Text: Louise Hagbard, Ph.D. **Photo:** Ritualen

STEM CELL INNOVATORS

FREDRIK LANNER

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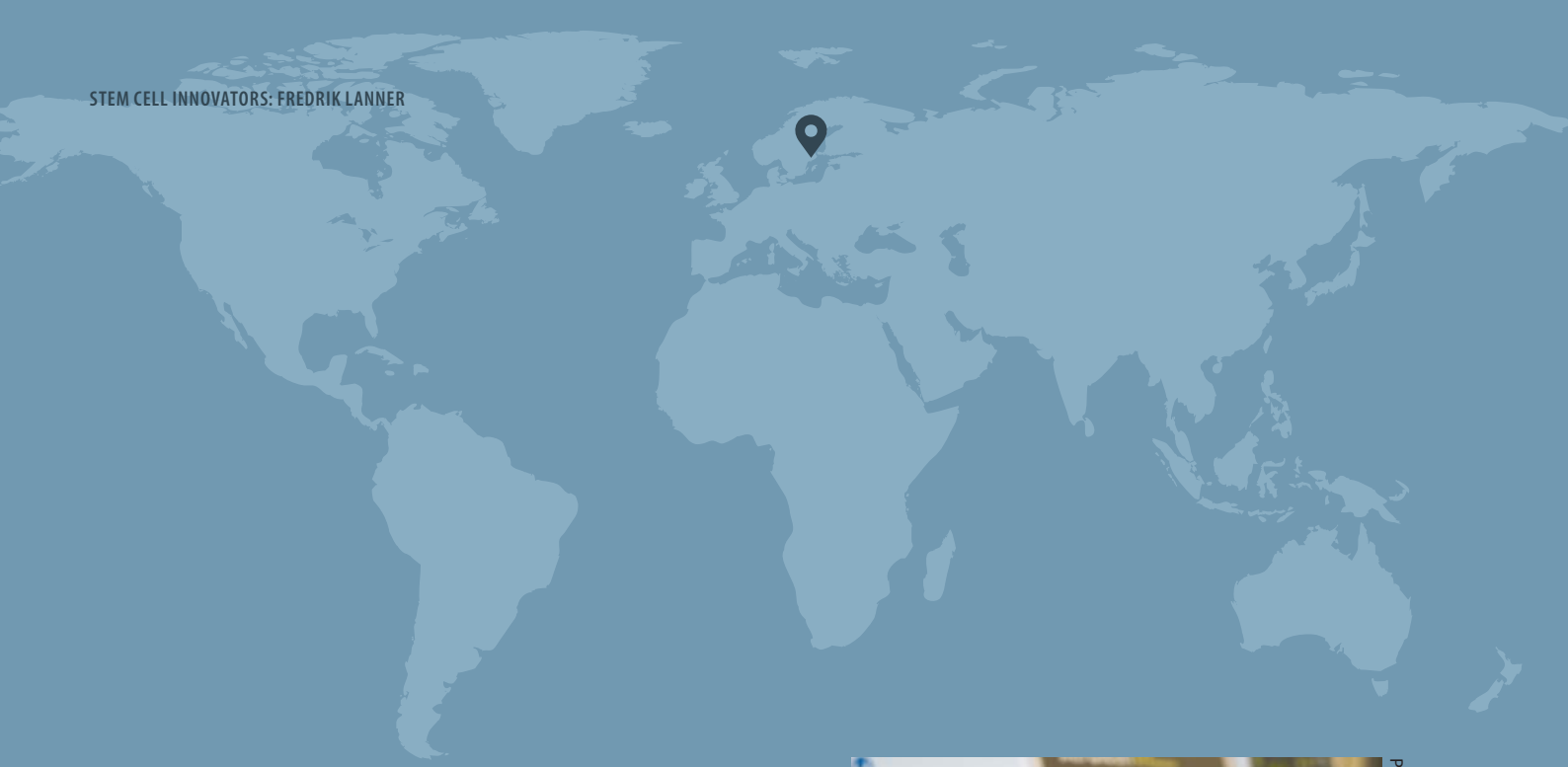


