Text: Louise Hagbard, Ph.D. **Photo:** Ola Jacobsen

STEM CELL INNOVATORS

TILO KUNATH

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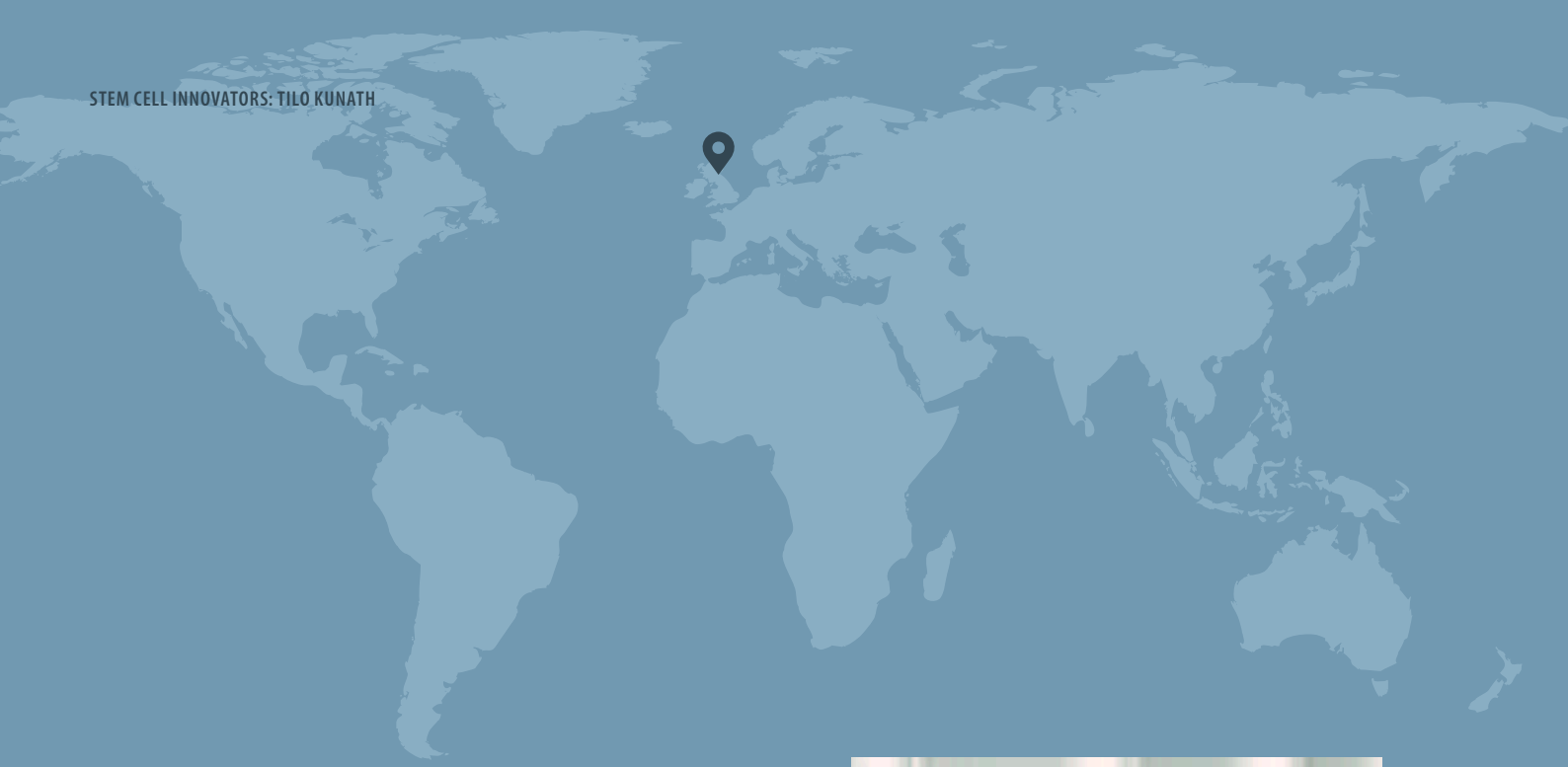
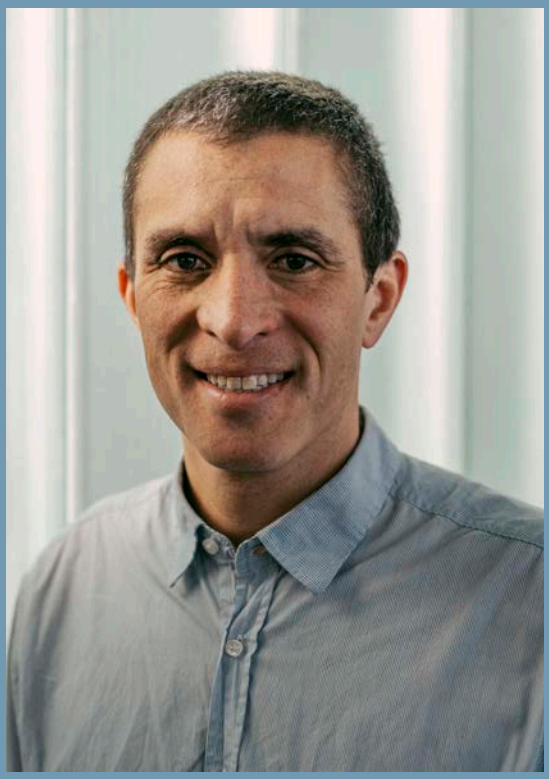


