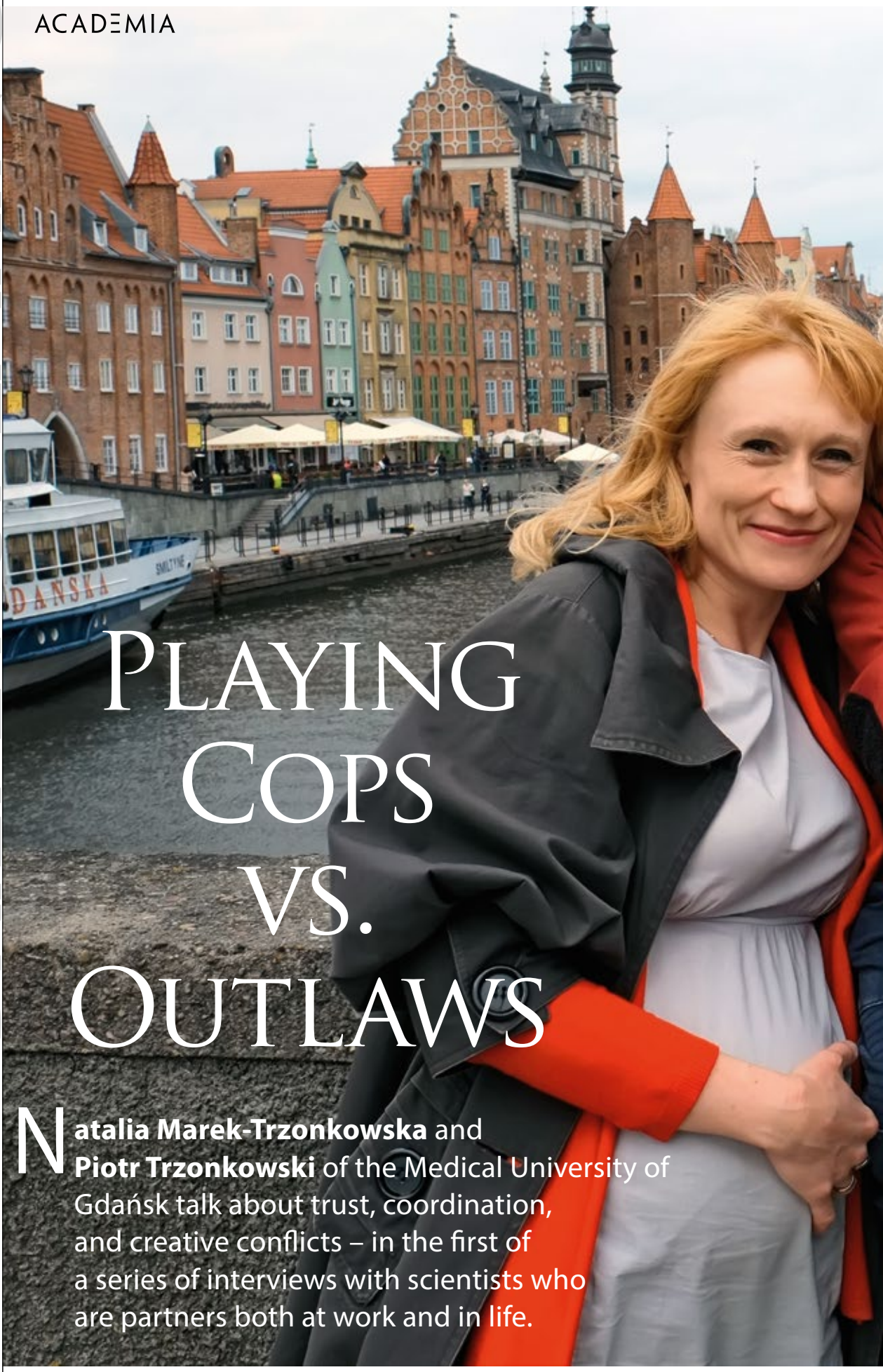

Playing Cops vs. Outlaws

Natalia Marek-Trzonkowska and **Piotr Trzonkowski** of the Medical University of Gdańsk talk about trust, coordination, and creative conflicts – in the first of a series of interviews with scientists who are partners both at work and in life.

NATALIA AND PIOTR TRZONKOWSCY

ACADEMIA: How did you start working together?

NATALIA MAREK-TRZONKOWSKA: We met at work, became a couple, and later started working together closely in research. Of course, this doesn't mean that we sit side by side all the time and pass each other test tubes, or that we do everything together at the same time. We conduct different studies, which nonetheless complement each other. Aside from that, we understand each other well. For example, if I know that Piotr will be sitting at work until midnight, doing experiments, I don't get anxious or call him ten times to ask who he's sitting there with. This works both ways. If I spend the night in the lab, Piotr takes care of our child, dog, and home.

