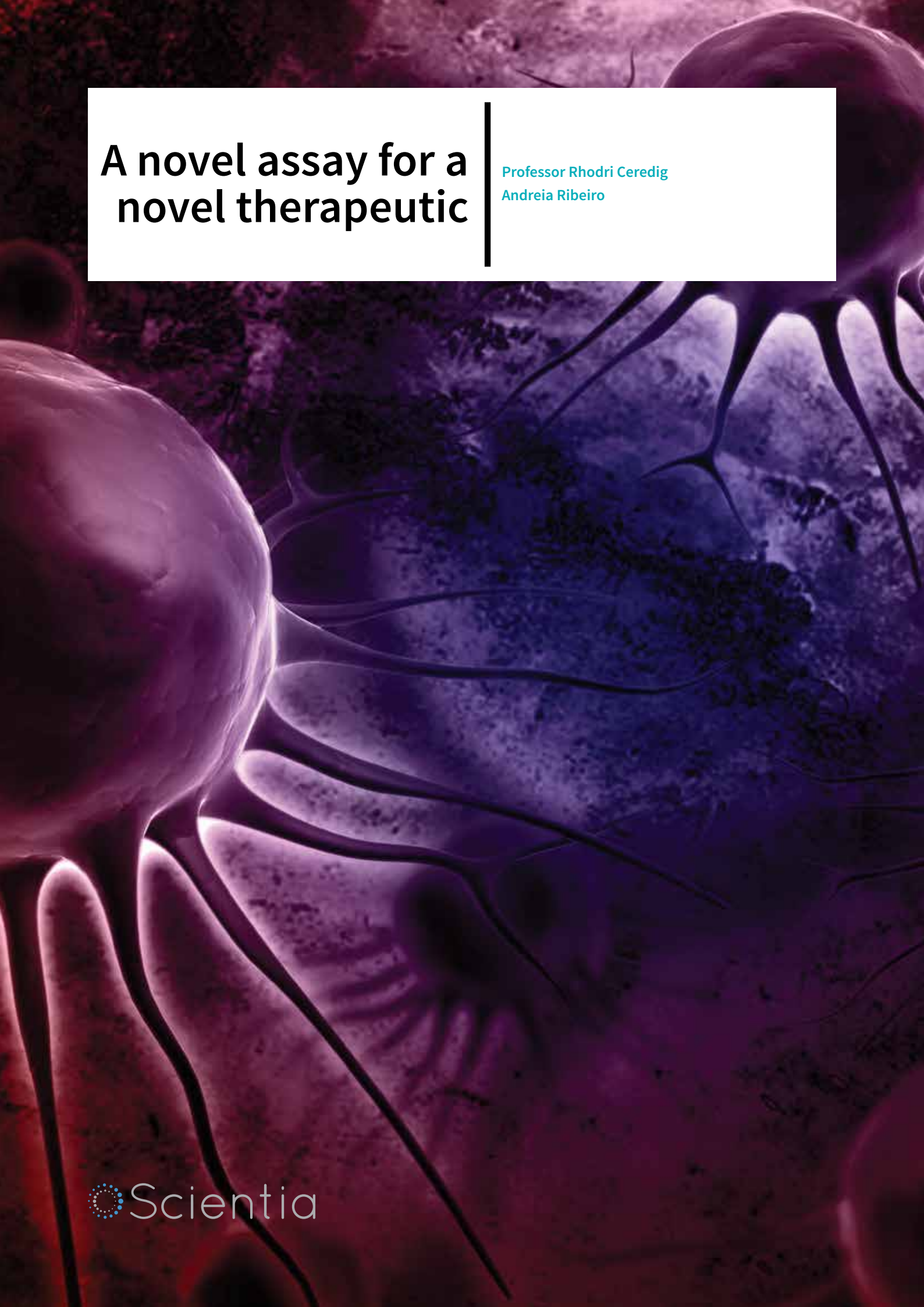
A novel assay for a novel therapeutic

Professor Rhodri Ceredig
Andreia Ribeiro



Scientia

A NOVEL ASSAY FOR A NOVEL THERAPEUTIC

Professor Rhodri Ceredig and his **PhD student Andreia Ribeiro** have developed a novel assay for measuring the immunosuppressive potency of MSC (mesenchymal stromal cells) that could revolutionise the use of this cell-based therapy

How did your research experience prior to this project lead to an interest in developing an assay to quantify MSC potency? How did you come to work together?

