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AU1 ▶

Reprogramming Stars #9: Spacing Out Cellular Reprogramming—An Interview with Dr. Valentina Fossati

AU2 ▶

Valentina Fossati[1] and Carlos-Filipe Pereira[2]

Introduction by Dr. Carlos-Filipe Pereira (Editor-in-Chief, *CELLULAR REPROGRAMMING*)

Dr. Pereira: Good afternoon. My name is Filipe Pereira, professor at Lund University and editor-in-chief of *Cellular Reprogramming*. I am very happy to bring you a new episode of Reprogramming Stars, our flagship series capturing the findings, projects, and ideas of the leaders in cellular reprogramming.

Today we have with us Dr. Valentina Fossati, a New York Stem Cell Foundation (NYSCF) Senior Investigator at The NYSCF Research Institute where she focuses on advancing preclinical studies of neurodegenerative and neuroinflammatory disorders, utilizing human-induced pluripotent stem cell (iPSC)-derived brain cells. Dr. Fossati received her undergraduate degree in pharmaceutical biotechnology and her PhD in stem cell biology from the University of Bologna where she studied mesenchymal stem cells.

She did her postdoctoral training, for which she received the NYSCF Druckenmiller fellowship, in the laboratory of Dr. Hans Willem Snoeck at the Black Family Stem Cell Institute at Mount Sinai where she investigated B cell development and identified new subpopulations of lymphoid and B cell progenitors. Her diagnosis of multiple sclerosis (MS) in 2009 disrupted her postdoctoral career and shifted her research interest toward better understanding the disease and in particular its neurodegenerative component, which is responsible for irreversible neurological disabilities. In 2011, she was recruited by the NYSCF Research Institute as principal investigator.

Bringing her stem cell expertise, Dr. Fossati has pioneered the development of human stem cell-based models to study the role of glia in neurodegeneration and neuroinflammation. Dr. Fossati established protocols to generate oligodendrocytes, astrocytes, microglia, and neuronal cell types. She is also developing organoids and coculture systems to identify and target the key pathogenic mechanisms leading to neurodegeneration and/or demyelination in progressive MS, Alzheimer's disease (AD), and other disorders of the CNS. Dr. Fossati receives funding from prestigious institutions in the United States such as the NIH and NYSTEM. Dr. Fossati, thank you so much for joining me today. It is a pleasure to have you featured as a Reprogramming Star.

Dr. Valentina Fossati

REPROGRAMMING STAR: ***Dr. Valentina Fossati*** *is a senior investigator at the New York Stem Cell Foundation Research Institute where she focuses on building human induced pluripotent stem cell (iPSC)-based models for studying progressive multiple sclerosis (MS) and other neurodegenerative diseases to investigate the role of glia in neuroinflammation and neurodegeneration. She has established protocols to generate human iPSC-derived oligodendrocytes, astrocytes, microglia, and neuronal cell types and is developing organoids and coculture systems to study the crosstalk between neurons and glial cells. Her ultimate goal is to identify and target key glia-driven pathogenic mechanisms leading to neurodegeneration in progressive MS, Alzheimer's disease, and other disorders of the central nervous system (CNS).*

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Dr. Fossati: It is an honor to be here. Thank you.

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Dr. Pereira: Your laboratory combines great expertise in human brain cell biology and cellular reprogramming. In the last years you have published new methods to generate oligodendrocytes, microglia, and astrocytes using iPSC differentiation and uncovered new important aspects of their biology in health and disease. I find this approach fascinating and wonder whether you could tell us how your journey started in this field.

Dr. Fossati: We need to go back to my early undergraduate studies: my interest in stem cells was the result of perfect timing, because the time when I started taking biology classes at my university coincided with the very beginning of the regenerative medicine era (*i.e.*, the seminal work to isolate the first human embryonic stem cell lines in 1998). I was so excited about the potential of this new field, and I continued to follow it closely, reading and learning as much about it as I could. As soon as I had the chance, I joined a laboratory working on adult stem cells for my master's thesis research. It was originally a hematopoietic stem cell laboratory, but we became one of the first groups to isolate human mesenchymal stem cells (MSCs) to explore their potential.