Being partners in a relationship is very important, especially for women scientists. For men, it's generally acceptable to have very demanding jobs. But if women are in a similar situation and additionally have small children, the social perception is different: they are neglecting their home, hurting their loved ones, and being overly ambitious. That's why having the support of your partner or your family is hugely important from the perspective of logistics as well as the female psyche.

> When we work together, we have differences of opinion about details. On the outside, this often looks very much like a quarrel. But that's very good for science.

PIOTR TRZONKOWSKI: When we work together, we have differences of opinion about details. On the outside, this often looks very much like a quarrel. We don't usually see eye to eye at work, and we've had silent days in the lab, too. But that's very good for science, because such arguments usually spark great results. Natalia is a great partner for me – we complement each other very well. Surely, we wouldn't have accomplished so much separately.

NMT: Just to set the record straight, we sometimes have different opinions, but I can't think of even one "silent day," because I couldn't just sit there and say nothing (laughs). For that matter, a difference of opinion can't be the cause for a real argument. If one of us can't convince the other of a certain opinion, the only thing we can do is agree or disagree and respect each other's views.

Do you argue at work so that things can be more peaceful at home?

PT: The passion we share helps us overcome certain things. I think we have difficult characters as scientists, because we have our opinions, and we try to push them through. An exchange of thoughts can be very emotional, but it makes you stop and think that maybe the other person is right. We sometimes find out who was right several months later, sometimes years later. I then feel like I've wasted so much time, as Natalia was telling me all along that we should have done this differently. We would have then made more progress.

NMT: In private life, we are very tolerant. We don't pay attention to trifles. We understand each other's behavior very well. Honestly, I don't understand why we would argue. We get along well, as if we had known each other for ages. Also, the dog and the kid constantly try our patience. We both have a lot of tolerance for messes, which helps (laughs).

Who was the first to come up with the idea of bringing together your research?

PT: We combined two approaches. I'm somewhat older than Natalia, so I earlier studied immunology, regulatory T cells, and tolerance. I came back to Poland after a research fellowship in the UK, and I started seeing Natalia. At some point, I started paying attention to what she was doing. She was studying type 1 diabetes, and she was interested in regulatory T cells in the context of this disease. Back then, she worked with Prof. Małgorzata Myśliwiec of the Department of Pediatrics and Diabetology, Medical University of Gdańsk.

At some point, the three of us began to talk about combining our research, because the cells I grew for a completely different project would fit their work to a considerable degree. More helpful inspiration for our project came from Prof. Anna Balcerska, who back then helped us a lot, and we got the project up and running. We started to use these cells to treat children with diabetes.

NMT: I'm not a doctor by profession, so it was more difficult for me to start such clinical trials. When I found out that Piotr was studying regulatory T cells in the therapeutic context, I was very keen to join the studies. Also, I saw a real chance for a therapy for diabetes, which I was studying back then, although those cells could be used practically in any autoimmune disease.

Let's talk about diabetes. What sort of a disease is it?

PT: It affects mainly children, who account for 90% of the patients with diagnosed type 1 diabetes. The onset

of the disease often occurs in early childhood, so these children don't remember a life without an insulin pen or insulin pump. But it's also a family disease. First of all, parents are very involved in the treatment process. Secondly, there are other problems. How will the children adapt at school? Can they go to any type of school? In Poland, there are some very good solutions, chiefly thanks to pediatric diabetologists. Teachers, and the public in general, know relatively much about diabetes. Unfortunately, there has been a considerable rise in the incidence of diabetes. Several years ago, Poland was mentioned at diabetes conferences as the European country with the fastest growth in the number of cases of diabetes.

NMT: In the case of small children, frequent measurements of blood glucose levels are certainly problematic, and so is the administration of insulin. That's less problematic for children who have insulin pumps. Teenagers, however, perceive not just the disease but especially the insulin pump as something embarrassing. At that age, we usually want to be like others, and we most certainly don't want to stand out in this way. If you go to the beach, the pump will be visible. You have to remove it when you go to the swimming pool. You constantly have to be remembering something, calculating something, and so on.

Also, people usually don't realize that stress, caused for example by a quiz or an exam, can cause changes in blood glucose levels. Such children must often leave class to check their glucose levels and take insulin. What is needed is knowledge and acceptance on the part of teachers and everyone at school to make sure such children are not discriminated against or excluded.