TILO KUNATH, Ph.D.

SENIOR RESEARCHER AND GROUP LEADER, MRC CENTRE FOR REGENERATIVE MEDICINE, UNIVERSITY OF EDINBURGH, UK

ABOUT: **Dr. Tilo Kunath is a group leader at the MRC Centre for Regenerative Medicine, University of Edinburgh, UK. His research group focuses on mechanisms of neurodegeneration with two main areas: to understand how the protein, alpha-synuclein, causes degeneration of neurons in Parkinson's, and to produce a cell-based therapy for Parkinson's from human pluripotent stem cells. The group also uses patient-derived material to generate induced pluripotent stem cells (iPSCs) with genetic mutations known to cause Parkinson's.**



MRC CENTRE FOR REGENERATIVE MEDICINE

The MRC Centre for Regenerative Medicine (CRM) is a part of the University of Edinburgh, located at the Edinburgh BioQuarter site. The Centre is led by the Centre Director, Prof. Stuart Forbes and is a world leading, state-of-the-art facility, and a working place for almost 300 scientists and clinicians studying stem cells, disease and tissue repair. Research at the CRM is aimed at developing an understanding of the mechanisms underlying stem cell self-renewal and differentiation processes, and at developing new treatments for major diseases including cancer, heart disease, liver failure, diabetes, and Parkinson's.

↑ "I have a great team which now consist of six people: four Ph.D. students and two postdocs. We work a lot with disease modelling for Parkinson's and we have a large focus on regenerative medicine."

What is your background story and why did you choose a career in neuroscience?

"I started my research career in developmental biology. I did my Ph.D. in Janet Rossant's lab in Toronto where I was working on placental development and placental stem cells. I then did my postdoc with Austin Smith here in Edinburgh with a focus on mouse ES cells and neural induction. Later, I transitioned into human embryonic stem cells, pluripotency as well as neural differentiation. It was at about this time when my research focus developed from basic research and the basic mechanisms of how pluripotent cells are maintained to dopaminergic neuronal differentiation and Parkinson's disease."

"I have chosen neurobiology for two reasons: first, I think the neural lineage is very interesting in terms of scientific questions. It seems that pluripotent cells, or ectoderm, develop to neural cells by default without any instructive signal. Just imagine, you do nothing to a pluripotent cell and it becomes neural. It's very interesting to see if and how your research can have an impact upon people's lives, and on society. So, the second reason for why I have chosen neurobiology is a translational one. With a growing life expectancy, neurodegenerative diseases, such as dementia and Parkinson's disease, become