I enjoyed isolating MSCs from various sources, including the dental pulp, the amniotic membrane, the placenta, and cord blood. The more I learnt about adult stem cells, the more I became interested in expanding my knowledge to embryonic stem cells (ESCs), but I knew it would not have been possible to pursue this research in Italy because of strict regulations that did not allow studies on human embryos. Attending a summer Stem Cell School at Cold Spring Harbor laboratory in 2004 changed my future.

There, I connected with an assistant professor working in New York City and I shared with him my enthusiasm for stem cells and my desire to work in the United States; we stayed in touch through email and at the 2005 International Society for Stem Cell Research (ISSCR) meeting, he offered me to join his laboratory as a visiting PhD student. Again, with seemingly perfect timing, I moved to New York in 2006, exactly when Yamanaka made the groundbreaking discovery of iPSCs, which was completely revolutionizing the way we could study and cure human diseases. I fully embraced this exciting and rapidly evolving field, and long story short, what was supposed to be a 1-year experience abroad, became my new life: and here I am, 16 years later.

During my postdoc I worked with both ESCs and iPSCs on a project to model thymic epithelial cells. Until that point, I had only worked on projects focused on the hematopoietic system. I never intended to work on the brain! Brain diseases had always scared me because they are so complex. In most cases, we do not know the etiology and we do not have cures yet. But in 2009, I was diagnosed with MS. It came completely out of the blue, a true shock! After my diagnosis, I began reading and reading.

I found a lot of information about the role of immune cells, mostly from animal models, but I realized that human models for studying oligodendrocytes and myelination were severely lacking. And that is when I became fascinated by the idea of leveraging my stem cell background and applying it to neuroscience, to enable us to build better models of the cells in the brain.

Dr. Pereira: What was your main motivation for focusing on oligodendrocytes, microglia, and astrocytes? Can you explain to the audience of *Cellular Reprogramming* to what extent you better understand now the process of demyelination and early inflammation in humans, having these models available?

Dr. Fossati: I think it is very important to have human models complementary to the rodent models that have traditionally been used to study this disease. MS has been studied for over a century, but we still know very little about the process of human myelination. Myelination is a truly fascinating and complex phenomenon. Myelination begins around birth and continues throughout life; it can be modulated by the environment, and thus it is a highly dynamic process. Furthermore, there may be differences between mice and humans that we still do not understand.

Ultimately, MS is a disease that only spontaneously occurs in humans, and although there are several animal models that mimic certain features of MS pathology, such as the lesions, they are not able to answer the critical question of what actually causes MS.

With human models we now have the opportunity to investigate human genetics. It is the combination of different approaches that will lead us to major advances in this field, and the way I see this research evolving is through integration of the *in vivo* study that is enabled by animal models and the *in vitro* study with human cells, where we can uniquely investigate the polymorphisms and mutations found within the human population and linked to the disease pathogenesis. MS and neurodegenerative diseases in general are complex diseases. There is not just one simple mutation that can be described as the root cause.

It is a combination of both environmental and genetic factors, and there are often multiple genetic factors, that may come into play. Some polymorphisms may be protective while others may increase the risk of disease. And now by making iPSC lines from people with MS, we can begin studies that correlate genotypic and phenotypic differences.

Dr. Pereira: It would be interesting to hear more about one of your recent contributions. For example, for the article you published in *Neuron* entitled "CD49F is a Novel Marker of Functional and Reactive Human IPS-Derived Astrocytes," what were the main findings and which avenues were opened from this study to better understand astrocyte biology?

Dr. Fossati: Astrocytes are the predominant type of glial brain cells, and they are in fact the most abundant cell type in the CNS. This population has been understudied for a long time, but there has been a growing interest in these cells over the past decade. We know that astrocytes are essential to preserve CNS homeostasis, but their role in diseases is less understood. I was really fascinated and

inspired by the studies of Ben Barres and other leaders in the astrocyte field, who showed how astrocytes respond to inflammatory signals, changing their state and becoming ''reactive.''

Reactive astrocytes have altered functions and the Barres laboratory showed in a seminal article in 2017 that a specific substate of reactive astrocytes become toxic to neurons. This discovery made astrocytes a novel and compelling target for therapeutic intervention.