What causes this disease?

PT: There are well-known genetic factors. People who are predisposed to develop diabetes carry certain genes, in particular specific HLA variants as well as genes that determine the function of regulatory T cells. In other words, if one child in a given family develops diabetes, other children in this family are also very likely to have it.

NMT: Sometimes, however, one identical twin has diabetes, while the other doesn't.

PT: As for the increasing numbers of cases, that's the price of the advancement of civilization. Diabetes occurs more frequently in societies that develop very rapidly. A rapid change of environmental conditions, a change of diet and the related change of gut flora, and greater exposure to synthetic components all cause the immune system to stop correctly identifying tissues as friends or foes and to start seeing the pancreas as foreign and start attacking it.

NMT: But we must stress that at the root of type 1 diabetes lies an autoimmune response, which means that blood cells start attacking the insulin producing cells in the pancreas.

Your research was aimed at boosting the immune system with a vaccine.

NMT: More at toning down the autoimmune response. We want to restore the balance in the immune system to control it better. That's the purpose of regulatory T cells. We compare them to the military police, who make sure that all the other soldiers – all other cells in the immune system – defend the body against bacteria and viruses, instead of attacking friendly tissue. However, if such autoaggressive "renegades" appear in the immune system and start damaging the body's own tissue, regulatory T cells can "arrest" them, even kill them. We take regulatory T cells from patients with diabetes, multiply them in the lab, and administer them back to the patients to help them gain the upper hand over the "renegades" damaging their pancreases.

> We understand each other very well and don't concern ourselves with trifles. We get along as if we had known each other for ages. Also, having a dog and a kid constantly tries our patience. Having a lot of tolerance for messes helps.

Do you do that yourselves?

NMT: We handled the first patients ourselves. The task is quite unusual, because it takes place in what is referred as a cleanroom, or a lab maintaining a very high level of air cleanliness. It is monitored, and only specially trained personnel can access it. We work in special sterile suits. We essentially look like aliens. It's more difficult to breathe and move in such clothes. It's usually hot in there, despite the air conditioning. For safety reasons, there should be at least two people working in such a lab. Initially, we did everything together. After that, we reached the conclusion that we were skilled enough to divide up the work. Piotr grew cells for one patient, I did the same thing for another patient, so we could work more efficiently.

No one realizes how difficult this work is. It appears simple: we take blood, we isolate and multiply something, and administer the product using a syringe. In

reality, however, we process liters of liquids by measuring doses of 100–200 µl every step of the way. Such isolation can take 12–24 hours. As a rule, we receive the material in the afternoon, so we finish work after midnight or the next morning. On the next day, we start culturing the cells every day for two weeks.

We need to passage the cells, which involves transferring them to further and further culture dishes. As they grow, there are more and more of them, which also means proportionally more work. Initially, that took us a lot of time. I'd sometimes walk into the lab at 3 p.m. on a Saturday, hoping to call it a day by 8 p.m.

Natalia Marek-Trzonkowska, PhD, DSc

is the principal investigator of a research team at the International Center for Cancer Vaccine Science, University of Gdańsk, and the head of the Laboratory of Immunoregulation and Cellular Therapies at the Department of Family Medicine, Medical University of Gdańsk. She studies cancer immunotherapy and ways of intercellular communication. She has won scientific prizes both in Poland and abroad.

Prof. Piotr Trzonkowski, MD, PhD

is the head of the Department of Clinical Immunology and Transplantology, Medical University of Gdańsk. He has won numerous scientific prizes, including the 2017 Prize of the Foundation for Polish Science in the life and earth sciences for studies on regulatory T cells and their pioneering use in cell therapy of human diseases.

and get some rest, but end up finishing work on Sunday morning. On the face of it, this work looks simple, but you need to concentrate, because if the culture gets infected, everything's over, the whole work goes to waste. It's very difficult to stay on your toes when you are tired.

PT: The good thing is that we already have a team, and our associates can now perform these tasks, too. We've just completed the recruitment of patients for another clinical trial. We are not isolating cells now, also because the standards of the production of such cells are constantly rising, so the old lab no longer meets the required criteria. That's why we are arranging for a new lab, but this is logistically and "politically" very challenging, because not everyone wants us to continue the project. Also, the work is now more automatic. As Natalia said, it's no longer so arduous.

NMT: At the beginning, however, it was arduous. I mean the first 20 or 30 patients. Now, we have a larger team, and I no longer grow cells for patients, which is probably also healthier for us as a couple, because we've divided up the work. Piotr deals with the clinical part, I conduct basic research.

How do you measure efficacy?

