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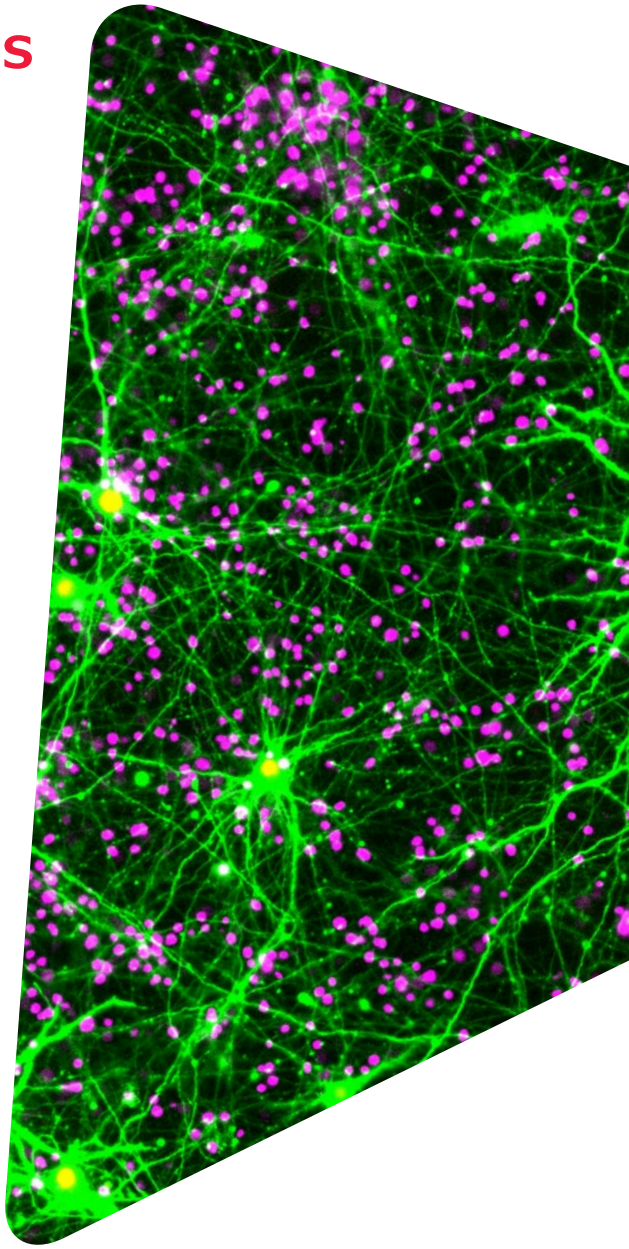
Case Study

Finding Novel Therapies for Neurodegenerative Diseases



Read about how Dr. Emmanouil Metzakopian and his team are successfully using the Sigma-Aldrich® CRISPR product portfolio to prioritize gene targets for drug development to treat neurodegenerative diseases. Based on an interview, this case study outlines the collaborative process between Dr. Metzakopian's research group and the Sigma-Aldrich® team in developing a new research tool.

"We started as…. having this, let's say, scientific and business interaction which ended up actually forming a beautiful friendship as well. It's a nice story; I'm happy to share it."

- Dr. Emmanouil Metzakopian
UK Dementia Research Institute

As a longtime user of Sigma-Aldrich® products, Dr. Emmanouil Metzakopian knows the value of access to high-quality reagents and customer support.
Dr. Metzakopian is a group leader at the UK Dementia Research Institute at Cambridge University, and his team studies the effects of environmental stress on the progression of neurodegenerative conditions such as Alzheimer's and Parkinson's Diseases.

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Keeping Neurons and Synapses Healthy

Neurological disorders are the leading cause of disability and are increasing in global prevalence.[1] More specifically, Alzheimer's Disease and Parkinson's Diseases represent a significant societal burden that researchers are striving to address.[2, 3] One approach to understanding these neurodegenerative diseases has been to conduct large-scale genetic studies to identify risk factors that predispose people to these conditions. These types of studies helped unravel underlying mechanisms and have led to important breakthroughs in our knowledge, but these diseases are not exclusively genetic. In order to fully understand these conditions, that research must be complemented with an examination of environmental effects.

Dr. Metzakopian's group seeks to do precisely that, exploring how genes interact with environmental factors such as different types of stressors in disease progression, with the ultimate goal of identifying ways to prevent neurodegeneration and preserve synaptic connections.