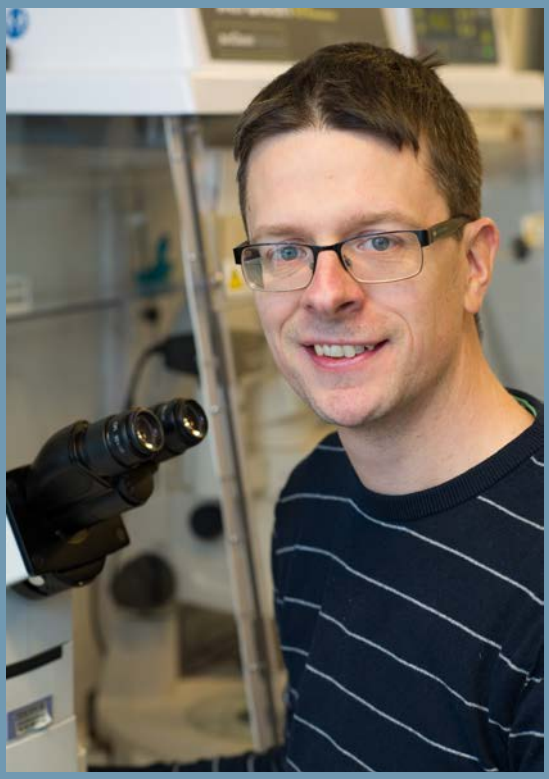


Photo: Ulf Sirborn

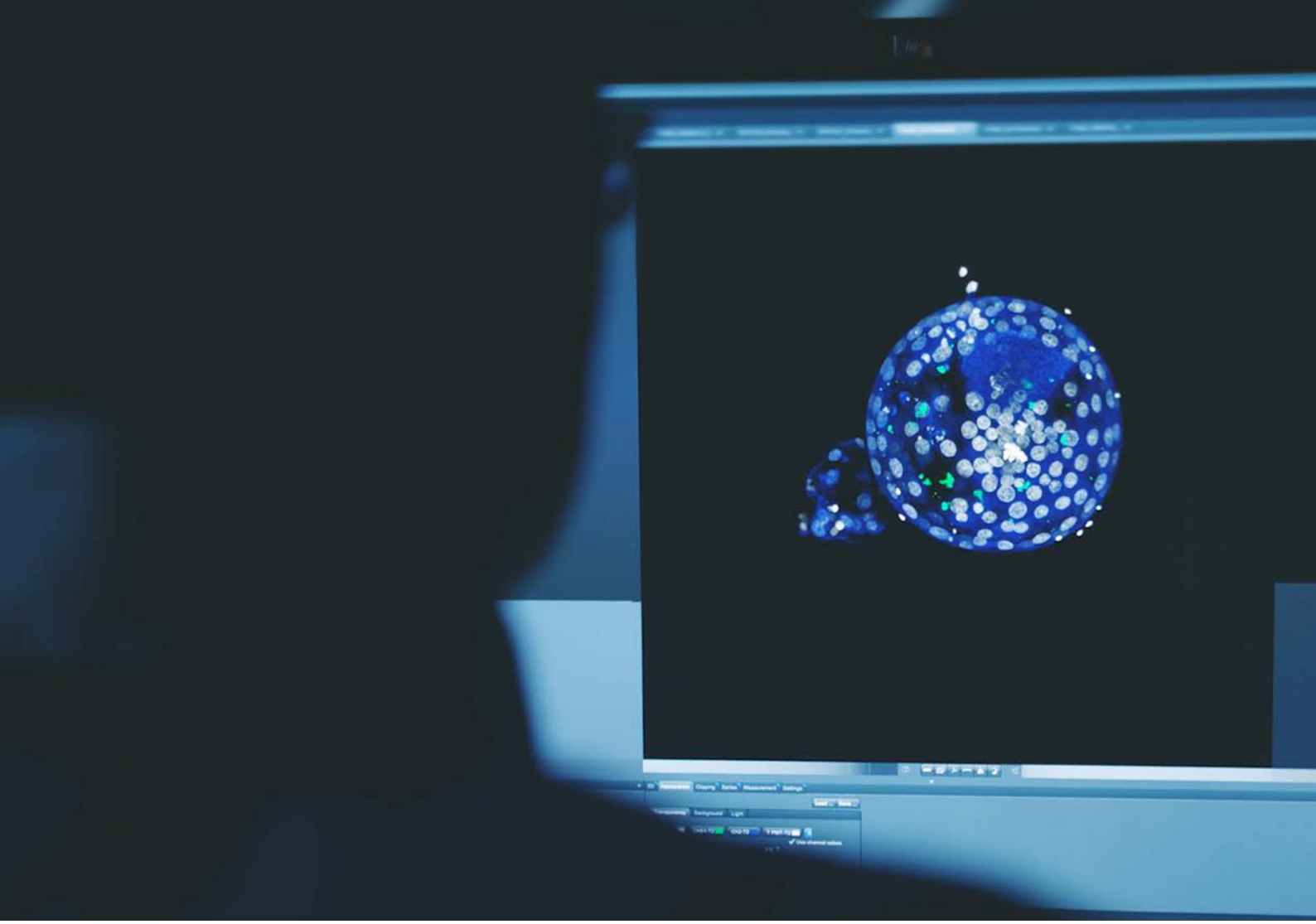
FREDRIK LANNER, Ph.D.