The original studies on reactive astrocytes were performed in rodent models, and I immediately wanted to validate them using human cells. I was also lucky that the first author of the 2017 study, Dr. Shane Liddelow, had just moved to New York University to establish his own laboratory. We began a collaboration with the goal of integrating his rodent models with my iPSC-based human models to investigate reactive astrocyte responses to various stimuli. It was extremely exciting to see that we could reproduce the specific astrocyte reactive state induced by inflammatory signals *in vitro*.

Critical to the success of our models was the use of serum-free media in every differentiation protocol that I have developed. The absence of serum is particularly important when studying astrocytes because serum components activate these cells, making it impossible for them to modulate their responses to different stimuli *in vitro*. By using chemically defined media, we were able to reproduce the work that was done *in vivo*, showing that iPSC-derived astrocytes become reactive and neurotoxic, inducing apoptosis of human iPSC-derived neurons.

We are currently expanding our studies to understand the role of reactive astrocytes specifically in MS and in AD. Is there a common mechanism underlying all these diseases, or what is different between each neurodegenerative disease? These are some of the questions we are beginning to address with our study.

Dr. Pereira: What are the signals astrocytes react to? Are they neuronal-derived signals?

Dr. Fossati: The neurotoxic reactive astrocytes are induced by inflammatory signals released by activated microglia. When microglia are stimulated (*e.g.*, with LPS), they release TNF, IL1 alpha, and C1Q, which act directly on astrocytes. Obviously, reactive responses are complex, and several substates exist, depending on time, location in the CNS, and type of stimuli. And not all reactive astrocytes are toxic. We think that there are some substates where the astrocytes play a protective role and are trying to preserve CNS homeostasis. They are trying to fight back inflammation, but at some point, they can no longer compensate the changes in the environment and become toxic.

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This is the direction that we (Dr. Liddelow and I) are taking now. We have characterized one substate of reactive astrocytes and we are expanding the characterization to different subtypes, focusing in particular on AD. In collaboration with Peter Calabresi at Johns Hopkins, I am also looking at the role of reactive astrocytes in MS. In this study, we looked at the effect of reactive astrocytes on human oligodendrocytes, the cells primarily damaged in MS, and we found that the reactive astrocytes do not kill the oligodendrocytes, but rather they inhibit the differentiation of oligodendrocyte progenitor cells (OPCs). OPCs play an important role in MS, enabling remyelination after oligodendrocytes have been damaged by immune cells.

To achieve remyelination, OPCs need to make new oligodendrocytes. However, we now know that this process is inhibited by the reactive astrocytes. This is very important because it means that to develop effective remyelinating strategies, we need to not only discover drugs that promote myelination, but also test that these drugs do not lose efficacy within the hostile environment of the lesions.

Dr. Pereira: This is one of the beauties of cellular reprogramming, allowing us to work with human cells which was not possible before! Can you generate these astrocytes that become reactive to TNF, and can you also model the interactions with oligodendrocytes and neurons? By coculturing them or by using more complex culture systems?

Dr. Fossati: Yes. I spent a decade focused on developing methods by which we can make the main cell types in the brain—oligodendrocytes, astrocytes, microglia, and neurons—one-by-one. More recently, we have begun combining these cell types together using coculture or organoid systems. Oligodendrocytes are the most difficult cell type to generate because it takes such a long time to differentiate them, mimicking their late appearance during fetal development. We established a serum-free protocol that generates neural spheres that are plated down to allow migration of neurons, astrocytes, and ultimately oligodendrocytes.

We have developed sorting strategies to purify the different cell types; for example, we can isolate astrocytes using a novel marker that we discovered (CD49f). Thanks to a collaboration with Dr. Paul Tesar at Case Western University, we have also established a protocol to generate cortical organoids that contain myelinating oligodendrocytes (it takes over 200 days to produce myelination) and we have developed techniques to also introduce microglia, which are of mesodermal origin, into these organoids.

We integrate microglial progenitors into early organoids, mimicking the migration of microglial progenitors from the yolk sac to the developing CNS during embryogenesis. We leverage the migratory capacity of microglia progenitors, and we follow them entering the organoids and differentiating into microglia. Now we have organoids with the full compendium of glia, which can persist in culture for over a year.

Depending on the downstream analysis we want to perform, we can either use 3D organoid models, or generate cocultures of specific cell types in monolayers, which are often better suited for functional studies such as testing the phagocytosis capabilities of astrocytes and microglia.