The Challenge: Developing the Tools for Large-scale Screening of Human and Mouse Genomes

A long-standing issue in neuroscience research is that animal models do not always recapitulate the features of human disorders, and obtaining sufficient human neural tissue for large-scale experimentation is not feasible. To address this, Dr. Metzakopian and his team use stem cell technology where more easily-obtained human cells such as fibroblasts are brought back into an undifferentiated state as human induced pluripotent stem cells (hIPSCs) which can be propagated in culture. From there, they can be differentiated into a number of different types of neurons. They can also be differentiated into glial cells, which both support neurons and are active players at synapses in their own right. With a culture system ready, the next step was to find a way to look at the genome in terms of stress responses.

In order to answer such broad questions, new tools were required. As an experienced user of the CRISPR/Cas9 system, Dr. Metzakopian looked to this technology to assess stress responses across the full genome of hIPSC-derived neurons. "We use CRISPR/Cas9 genetic screens to knock out genes and see which genes are beneficial for the disease model," he explains. "We can use either a small group of druggable genes, or we can interrogate the whole genome."

In order to carry out these ambitious experiments, Dr. Metzakopian's group needed oligonucleotides to clone into expression constructs for every protein-coding gene to produce a library of CRISPR guides for a loss-of-function screen. In addition, they wanted to produce a library of similar sgRNA expression constructs for the mouse genome to expand the models that could be tested. Neither of these libraries existed at the time, and producing them was beyond the capacity of an academic laboratory to accomplish on its own both in terms of scale and the chemistry required to synthesize the necessary nucleic acids.

An industry partnership emerged as a way to tackle the project. The vast scale was unmanageable for some that Dr. Metzakopian approached: studying roughly 20,000 protein-coding genes, with two guides per genes, and two full genomes resulted in a requirement of 160,000 guides and corresponding oligonucleotides. "It's conceptually a very simple process, but when you upscale to this size… everything has to be done perfectly." With the requisite in-house experience and capacity to design and produce the required oligonucleotides, the Sigma-Aldrich® team joined Dr. Metzakopian's to take on the challenge.

Building a Successful Scientific Partnership

Through an effective scientific rapport, the Sigma-Aldrich® team worked with Dr. Metzakopian's group, providing input to help set the parameters of the experiments that would maximize the probability of success as well as supply products in the most appropriate format. During these discussions, details like the concentration of oligos, whether they were annealed, and the plate format were determined. This cooperative effort resulted in the first individually-cloned CRISPR/Cas9 genome-wide arrayed sgRNA libraries covering 17,166 human and 20,430 mouse genes.[4] These are now available as part of the Sigma-Aldrich® portfolio as the Sanger Arrayed Whole Genome Lentiviral CRISPR Libraries for the human and mouse genome. This interaction was not only a supplier-consumer relationship, but also a true scientific collaboration, with five Sigma-Aldrich® scientists listed as authors on the research report with Dr. Metzakopian's team. "That required very deep understanding of our cloning process and where we wanted to end up," he commented, "which is here, having [expression plasmids] for guides that can be used as either a virus or transposon."

The collaboration did not end with the production of the libraries. It was important to Dr. Metzakopian that the libraries be available for others to utilize in their own research. "I have it for myself," he said. "There are people asking for it, but I can't really deliver it. I don't know how to do that. And [the Sigma-Aldrich® team] said, 'We can do it for you,' and they took care of distribution worldwide." As a result, these important tools became the first CRISPR-Cas9 arrayed libraries distributed globally.