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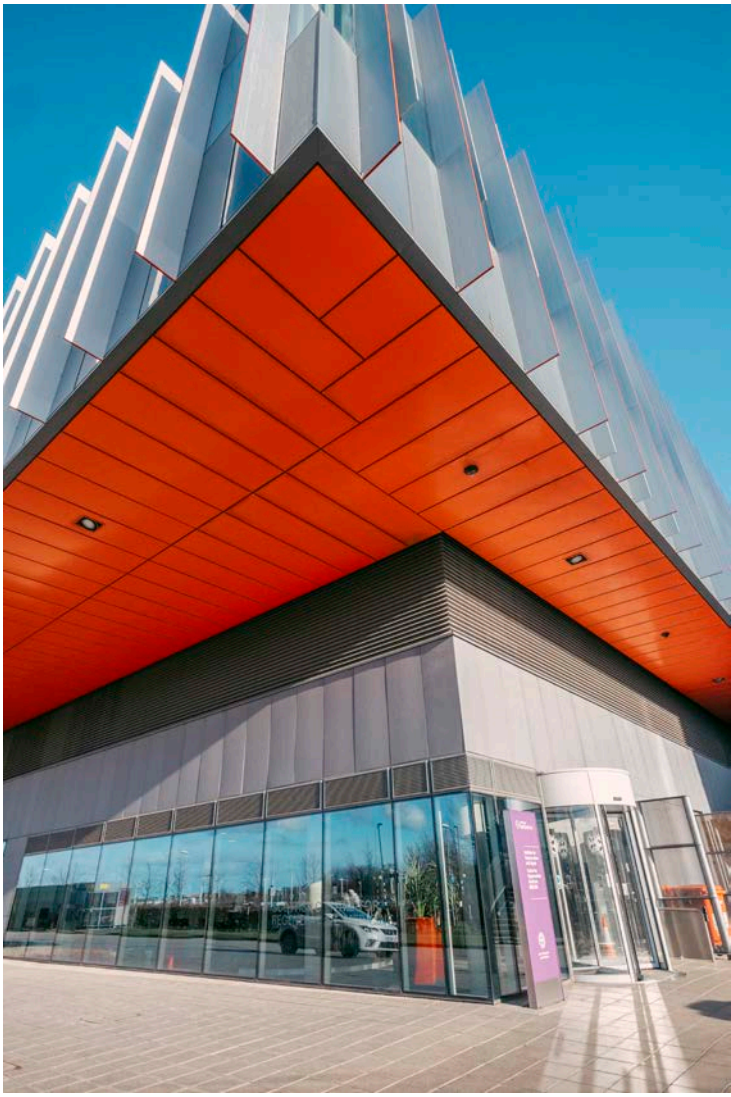
more imminent. How can we apply our knowledge about the nature of neural cells and the differentiation process of stem cells to find a cure for these threatening diseases?”

What made you choose Edinburgh as a location?
“I really wanted to work with the best stem cell scientist in the world at the time. I believed and I still believe that Austin Smith was that person. He was doing the best stem cell research in the world in 2003, which is when I was looking for a postdoc. I wasn’t concerned where he was. I just really liked his work, his papers; he is an incredibly precise thinker. He was the man. I wanted to work with him. When Austin left for Cambridge, I stayed on to set up my own lab here. Edinburgh is a fantastic place to work, with a fantastic university.”

How is your lab set up and what do you currently work on?
“My group right now consists of six people: four Ph.D. students and two postdocs. We work a lot on disease modelling for Parkinson’s and we also have a large focus on regenerative medicine. I would say that half the lab is looking at disease mechanisms and the biology of α-synuclein. Although I am focused on Parkinson’s disease, I am convinced that what we learn there will be applicable to other neurological conditions and neurodegenerative conditions as well.”

What is your twist to your protocols? What is it that makes them unique?
“Our standard differentiation protocols are very robust. We homogeneously differentiate cells from multiple cell lines with a very high purity of over 95% CORIN-positive cells. At this level of purity, there is no need to sort your cells anymore which allows us to skip the cell sorting. We just published a lot of this work in a great paper in the European Journal of Neuroscience for which we used a lot of Biolaminins by the way. Currently these cells are



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used for disease modelling. But our next paper we will be showing that they are very well suited for transplantation as well. We have been transplanting our CORIN-positive cells into the 6-hydroxydopamine lesion rat model, which is a very well-established Parkinson's model. That is work we are doing in collaboration with Dr Mariah Lelos at Cardiff University."

Will there be an off-the-shelf, cell-based treatment for Parkinson's disease?