Dr. Pereira: I believe you would have regional differences within these cultures, right? Including protective cells

and other cells that release toxins damaging to the neurons, how do you resolve heterogeneity in the cultures?

Dr. Fossati: Yes, we must always remember that we are modeling complex cellular systems, which display regional differences and heterogenous responses that are dependent on spatiotemporal factors. That is why we need to plan the experiments with rigor. As we know, these are models, but if we acknowledge their limits as well as their strengths, and use the appropriate controls, we can use them more effectively. Our ongoing studies with organoids are focusing on novel drivers of AD pathogenesis, identified through collaborative study that involves Dr. Scott Noggle and myself at NYSCF and many other groups, led by Dr. Bin Zhang at Icahn School of Medicine at Sinai.

These projects are funded by NIA and by the AMP-AD program (Accelerating Medicines Partnership—AD: a public–private partnership between the NIH, FDA, multiple biopharmaceutical and life science companies, and nonprofit organizations aimed at transforming the current model for developing new diagnostics and treatments for AD).

The analyses of several omics studies converged on a few hits for key drivers in AD, which, not surprisingly, are primarily expressed in glial cells, mostly microglia and astrocytes, again pointing toward an important role for glia in neurodegeneration. We are now generating CRISPR/Cas9 edited lines to investigate the effect of knocking down and overexpressing interesting genes and we are making organoids where we combine, for example, knockout microglia with isogenic wild-type neural cells. Our ultimate goal is to understand the impact of these genes in neurodegeneration and to identify new targets for therapies.

Dr. Pereira: That sounds exciting. You mentioned as well that these protocols are complex, and the need to adapt to serum-free conditions that require a good understanding on the signals to introduce, so cells differentiate in the right direction. This takes a long time–many weeks, right? I'm wondering whether you're considering using transcription factors to speed up the process of differentiation into these lineages?

Dr. Fossati: Yes, absolutely. As you know, transcription factor-driven protocols have been very successful for generating neurons. There are the so-called NGN2 neurons, which are used by many laboratories, because the protocol is robust and short: ideal for high-throughput studies. This is ultimately what we want to achieve with glia. Some studies have identified key transcription factors to drive astrocyte, oligodendrocyte, and microglia commitment. However, I believe there is still a lot of room for improvement, especially for the oligodendrocytes.

AU7 ► I think that both approaches are important: the TF-driven protocols may become more useful for high-throughput studies, such as drug screening; however, the traditional patterning through reagents added to the media allows us to dissect each step along the differentiation process, which may be important for disease modeling to identify disease phenotypes that would otherwise be missed with an accelerated protocol. We would ideally like to have both approaches available to us. There is a dedicated team at NYSCF, led by my former postdocs, that is working exactly on refining TF-driven protocols for CNS cells.

Dr. Pereira: In the organoids you are going to have the diversity of cells, a complexity that's established through developmental processes that take a long time. For the transcription factor-driven approach, are you envisioning to generate those cell types mainly for disease modeling?

Dr. Fossati: The transcription factor approach, it is sort of forcing the cells to become mature, and this accelerated differentiation may mask a disease phenotype. If we are interested in modeling a disease, we prefer to use the traditional longer methods, however, as the protocols are continuously evolving and improving, so is our approach. We may transition to TF-driven microglia differentiation within cortical organoids in the future, for example.

Dr. Pereira: I am wondering whether you could tell us a little bit more what you are doing in the laboratory right now. Can you give us a flavor of the projects that you are running now?

Dr. Fossati: We are continuing to expand our study on the roles of reactive astrocytes in AD and MS. We are investigating the roles of specific genes expressed by microglia to understand and target them in AD. Last but not least, over the past few years, I have literally expanded my horizons performing pioneering experiments in low Earth orbit (LEO). These studies are complementary to the study that we are performing on Earth about neurodegeneration, but also leveraging the additional environmental context of microgravity. This has been an exciting and completely new experience.

I have always been excited to lead pioneering experiments in the stem cell field: performing the early experiments on MSCs, generating the first iPSC lines from people with progressive MS, developing some of the early protocols for glia differentiation, and now I am also developing the first long-term cultures of organoids in space. This project was made possible thanks to Dr. Paula Grisanti at the National Stem Cell Foundation and Space Tango, which provided software and hardware tools to ship our cultures and maintain them onboard of the International Space Station (ISS).

