in the study. "Brazil is one of the signatories of the Helsinki Declaration (a chart of

ethical principles in scientific research sponsored by the World Medical Association) and the initial trials of this therapy should not have included children, only adults", states Lainie.

"Furthermore, there should have been a control group (patients who underwent conventional treatment for type 1 diabetes and whose clinical evolution would form the basis for comparing the effectiveness of the alternative therapy)." Eight of the fifteen people treated were under 18 at the time when the therapeutic scheme was adopted. These children, to her mind, should only have taken part in the experiment later, once it had been clearly demonstrated among adults that the alternative therapy is better than the conventional one. Lainie also feels that the experiment is very dangerous for its participants and alludes to the increased risk of cancer, infertility and even death as a result of using such an aggressive treatment for diabetes. In Brazil too there are researchers who objected to the CTC experiment, although more affably and without detracting from the study's merit. "I have great admiration for doctor Julio's bold and courageous work", ponders Mari Cleide Sogayar, from the USP Chemical Institute, another scholar who focuses on diabetes. "However, the proposed treatment is a heterodox step and one must evaluate its cost/benefit effect well."

The CTC team is the first to admit the risks and limitations of the therapeutic scheme under study. It even refers to this in the very text of the *JAMA* article. Still, the scientists do not exempt themselves from responding to the criticism and advocating the ethical correctness of the experiment. According to Voltarelli, the clinical study fulfilled all the moral and legal requirements in force in the country and took more than one year to gain approval from the National Commission of Research Ethics (*CONEP – Comissão Nacional de Ética em Pesquisa*), a body of the Ministry of Health that authorizes this type of work. "The Commission is more demanding than the US Food and Drug Administration (which is in charge of the quality of food and drugs, besides regulating clinical studies)", argues the CTC immunologist, giving one to understand that part of the criticism of foreign researchers is driven by the fact that the study was conducted by a group that was not from one of the major centers of world science. He considers using minors in the study justified because the disease manifests itself in different ways among children and adults. Voltarelli also tells us that he tried to set up a control group, but was unable to find a sufficient number of interested parties. "But we will have to form a control group for the next studies", he admits. Regarding the health problems that the experimental treatment could cause the patients, the CTC team also embraces a policy of total transparence. "We talked about everything during the process of selecting candidates for the experiment, even the possibility of death", says Couri. "It is minimal, but it does exist. So much so that most interviewed patients chose not to go through with the treatment."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One of the CTC team's chief concerns is not to give false recovery hopes to type 1 diabetics. Since the positive results were published by the media, Voltarelli has been receiving 200 e-mails a day from patients interested in undergoing this therapeutic scheme. "From the USA alone there are ten a day", the immunologist tells us. The researchers are well aware that the experimental treatment is not a definitive solution for the disease. Besides the doubts that haunt the therapy's action mechanism and the issue of how long its benefits will last, Voltarelli points out that the treatment is too expensive and risky to be proposed as a standard procedure for the world's millions of type 1 diabetics. At present, each patient treated at USP in Riberão Preto costs some R$ 20 to 30 thousand and must remain within an isolation unit for at least 20 days under intensive care in the bone marrow transplant center. In other words, the procedures that are necessary to perform the treatment that CTC is testing can only be carried out at highly specialized hospitals. The researcher's dream is to achieve an effective but less aggressive and cheaper treatment for diabetes. One of the CTC team's hopes center on mesenchymal stem cells, another primitive type of cell also found in the bone marrow. These cells seem to be able to depress the immune system. "Perhaps with them we will manage to do without chemotherapy, the treatment's most aggressive stage", says Voltarelli.