"If dopaminergic precursor cells can be cryopreserved (i.e. frozen), then yes, I think there will be an off-the-shelf cell replacement therapy for Parkinson's in the future. The dilemma for a cell therapy for Parkinson's is who should be receiving it. Since patients display symptoms in form of tremors only after a substantial number, 50%-70%, of the neurons has been already lost, it could be argued the newly-diagnosing patients would benefit most from a cell therapy. Because of this substantial nerve loss, I think a definitive cure will need to include some form of cell replacement in the region that the neurons lost in order to regain full functionality."

What do you think will be the outcome from the first clinical trials with cell therapy for Parkinson's disease?

"The first patient was transplanted last year in Japan. To see if a cell therapy for Parkinson's is successful or not, it will take two to three years after transplantation. This is the time it takes for the transplanted dopaminergic precursor cells to mature. It may take until the year 2022 or later before the first results are published. What the scientific community is hoping to see is the survival of the transplanted cells, and an increase in fluorodopa signal in the striatum that is time-dependent. In patients with Parkinson's disease, the fluorodopa signal is getting smaller as the condition progresses. And of course, we want to see a significant reduction of motor symptoms, reduced dependence on L-dopa, and an improved quality of life. What we don't want to see, is side effects such as graft-induced dyskinesias which is a type of uncontrolled movement that was sometimes caused by the fetal grafts. Q: Do you see any difference between iPS cells and hES cells with regards to their suitability in a clinical setting?

HIGHLIGHTED PUBLICATION

Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus
Devine M.J. et al.
Nature Communications, 2011, doi: 10.1038/ncomms1453

Here, the authors developed a new experimental system to identify compounds that reduce levels of α-synuclein and to investigate the mechanistic basis of neurodegeneration caused by α-synuclein dysfunction. Triplication of SNCA, encoding α-synuclein, causes a fully penetrant, aggressive form of Parkinson's disease with dementia.

Engineering synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated deletion of the SNCA gene
Chen Y. et
European journal of Neuroscience, 2018, doi: 10.1111/ejn.14286

In this article, the authors generate SNCA+/– and SNCA–/– cell lines with the use of use CRISPR/Cas9n technology to delete the endogenous SNCA gene in a clinical-grade hESC line. The work demonstrates that reducing or completely removing SNCA alleles by CRISPR/Cas9n-mediated gene editing confers a measure of resistance to Lewy pathology.

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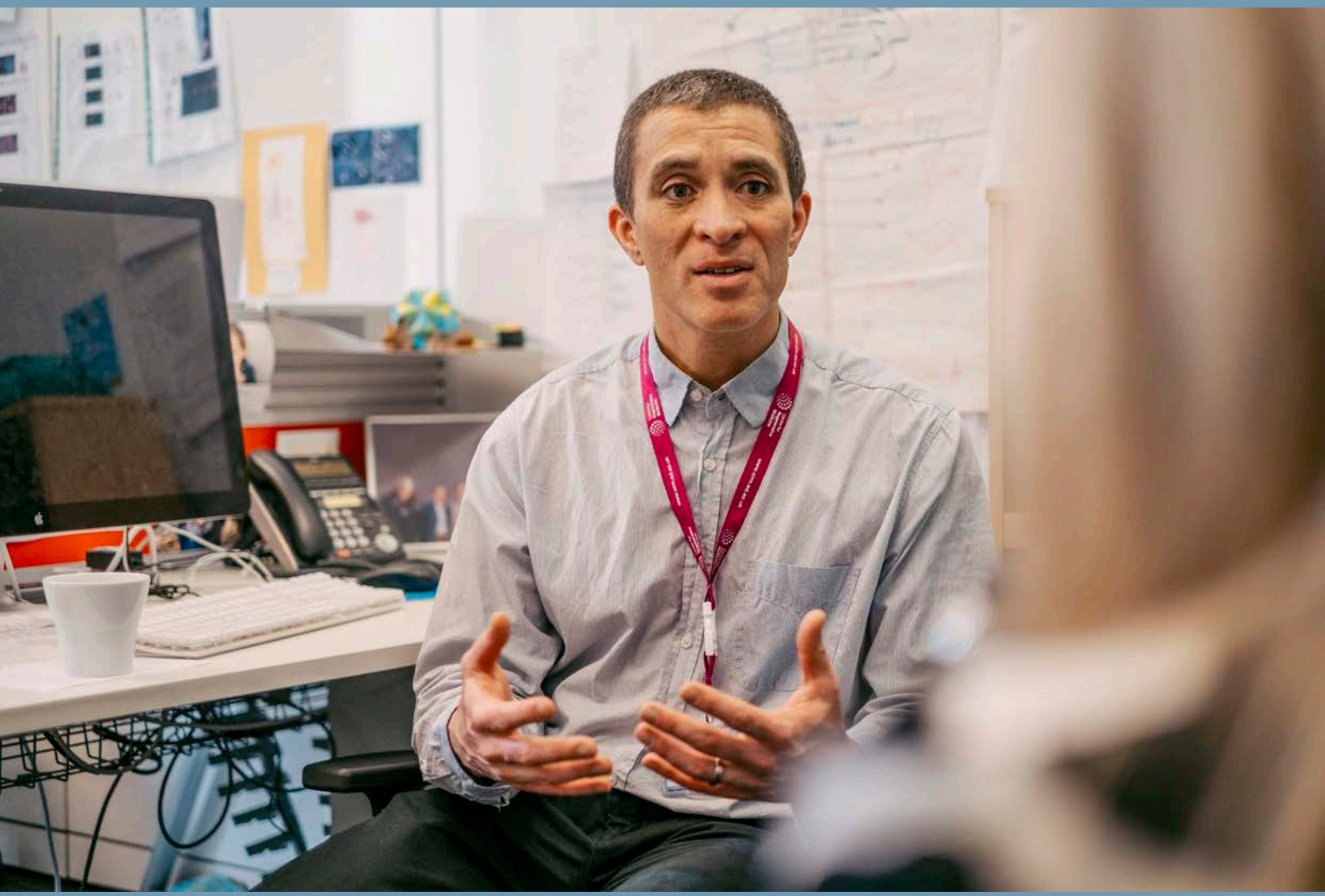