PT: That's why we have good manufacturing practices, which dictate the criteria that such preparations must meet. As for the preparation's efficacy in a specific disease, that depends on the disease. In the final analysis, we'd like to have a healthy person, or at least reduce the symptoms of the disease and its complications. In diabetes, we're interested in the secretion of insulin, but we assess this by measuring the levels of C-peptide, which is part of the pro-insulin molecule secreted together with insulin. If someone receives exogenous insulin, or insulin injections, we can't measure insulin levels in the blood, because we'd then be measuring the patient's own insulin and the insulin coming from external sources. C-peptide, in turn, always comes only from a patient's pancreas and is secreted in the same amount as the patient's own insulin. A child with diabetes is given a very standardized meal. After the meal, the level of insulin and C-peptide should rise, like in every person. We monitor the level to which they do increase, and that's the best measurement of the progress of the disease and the efficacy of the therapy.

NMT: Patients who have never received regulatory T cells practically produce no insulin after two or three years, so we can see no increase in C-peptide levels after a meal. In turn, in patients who received such cells, even after such a long period, we can still observe the release of insulin after a meal, which offers proof that the pancreas still works. It's not as high as in healthy people, but it is significant. That's very important for patients with diabetes, because even if they already take insulin in injections, their own insulin, produced in the pancreas, and C-peptide alone allow a lot more precise control of glucose levels in the blood, which protects against complications. And complications are what currently poses a problem for diabetic patients. In the course of the disease, the patients develop eye, kidney, and blood circulation problems, and the insulin produced by their bodies, even if these are small amounts, allows the postponement of the onset of such complications for a certain number of years. In a sense, we are fighting for the quality of our patients' lives in the future.

NMT: Moreover, we know that the secretion of even small amounts of insulin protects patients against life-threatening hypoglycemia, which is a rapid drop in blood glucose levels. It may even occur at night, when patients are unaware.

PT: We started off with one of the complications of bone marrow transplantation: the graft-versus-host disease, which is a cause of a large share of deaths after the procedure. In this disease, the transplanted immune system recognizes the host's tissue as foreign and attacks it. The mechanism is similar to that of the autoimmune process: the damage is done by "renegades," in other words by the donor's lymphocytes sensitized to the host, while the defense is offered by "cops," or regulatory T cells. In this disease, efficacy is measured by the improvement of symptoms and vital parameters and the possibility that such patients may stop taking immunosuppressive drugs.

In multiple sclerosis, in turn, we monitor the progression of the disease as such. For the time being, these are safety trials, but we also monitor the pace of the progression after such cells are administered. In a year, we will complete phase one, and we'll be able to say something more.

I think that it's important to stress that the role of the clinicians who look after such patients on a daily basis can't be overestimated. Their observations regarding efficacy are a mine of information about what we could improve in the future. It's always about teamwork.

You conduct clinical trials. What are the potential negative consequences for patients or for you? How are you and your patients protected against them?

PT: That's an important issue. Clinical trials have a specific framework. It determines very precisely the role of investigators, patients, and preparations, the rights and the obligations. Everything starts with a bioethics committee, which must approve such a study, concluding that it will not harm patients. After that, we must describe the production of the preparations in detail. And we must write out a plan for the study. It's also very detailed: we describe what we will be doing every day during the trial. We even specify how many samples of blood we will collect on a given day. That's the clinical trial protocol, which is

chiefly for the doctors who look after the patients. We also write information for patients, where we write exactly the same things as in the clinical program, but in a simple language – what we will be doing, why we do it, what the potential benefits are, and what losses the patients could potentially suffer. Let's face it, there are no medicines that only have beneficial effects. By definition, every substance we administer may have certain side effects that we can anticipate. For such a trial to be conducted, it needs to be insured. Much in the same way we'd insure a house or a car. In addition, clinical trials always include a clause allowing patients to withdraw at any time, without giving reasons. For children, there is obviously the additional requirement of the consent of the legal guardian. It's typically given by parents and, in the case of older children, also by the children themselves. We always talk to them to check if they are convinced that they want to do this or perhaps being pushed by their parents. If they don't want to, they don't participate in the program.

Do parents sometimes say "no," like in the case of vaccines?

PT: The term "vaccine" is somewhat confusing. It doesn't mean a vaccine in the general understanding of the word. This's a cell preparation that has immunosuppressive effects, as opposed to immunizing ones. We therefore don't meet with the negative social reactions that often occur when immunizing vaccines are discussed.

NMT: That's because what we administer to patients doesn't include anything foreign, there are no bacteria or viruses, unlike in vaccines. We only give them what was previously in their bodies. We only change the proportions slightly.