ASSISTANT PROFESSOR AT THE DEPARTMENT OF CLINICAL SCIENCE, INTERVENTION AND TECHNOLOGY (CLINTEC), KAROLINSKA INSTITUTET AND KAROLINSKA UNIVERSITY HOSPITAL, SWEDEN

ABOUT: **Assistant Professor Fredrik Lanner undertook his Ph.D. thesis at the department of Cell and Molecular Biology (CMB) Karolinska Institutet, Sweden. After his postdoctoral research in Janet Rossant's lab at The Hospital for Sick Children in Toronto, he returned to Karolinska Institute and established his independent research lab exploring how pluripotent stem cells are regulated in the human embryo and how embryonic stem cells (ESCs) can be used for treatment of age-related macular degeneration.**

KAROLINSKA INSTITUTET

Karolinska Institutet was founded in 1810 and is Sweden's single largest centre of medical academic research and offers the country's widest range of medical courses and programmes. It is one of the world's foremost medical universities and since 1901 the Nobel Assembly at Karolinska Institutet has selected the Nobel laureates in Physiology or Medicine.



↑ Early developing embryo. Photo: Ritualen

How did you end up working with human pluripotent stem cell research?

"My motivation to do research has always been to try to understand how the human embryo is controlled, and how can we go from a single, fertilized cell to the thousands of cell types that we have in the adult body. During my Ph.D. studies I studied the formation of different blood vessels and I used embryonic stem cells from mouse to makes arteries and veins. I wanted to get a deeper understand in cell development and how different cell types are formed. So, I moved to Toronto to study mouse preimplantation development in Janet Rossant's lab. The work I did there was the background to my work generated here at Karolinska Institutet on human embryonic development. What became evident was that the early embryonic development in mice is very different from human embryonic development and that the mouse probably is not such a great model system to understand human preimplantation development. So, the human embryo is relatively unexplored and I wanted to expand my knowledge of the molecular and cellular mechanisms that govern the early embryo and early human developmental biology. So, I moved back to Sweden, to Karolinska Institutet, to set up my own independent

"Contrary to what everyone believes, the 'classical' embryonic stem cells, the ones that everyone is using and planning to take to clinical applications, do not really correspond to the embryonic cells of the blastocyst."

research group. In 2016, we published an article in Cell where we provide a single cell transcriptional atlas of the human preimplantation embryo with the aim to investigate human development and embryonic stem cells. We did single-cell RNA sequencing where we could study virtually all of the approximately 20,000 genes in each individual cell, to see which ones are switched on at each stage in early human embryonic development. We then built a transcriptional roadmap of how the first cell types emerge during the first week of human development. What we showed was, contrary to what everyone believes, the "classical" embryonic stem cells, the ones that everyone is using and planning to take to clinical applications, do not really correspond to the embryonic cells of the blastocyst. Actually, they rather correspond to a stage later in human embryonic development, perhaps up to a week later. In recent years, researchers have succeeded in establishing a stem cell type that is from an earlier stage than the classic, which is believed to correspond to the one in the blastocyst. What we did was to characterize these two types of human pluripotent stem cell types, the naive and the primed cell type, and in 2017 we published an article in Cell Stem Cell where we describe a comprehensive profiling of cell surface proteins in naive and primed human PSCs. These data provide a standardized and straightforward approach to defining and characterizing state-specific human pluripotent cells and will hopefully help us understand embryonic development better and tell us more about how pluripotent cells are formed and regulated in the early stages. This knowledge is important for stem cell biology and for the selection of stem cells for clinical applications, to understand what these cells really are. It is also important knowledge that could be important to understand causes of infertility, why some patients have difficulty getting pregnant and why not even IVF treatment helps in some cases."