We have successfully completed three missions, sending organoids derived from MS and Parkinson's iPSC lines. Our study has been the first to send human patient lines to the ISS, maintaining cultures for 1 month. We are now analyzing the data to understand how the biology of neural cells changes in microgravity.

These experiments are incredibly challenging; it is a completely different way of doing research with many more variables to consider. It requires a lot of troubleshooting and until the very end many steps can go wrong, but this makes the study only more exciting. Research in space is a field in rapid expansion, and I predict we will only hear more and

more about biomedical studies conducted onboard of the ISS. In fact, several conferences (including ISSCR) now have dedicated sessions to space research.

Many people are probably familiar with the effect of microgravity on the musculoskeletal system, or on body fluids, however, less is understood about the effect on the CNS and the immune system (which include microglia). Some animal studies and analyses of peripheral blood of astronauts suggest that microgravity may accelerate neurodegeneration and enhance inflammation, making this a compelling model to dissect the pathogenic mechanisms linked to those processes. Perhaps we can identify some molecular mechanisms that are hidden or difficult to detect working in traditional laboratories on Earth.

Dr. Pereira: This is really taking cellular reprogramming to another world!

Dr. Fossati: Yes, we could say that!

Dr. Pereira: How does it work in practical terms? Do the astronauts change media, or do you send the organoids in a closed container? How do you culture cells in space?

Dr. Fossati: The experimental strategy requires a lot of planning. For our first experiments launched with SpaceX-CRS19, SpaceX-CRS24, and SpaceX-CRS25, we sent organoids in static conditions within a temperature-controlled container (a sort of miniaturized incubator). They were kept inside vials for the entire duration of the experiment, without any media changes. We were quite surprised to find that the cells survived for the whole month! They were perfectly fine: they returned to Earth alive, we were able to plate the organoids into coated dishes and observe neurite growth.

For future studies, we are working on fluidic systems, and we may need some help from the astronauts. Obviously, our first goal was to establish the minimal successful conditions to enable organoid cultures in LEO. Now that we have achieved that, we are expanding the studies further, for example, by testing organoids at later stages of the differentiation, or challenging the cells with drugs, and by developing downstream analyses directly onboard of the ISS.

Dr. Pereira: Are you going to start doing experiments in space as well?

Dr. Fossati: Yes, that is the goal! The idea is to equip the ISS with microscopes, and many other tools and instruments. NASA has been investing a considerable amount in this research and improvements are happening rapidly. I anticipate we will read more and more articles about those studies. It is possible that this journal will publish some studies on reprogramming in space!

Dr. Pereira: That would be an interesting experiment. Can you reprogram cells in space without gravity, and is this or isn't it translated into a change in reprogramming efficiency? This is a completely unexplored idea, it is fascinating! Now I am curious about the control of such experiments. Do you send organoids to space–is the control keeping organoids on Earth or is the control sending organoids into space where you introduce artificial gravity?

Dr. Fossati: We have two equivalent sets of organoids inside two miniaturized incubators. One set is loaded into the rocket and then shipped to space, one stays on Earth as a ground control, and at the end of the mission we compare the two groups. I would also like to mention that we have taken steps to consider the potential effects of radiation, in addition to microgravity. We were able to measure the cumulative radiation received by the organoids and concluded that the levels were likely not sufficient to affect the cells.

Eventually, we would like to include radiation in the ground control experiments to simulate the exposure, but this is a more sophisticated system that will require more development. What we have successfully performed was already complicated: just imagine that we originally grow the cells in NYC, then we ship them to the laboratory at the ◀AU8
Kennedy Space Center, then we load them into the rocket and hope for a successful launch. Most of the time the launch is delayed by one or several days, so we also need to ensure we have backups. Nothing is sure until the very end. I cried with joy the first time that I saw the launch, it really is emotional.

Dr. Pereira: Yes, I can see that. What are the first assays you will perform to look at those organoids?

Dr. Fossati: Mostly RNA.

Dr. Pereira: Single cell RNA sequencing or bulk RNA sequencing?