You've mentioned the losses that patients could potentially suffer. What could happen?

PT: As in every immunosuppressive therapy, the activity of the immune system is being inhibited so infections are more frequent. We have results, so we know that these infections are quite trivial, but there's lower immunity, and we diligently say so in advance. Theoretically – I repeat, theoretically – there's is a possibility of the progression of an existing cancer that we do not know of at the time the preparation is administered.

When we started, we foresaw this possibility, but we still weren't 100% sure what would happen. Right now, there are around 40 ongoing studies involving regulatory T cells in the world, and no such cancer can be observed. What we've always thought has been confirmed – these cells are smart immunosuppressants. In fact, they only act when they should act, unlike pharmacological immunosuppressants, which…

NMT: … always have the same mechanism of action and always have side effects. Our cells adjust to the situation in the body. Side effects are possible – long-term side effects, which we've mentioned, and short-term side effects, for example like with every IV drip. Anything that is administered to a patient may have side effects, including shock. We've had several infections, but we've had no side effects more serious than that. Here, I'd like to thank our clinical cooperators, who put a lot of effort into reducing those negative consequences. It's very important that the doctors have contact with the patients, because the hours they spend with patients, explaining to them what will happen, prevent patients from approaching the therapy emotionally and help them see it as another treatment.

Is the treatment patented?

PT: There are several patent applications. We've been working on this therapy for many years, so we've sought patent protection for certain elements. But patents and all these commercial things are not science. I think that's another story, not necessarily an optimistic one, to talk about on another occasion.

Is there similar work being done in the world?

PT: Currently, there are around 40 clinical studies involving regulatory T cells. They started around 2011. Initially, that was great, because the medical environment was enthusiastic, and this was being done by universities in an academic way that allowed for rapid development. Whether it's good or bad, pharmaceutical companies spotted a business opportunity, and things started to head in a very commercial direction. Commercial trials have started, and the purpose is to sell these cells as medicines at some point. To me, what is important is also the development of this therapy and its availability to patients, so I constantly wonder where the boundaries should be set between science, patients, and commercial businesses. These latter stakeholders, in particular, have no sense of such boundaries.

NMT: Nevertheless, our team was the first to administer these cells to humans. The beginnings were very difficult. We had the first clinical report ready in 2008, and it was difficult to publish it, because the scientific world did not believe us. Poland is still stereotypically perceived as a country that is not fully prepared to conduct professional clinical trials, and we were probably not credible in the eyes of many people. Also, it was hard to accept that we really had achieved something earlier than good hospitals in the West.

PT: On the other hand, there are indeed very few non-commercial clinical trials in Poland, which means trials conducted by universities. There are various reasons for this, one of them probably being that we have no tradition of such research. Also, such trials pose a considerable logistic, administrative, and bureau-

cratic burden, and our medical universities are unprepared for that.

NMT: We had to do everything ourselves. For such a small team as ours, it was a considerable burden – in addition to research and logistics, we had to deal with an enormous amount of paperwork. That could put off even the toughest people!

Since we started off by asking you how all of this began, let's finish by asking what you want to achieve now.

NMT: It may be too early to talk about this, because it's now at the stage of plans and preparations. I will be studying cancer immunology. This means that I'm shifting to a different field of research, in a sense the other side of the same coin. When we study autoimmune diseases and cancers, we try to achieve different effects on the immune system, but we use very similar research techniques. I've always wanted to study this. For that matter, I don't know a scientist who never wanted to cure cancer, at least in his or her youth. Now, there is a real chance for that, and I have no doubt that this is the direction I want to follow.

PT: I'd like bring this to a point in which we could offer this treatment as a standard therapy. That's my short-term dream. I hope that we will soon have another way of treating insulin-dependent diabetes here in Gdańsk. I mean islet transplantation. We're training, and I hope we'll start this treatment in the next several months.

As for long-term goals, I'm also interested in cancer research, but I don't know if I'll manage that. But regenerative medicine, also clinical medicine, is a great field. Stem cells administered in a safe way, because we have to remember that there's a lot of charlatanry also in stem cell medicine. In order to start in a responsible way, we need to filter out and reject certain reports. We must not give patients false hope. I'd like to prepare an evidence-based research program first. Such studies must be done in a way that is responsible and safe for patients.

So your professional paths are parting slightly.

PT: I'm not sure that's true.

NMT: I think my husband will ultimately follow me.

Interview by **Anna Zawadzka** and **Katarzyna Czarnecka**
photography by **Jakub Ostałowski**