What is your aspiration for your research field? Tell me a bit about your research?

"The overall goal of my research is to understand how and when the pluripotent stem cells become locked to the respective cell type in the early embryo and also investigate how human embryonic stem cells are established. The two articles mentioned both got a lot of attention which was great. So, after having identified which genes are activated in the different cell types in the early human embryo, we are now using CRISPR/Cas technology to ex-

HIGHLIGHTED PUBLICATIONS

Single-cell RNA-seq reveals lineage and X chromosome dynamics in human preimplantation embryos
Petropoulos S., et al.
Cell, 2016, doi: 10.1016/j.cell.2016.03.023

In this article, the authors provide a comprehensive single cell transcriptional roadmap of human embryo development, including the sequenced transcriptomes of 1,529 individual cells from 88 human preimplantation embryos.

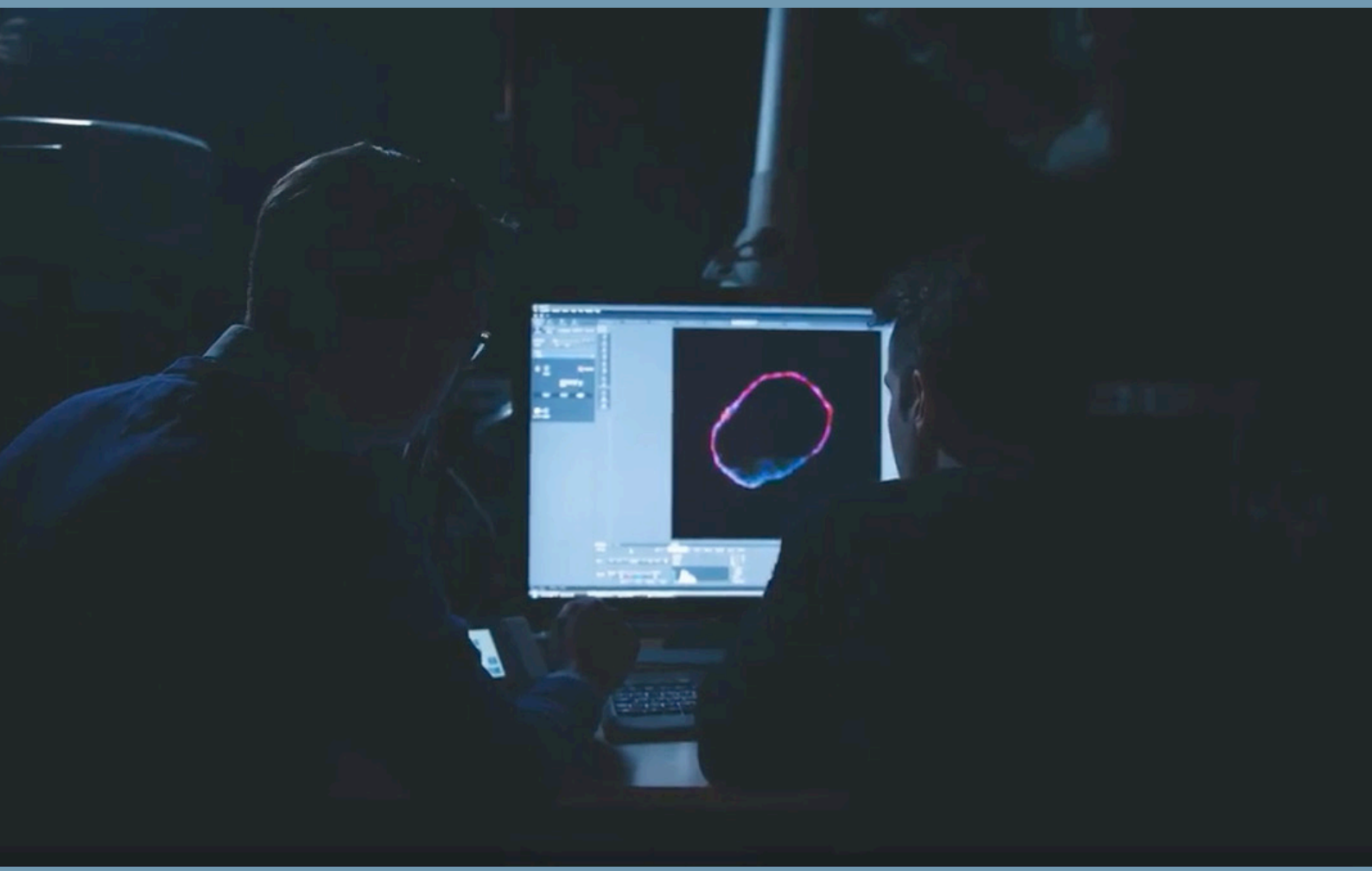
Comprehensive Cell Surface Protein Profiling Identifies Specific Markers of Human Naive and Primed Pluripotent States
Collier A.J. et al.
Cell Stem Cell, 2017 doi: 10.1016/j.stem.2017.02.014