Dr. Fossati: We have done bulk for now. We are performing bulk RNA sequencing and histology on cryosections. Because we used static cultures without media changes, we were also able to collect the supernatant for secretome analysis. Again, the plan for the future missions is to get access to more analysis that can be done directly on the ISS. We are preparing an article describing the first results, look out for it!

Dr. Pereira: Very nice. We are looking forward to seeing the results of these experiments. As we approach the end of the interview, I was wondering whether you can highlight your main challenges, from your experience on IPS differentiation of brain cells, to understand in MS, ◀AU9 what are the limitations? What are your main challenges to develop new interventions?

Dr. Fossati: There are a lot of challenges. First, it took me 10 years to get all the protocols working robustly. There were no efficient protocols for glia cells when I started in 2011, and still, for the oligodendrocytes for example, the protocols are not optimal. It has also been challenging to receive funding for iPSC modeling, because the field initially faced a lot of skepticism from the neuroscience community. It is improving now, but still our projects are considered a high risk/high reward which is typically not funded by traditional NIH-based mechanisms.

Moreover, there is the problem of line-to-line variability and reproducibility. When applying iPSC-based models to neurodegenerative disease, we need to validate the findings with multiple lines, and that is where working at NYSCF has been incredibly valuable because I have access to a huge repository of iPSC lines.

In the first decade of iPSC modeling, the field was mainly focused on performing proof-of-principle experiments. The challenge now is to expand the study to population-scale studies, using multiple lines to understand how polymorphisms and mutations affect disease pathogenesis and progression. This is something important in MS, AD, and all neurodegenerative and neurodevelopmental disorders. We now have the opportunity to unravel differences linked to gender and ethnicity, moving toward precision medicine, that will ultimately bring the optimal treatment to each patient.

Dr. Pereira: Each cell is a cell, right? We need to take it one by one if we want to advance…. Multiple pathways govern lineage commitment in a step-wise manner, therefore the protocols to generate these cells will be unique. We need to first understand the regulatory networks, right?

Dr. Fossati: Yes, absolutely.

Dr. Pereira: I was wondering to what extent the NYSCF Research Institute has been critical for you to overcome these challenges?

Dr. Fossati: NYSCF and in particular Susan Solomon, our founder CEO, were essential to my work and my success. I want to dedicate this interview to her memory, because she has been my strongest support and source of inspiration, from the first time I met her, in 2009. When Susan recruited me to join the NYSCF Research Institute, she was aware of my MS diagnosis and we discussed my desire to develop human models of the brain, despite my lack of background in neuroscience. Not only did Susan fully support my research plans, but she also taught me to achieve the impossible, by working hard, being perseverant, and remaining strong even through the hardest of challenges.

NYSCF has notoriously funded high-risk/high-reward projects, propelling the iPSC field from the very beginning. The environment that I found at NYSCF is unique: a multidisciplinary team that includes biologists, computer scientists, software, and hardware engineers. Thanks to Susan's vision, NYSCF built a fully automated platform for iPSC generations, which has made hundreds of iPSC lines, including a cohort of MS lines for my studies, and now we are expanding automation to perform differentiations. I work on neurodegenerative diseases, and I have colleagues working on diabetes, cancer, and immune cells, which is a constant source of inspiration.

In addition to the robotics platform, NYSCF is also investing AU10► in artificial intelligence (AI). My colleagues have recently performed groundbreaking studies that use AI to recognize fibroblasts derived from people with Parkinson's disease AU11► versus healthy people (Schiff et al, Nat Commun. 2022). I think that the application of AI to biology will expand enormously, and AI will help us to analyze data as well as perform diagnoses. I feel very privileged being at NYSCF and having access to all these state-of-the art technologies.

Dr. Pereira: Just to finalize, can you tell us a little more about your ambition and visions for the field of cellular reprogramming? What will be the focus of your research for the next 10 years, and where do you think the field will be going beyond that?

Dr. Fossati: I think that in the first two decades of ESC/iPSC technology, we have made tremendous progress, to the point that there are currently clinical trials in progress using pluripotent stem cells for cell therapies for Parkinson's disease and for macular degeneration, to name just a few. I am hopeful that in the next decade we will have made important progress toward translational studies in MS. I am interested in drug discovery for remyelination and neuroprotection, targeting glia-driven inflammation and degeneration. After investing a decade in building all of the differentiation protocols, tools, and assays, I look forward to a decade of translational studies for MS, AD, and in general diseases that involve neuroinflammation.