“Parkinson’s is the only cell therapy that requires a fetal cell type, which is easily made by ES and iPS cells in the lab.”

I don’t see a mayor difference between them. I think iPS cells or ES cells are functionally equivalent. In my view, as long as your iPS cell line is of good quality it is fine. As for hES cells, the cell line that we used the most is the RC17. This is also the cell line we used in our recent publication. We tested many hES cell lines and we have chosen this one because it makes dopaminergic neurons very well.”

What do you reckon is the highlight of your career so far?

“It has to be the establishment of the first iPS cells that we made from Parkinson’s patients. We published this work in Nature Communications in 2011. We had an opportunity to make our first human iPS cells here at the Center for Regenerative Medicine. I had just established my group, but I was still working a lot in the lab at that time and it was when human iPS cells had not been made in Scotland yet. We, Mike Devine, a former Ph.D. student and I, had a big race on our hands, because we knew that multiple labs had the same source material as we were working on (fibroblasts from a triplication SNCA family) and all labs were trying to publish. But we were the first. That single paper, without my Ph.D. or post-doctoral mentors, is when I established myself as an independent researcher. It was also at that time where the shift happened and instead of being a 50% Parkinson’s researcher, I became a 100% Parkinson’s researcher.”

What has been your biggest inspiration in your research career?

“The first inspiration was the research they did in Lund, Sweden, where they showed the concept of a treatment for Parkinson’s by cell therapy using fetal tissue. What is very interesting about that work is that it showed that the age of the fetus is of great importance. If the fetus was too mature, the transplantation didn’t work, at least not in the rat model. In my opinion, Parkinson’s is the only cell therapy that requires a fetal cell type, which is easily made by ES and iPS cells in the lab, because that is what the cells are programmed to make first.”

“We will continue to focus on refining tools and technologies for cell replacement therapies for Parkinson’s.”