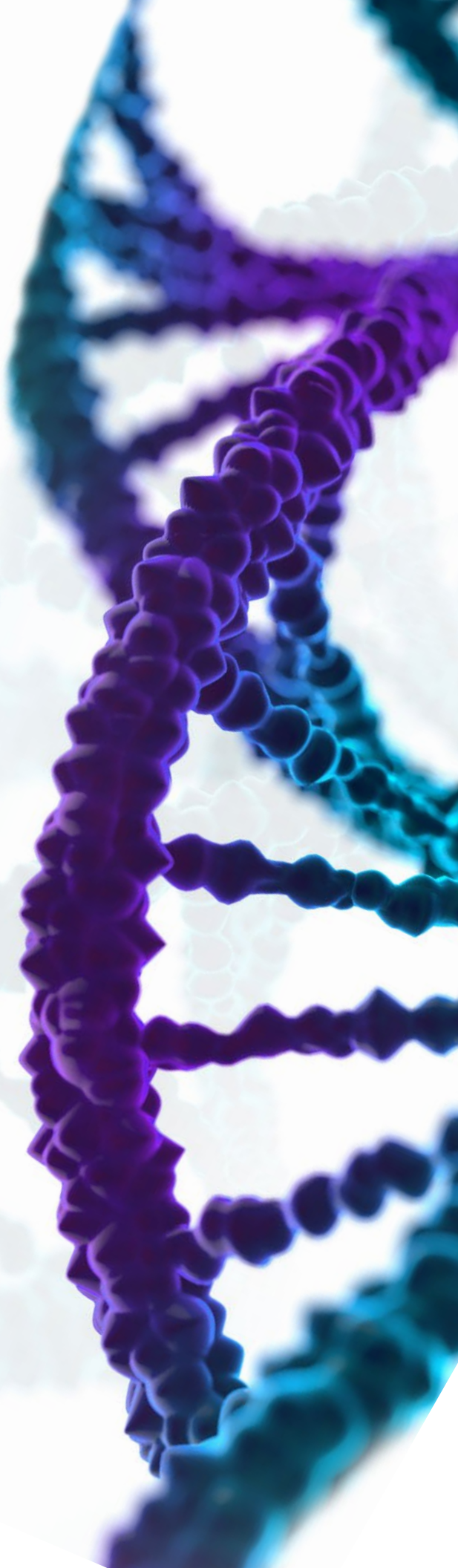
A Customer-Focused Culture

While high-quality science is a clear priority in Dr. Metzakopian's work, there are other considerations necessary for a successful collaboration. One important point for him was customer service. "[Sigma-Aldrich®] customer service is very friendly, [with] quick replies. There is a lot of… scientific support; you talk to intelligent people that can help if we're stuck. We [get] feedback," he explained. Delving deeper into the subject, Dr. Metzakopian described how he felt the Sigma-Aldrich® team was invested in understanding his needs. "They sit down with you and say, 'What do you want? And why do you want it?' They really care about that, and that's really important." He further commented that he appreciated that there was a sensitivity to budget, which is key, especially for an academic lab.

This focus on the customer extended beyond interactions with the principal investigator: whether communicating with undergraduate researchers, graduate students, or postdoctoral researchers, interactions have been positive for all members of Dr. Metzakopian's team. "It's a culture thing, that's for sure."

Putting the New Tool to Work

The goal of Dr. Metzakopian's work is to find genes that protect neurons from degeneration. Armed with new, genome-wide, arrayed CRISPR-Cas9 knockout libraries, his team is currently looking for factors that protect hIPSC-derived neurons and maintain synaptic connections when subjected to different stressors involved in Alzheimer's Disease. Using compounds that cause oxidative stress, endoplasmic reticulum (ER) stress, and lipid peroxidation, they induce neurodegeneration and use the knockout library to then look for genes that rescue cell death. Additionally, they look for genes modulating stressor effects—either increasing or decreasing them—using fluorescent stress reporters. So far, they have successfully



identified 32 novel, high-priority, validated targets that they can study further with a multi-faceted approach involving imaging studies, electrophysiological measurements of neurotransmission, and more.

Dr. Metzakopian's team also has an ongoing project examining neurodegeneration in Parkinson's disease. One of the main, nonmotor symptoms of this disorder is constipation resulting from degeneration of gut neurons.[5] With the aim of replacing them, the team is using the whole genome CRISPR array to identify factors that allow hIPSC-derived neurons to integrate with intestinal organoids, a model of the gut. Replacing degenerated neurons would ameliorate an often-debilitating symptom present in more than half of patients.

When asked what his long-term plans are, Dr. Metzakopian articulated that his hope for the research is to design and produce new and better disease models. "Better models will lead to better therapies, better targets. You cannot take every target all the way to the clinic. You can prioritize targets better this way," he said. "Prioritize a few targets and hopefully one or [several] of them would benefit some people." With ambitious projects using innovative approaches to contribute to our understanding of neurodegeneration, there is no doubt that Dr. Metzakopian and his team will succeed in that goal.

About Dr. Metzakopian

Dr Emmanouil Metzakopian holds a BSc in Biochemistry and Biotechnology from the University of Thessaly, Greece. He received his PhD in midbrain development from University College London under the supervision of Dr. Siew-Lan Ang at the National Institute for Medical Research. Later Dr. Metzakopian had been working on genome scale genetic screens using the CRISPR-Cas9 gene editing tool in the Lab of Dr. Allan Bradley at the Wellcome Trust Sanger Institute. Dr. Metzakopian now leads a team at the UK Dementia Research Institute (UK DRI) in Cambridge. The aim of his projects is to understand the role of oxidative stress in neurodegeneration and to identify protective therapeutic targets. In parallel Dr. Metzakopian has been recently appointed as the head of Innovation at Bit Bio an ambitious start up to discover the transcription factor reprogramming code of disease relevant cell types.

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