Here, the authors report a comprehensive profiling of cell surface proteins in naive and primed human PSCs.

Xeno-free and defined human embryonic stem cell-derived retinal pigment epithelial cells functionally integrate in a large-eyed preclinical model
Reyes A.P. et al.,
Stem Cell reports, 2016, doi: 10.1016/j.stemcr.2015

Here, the authors have developed a xeno-free and defined hESC-RPE differentiation method and present evidence of functional integration of clinically compliant hESC-RPE in a large-eyed disease model.

“The mouse probably is not such a great model system to understand human preimplantation development.”



plore the potential of human embryo genome editing in fundamental research and to study which of these genes are important for early human development and pluripotent stem cells. This CRISPR'ing project is very exciting and it has generated a great deal of attention."

"The whole regenerative medicine field is also super exciting. When I came back to Karolinska Institutet, I saw that there were great opportunities to work translationally, to generate cell therapies. This was much owed to the nice organization that Outi Hovatta built up. We had good access to human embryos, we had the knowledge of how to generate human embryonic stem cells of good quality, and we had a GMP lab on the hospital site which we could use. So, we have established new GMP hES cells, a resource that we hope could be of benefit to many research teams allowing them to make the move from bench to bedside within the field of reparative medicine. Moreover, we had good connections to clinicians in the field of ophthalmology and the clinical benefits of developing a cell therapy for macular degeneration. We have developed a xeno-free and defined protocol to generate ESCs derived retinal cells which we published in 2016 and we work towards the use these cells in clinical trials addressing age-related macular degeneration. Now we can produce and deliver functional RPE cells of high quality, acceptable for clinical use. We have also come a long way in diabetes, a project that we started only a few years ago. So, what we have established here can be applied to

"Now we can produce and deliver functional RPE cells of high quality, acceptable for clinical use."

DRY AGE-RELATED MACULAR DEGENERATION (AMD)

Dry AMD is a major-as of yet-incurable disease of aging leading eye disease responsible for visual impairment of the elderly. Dry AMD is characterized by drusen, deposits buildup of waste materials beneath the retina which damages the Retinal Pigment Epithelium (RPE) cells. The Photoreceptors depend on RPE cells for nourishment, recycling of visual pigment and waste disposal. When RPE cells die, the photoreceptors also die and central vision is lost.