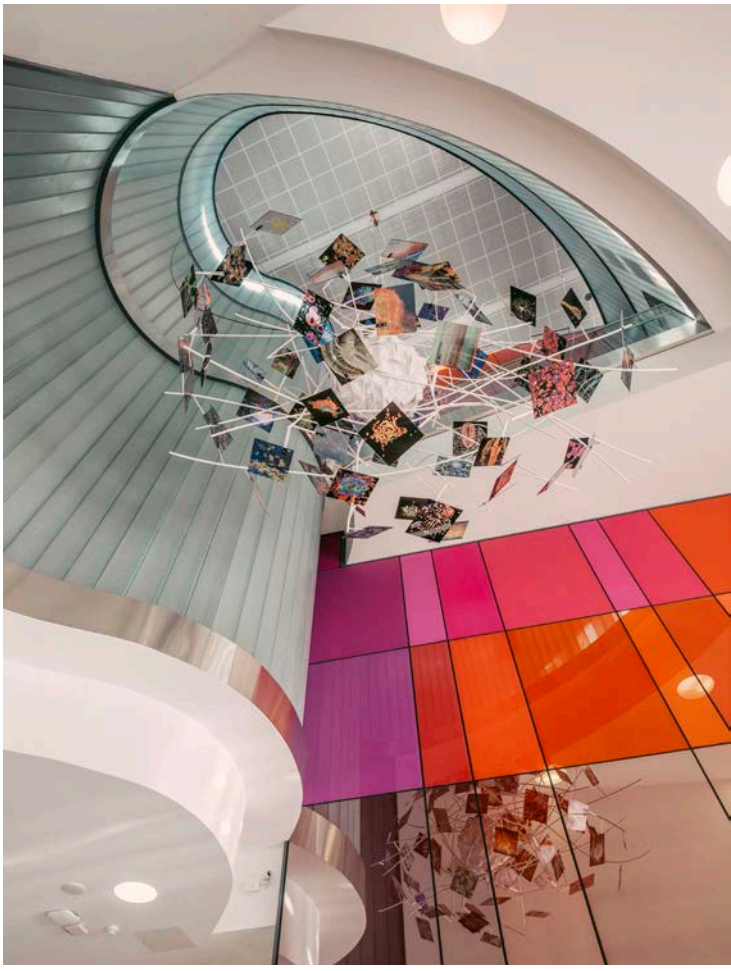
“My second inspiration is a person: Tom Isaacs, whom I have a photograph of on my desk right here. Tom is a founder of a charity called The Cure Parkinson’s Trust. He founded this charity around 10 years ago with funds that he raised by walking around the coast of the UK with quite severe Parkinson’s. Unfortunately, Tom died last year at a fairly young age. I was lucky to interact and work with Tom on many occasions. He was incredibly charismatic and inspirational and is a major hero of mine.”

If you had unlimited amount of funding, how would you spend it?

“I would certainly spend a lot of it on cell therapy optimization, understanding how to get the best cells, and understanding how the transplantation would be beneficial to patients. I think there is a lot of fine tuning to be done. Also, I would spend loads of time on understanding disease mechanisms and getting brilliant animal models to test as many promising drugs as possible. It is also worth looking into alternative types of therapies. We know that different types of exercise, different microbiomes and even different types of diets influence the progression of Parkinson’s. Can these be modeled in animals?”

For next coming years, what is the biggest focus for you?

“We will continue to focus on refining tools and technologies for cell replacement therapies for Parkinson’s. We are also setting up drug screening for repurposed drugs and novel compounds in our neuronal cell culture models. The evidence for repurposed drugs is usually quite diverse, and they’re often associated with one particular model. The idea is to develop disease-relevant human cell models, which take advantage of our efficient human ES and iPS cell differentiation into dopaminergic neurons. We hope data from these models will have a greater chance of succeeding in clinical trials.” •



↑ Research at CRM is aimed at gaining fundamental understanding of stem cells at to use this knowledge to develop new treatments for major diseases including cancer, heart disease, liver failure, diabetes, multiple sclerosis and Parkinson’s.

TILO KUNATH ON RESEARCH IMPACT

? In your opinion, what has been the biggest research impact during the last decades?

! "In biology, things are usually easier in mouse models than in human cells, but in the case of neural induction from pluripotent cells, it's the opposite. A big surprise to me is one of Austin Smith's concepts, that mouse ES cells and human ES cells are in different states. Mouse ES cells are in a naïve state and human ES cells are in a primed state. For making neural cells, human ES cells get more efficient conversion and better purity of neurons. In terms of a practical application of the cells, the human cells are in an ideal state, because they are already in a primed state in comparison to ES cells from mouse. The 2006 Cell paper by Kazutoshi Takahashi and Shinya Yamanaka was pretty significant as well."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