I am optimistic because this is an exciting time in which we have many new technologies at our disposal. For example, think about single cell RNA sequencing and other single cell -omics analyses, combined with AI. This is the era of big data. We are generating so much data and the challenge now will be to interpret it accurately. These studies will likely raise new questions, but they will also answer questions that were previously unfathomable, even just 10 years ago. When I studied biology, what I am doing now was considered science fiction, and here we are now, living a true revolution.

Dr. Pereira: Do you have any advice for the younger reprogramming scientists who are starting their career right now, looking to starting a PhD and a career in the field?

Dr. Fossati: Several of my mentees are undergraduate students who are looking to gain experience in research before continuing their studies in PhD or MD-PhD programs. What I always tell them is do not worry too much about choosing just one path or making the wrong decision on what to study. I think that now more than ever, science is interdisciplinary, and many fields have multiple possibilities to overlap and produce fruitful collaborations. One can train as physicist, mathematician, engineer, and end up working in the stem cell field.

Choose something that you are passionate about, choose good mentors, and just let your enthusiasm lead you. No avenue of research results in a dead-end road, rather scientific research comprises an intricate system of connected channels. I also never thought I would study neuroscience, and here I am.

The second, more practical, advice I would share with young students entering biomedical research is to study

bioinformatics. We are now dependent on sophisticated technologies and computers to acquire and analyze any sort of data. Training in both biology and computer science provides an important set of complementary skills that will enable you to design, perform, and analyze your own data. Bioinformaticians with a strong background in biology are in high demand now, both in academia and in biotech industries. These are the scientists who will lead the stem cell field in the next decade.

Dr. Pereira: I think that's a great piece of advice. Bioinformatics and the field of AI are growing massively. This will become more and more important, but it also applies to other disciplines such as engineering. If you can work at the interface of research fields from the beginning, it will enrich your career in the future. Even at the interface of business and science, and more….

Dr. Fossati: Absolutely.

Dr. Pereira: This is going to become relevant because we need also to train the CEOs of the emerging biotechs which are expanding around the world. I think that's a very critical point in what you said. Grow at the interface of fields. To close the interview, I'd like to ask you two questions, not strictly related to your science and research, but that will help the audience of *Cellular Reprogramming* to know you better. The first one is, if you could answer any single scientific question, regardless of your experience or expertise, what would that be?

Dr. Fossati: I would love to understand the process of aging. Aging plays a major role in the worsening of many pathologies, especially neurodegenerative diseases, and also in the development of cancers. If we could find ways to slow aging, there would be tremendous benefit. Life expectancy has increased significantly over the past few decades but, as I see it, reaching 100 years old is only a worthwhile goal if we are in good shape, both mentally and physically. Even if I am only indirectly working on it, it is good to know that many scientists are investing their efforts on truly understanding aging.

Dr. Pereira: Not to find the fountain of youth, but to find the fountain of healthy aging, right?

Dr. Fossati: Yes, exactly. I am speaking especially as a chronic patient. I wish my health conditions would not escalate after I pass the 50s.

Dr. Pereira: Now, if you were not a scientist, what would you be?

Dr. Fossati: I have often asked myself this! I think it is likely that I would have ended up somewhere in the world of education and teaching—teaching is something I have always felt passionate about. I enjoy very much when NYSCF organized seminars and events for lay people and for kids. I could also see myself working on science communication, perhaps within a patient advocate foundation. If we look completely outside of the field of science, since I was a kid, I dreamt of opening my own ice cream shop, making Italian gelato. Ice cream has the power to make people happy, you can eat it to feel better when you are down, you can eat it to celebrate when you are happy. In a way, it is still a job that helps people!

Dr. Pereira: I love that too.

Dr. Fossati: Yes, everybody loves ice cream, right? You cannot go wrong!

Dr. Pereira: Thank you for your perspective, and thank you so much for joining me today and for your time. It was great to learn more about yourself and about your science.

Dr. Fossati: Thank you for the invitation. It was a pleasure to be here.

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