→ hESC-derived retinal pigmented epithelial cells. Photo: Fredrik Lanner

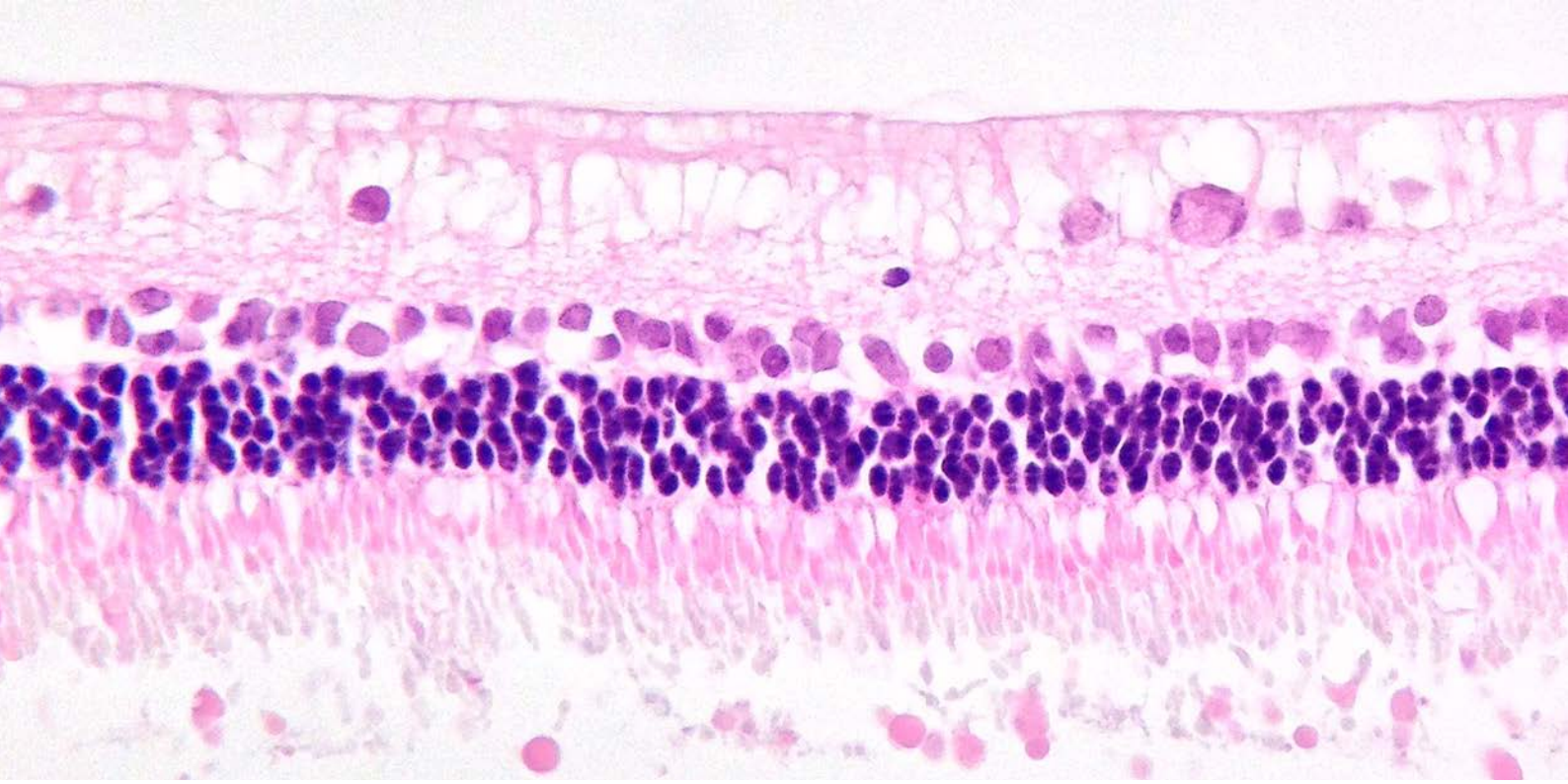
"There are many obstacles, but I think immune rejection is one of the big problems we have. When the iPS technology came, many believed that we would not have to deal with this anymore."

many other diseases and I feel very enthusiastic about the opportunities ahead. Many have advised me to invest in only one track but being able to work with both basic science and applied research in parallel I feel is essential if you want to develop a successful therapy. Moreover, we have been able to finance the research in a more stable way than we could have done otherwise."

Which do you feel is the single biggest hurdle to overcome to reach the clinic with a cell treatment?
"There are many obstacles, but I think immune rejection is one of the big problems we have. When the iPS technology came, many believed that we would not have to deal with this anymore. However, now they have realized that it will be too expensive to do individualized treatment so they are working on other strategies, such as banking a lot of different lines that one should be able to match to different individuals. But I think that will probably also be very expensive, and I think you have to look at other solutions. I believe that the CRISPR technology could be a strategy to use to create cells that are not rejected. Treatment of macular degeneration is a good example where it could be beneficial. The disease is not life-threatening, and it could come to a scenario where the benefits of treatment do not weigh up to the problems. Creating cells for treatment that are not rejected could be a great advantage there. Another thing is that we have to be able to show that the treatment is effective. The first clinical trials for macular degeneration that have been done have not yet given that impressive functional effects. Then the production-related part is another challenge, being able to get enough cells of the right quality. So, there are many obstacles to handle."

Do you think pluripotent cell therapy eventually will be an off the shelf treatment?
"Yes, I believe it will. There is so much progress being made in this field. It is really exciting to be involved. From my perspective, I think there has been surprisingly little interest from the pharmaceutical industry, up until perhaps three years ago. Now more and more are becoming involved and are really pushing things onward."

Gene editing of human embryos has generated a lot of attention, but it also raises many questions about ethics and security. What system and processes would you like to have in place to balance public society concerns?
"We think we already have clear legislation for what to do and how to behave. The Swedish law is clear, genome editing is only allowed within the first 14 days, and heritable genome editing for clinical purposes is forbidden. I think that is good. It's way too early to embark on curing genetic diseases. However, it is important that we are allowed to do basic research, to be able to investigate whether this can be safe and effective in the future."



↑ HE staining of neurosensory retina in normal albino rabbit. Photo: Fredrik Lanner

You have chosen to work mainly with embryonic stem cells but if you compare hES with and iPS cells, what do you think are the pros and cons?

"In my research career, embryonic stem cells were what was most assessable to me and, as I study early embryonic development, it has been natural to work with embryonic stem cells. Then I also have this the feeling that embryonic stem cells have the advantage that they come directly from an embryo, a natural environment, compared to iPS cells that have been grown and manipulated. However, when it comes to clinical use, I think both cell types are equally possible. Also, what I have started to think about more and more is the rejection effect and there I see greater opportunities for iPSCs as they create these HLA-matched cell banks. Moreover, iPS cells comes from an adult who has lived a normal life whereas the embryo has not been tested through the development process in the same way. So, there may actually be a higher genetic risk working with embryonic stem cells. On the other hand, iPS cells originate from cells that have been sitting in the skin and may have accumulated mutations that could be harmful. So, it's really hard to say. I see the pros and cons of both cell types and I think it's important to run both tracks. We should absolutely not abandon any of the cell systems. I myself am not completely locked on hES cells, and we may start working with iPS cells as well."

What has been your strategy for developing defined protocols?

"I think it is obvious that you should use defined reagents as much as you can, and to set defined protocols early, partly from a regulatory point of view. To grow something on Matrigel that you then intend to transplant I think is madness. Another aspect that we've noticed is that the work you put into finding and setting defined substrates is often rewarded in the long run. We see this time and again. The protocol becomes more robust and eliminates variables so you benefit both from a research perspective and you also get protocols that more easily can be taken to clinical use. Because a great challenge when moving from a research lab into a GMP facility is that you have to evaluate all the components that you are using and make sure that they both have traceability and that they are safe to work with."

What has been your biggest inspiration in your research career?

"I am inspired by people all the time. Research is based on collaboration where everyone has their skills and are good at what they do. I have evolved by being curious of how we, humans, work and why we have the different cell types that we have and how they are being generated. And then to see how we use that knowledge to cure diseases, that is my major driving force."

For next coming years, what progress do you hope to see within the regenerative medicine field?

"I would like to see major clinical studies and I hope to see positive results. You start to see the first studies getting started. Within the eye field, there have already started about ten clinical studies, and in other fields as well. I do not think that we will have a redy-to-use cell therapy any time soon but I would at least like to see some positive results."

FREDRIK LANNER ON FUNDING

What would you do with a $100 million grant?

"I would invest the money in generating cell therapies within a more academic sphere, something that everyone says that one should not do. Within the academic sector, we do not have the resources to operate completely ourselves. If you look at blood banks and spinal cord transplants, is not done through large companies, but it is the society that carries those costs. With that amount of money, one could make a real effort to establish cell lines and to develop protocols, generate products that could be used in health care, and run it entirely academically. It would be exciting to have such an opportunity."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