Rhodri Ceredig: The main topic of my research career has been to study the development of cells of the specific immune system, namely lymphocytes, in mice. My doctoral and post-doctoral research focused on the development of functional T cells in the embryonic and neonatal mouse thymus. After creating a transgenic mouse line that over-expressed interleukin-7 where animals routinely developed B/myeloid lymphomas I later became interested in B cells. To further study these cells, a colleague in my lab, Dr Amanda Fisher, generated a clone of bone marrow-derived MSC. Still interested in B cells, I joined the laboratory of Prof. Ton Rolink at the Basel Institute for Immunology; Rolink was using MSC-based culture systems to study B cell development. While at Basel I also met Prof. Alan Tyndall, a rheumatologist with interest in using MSC to treat autoimmune disease. I became interested in characterising MSC via flow cytometry and began collaborating with Tyndall. I came to Galway in 2008 as an immunologist to join Prof. Tim O'Brien's Regenerative Medicine Institute (REMEDI). In collaboration with Prof. Noel Lowndes, my primary focus is the understanding of the DNA damage response of MSC, particularly in the context of hypoxia. With my colleagues Prof. Thomas Ritter and Prof. Matthew Griffin, we answered a funding call from Science Foundation Ireland to investigate the potential of developing a novel flow cytometry based assay with which to measure MSC immunosuppression; this funding included financing for a technical assistant, and Andreia was chosen based on her past experience with MSC and flow cytometry. Andreia has continued this work as part of her PhD funded by a EU Marie Curie Skłodowska Initial Training Network.

Andreia Ribeiro: I worked in a flow cytometry diagnostic laboratory where I had the opportunity to study lymphocyte response to MSC isolated from different sources. I developed a strong interest in MSC and found myself with many unanswered questions at the end of the project. I applied to Prof. Ceredig's lab in the hope of pursuing some of those questions.

Why do we need an assay to quantify MSC potency?

Compared to our knowledge of blood cell development, our understanding of MSC is relatively crude. However, because of their differentiation and immunosuppressive properties, MSC show promise as a therapeutic modality for the treatment of autoimmune and/or degenerative diseases. Many experiments have demonstrated the immunosuppressive properties of MSC, yet their mechanism of action is unknown. Further, there is a glaring commercial and scientific need for a potency assay with which to test whether individual batches of MSC have equivalent immunosuppressive effects and more importantly, whether particular MSC batches will have immunosuppressive activity on a potential recipients' immune cells in a medical setting.

What impacts do you hope this MSC assay will have on medical research? Do you plan to extend this work further?

Rhodri Ceredig: We hope to see this assay used in clinical trials where MSC-based therapies are being attempted. Currently we do not have any correlation between results from our in vitro potency assay and in vivo clinical outcomes, but such clinical trials are being initiated in Galway. We await their outcome with anticipation.

I am coming to the end of my research career, during which I have been lucky to work alongside many talented, rigorous scientists. I came to Galway with the aim of passing on some of the experience I have gleaned from laboratories around the world to the next generation of scientists. I am excited to see upcoming scientists, such as Andreia, take up the mantle of MSC and immunology research.

Andreia Ribeiro: If clinical trials demonstrate a correlation between in vitro and in vivo results, this assay will ideally go on to be used as a tool to provide personalised medicine. It would be possible to predict if a certain batch of MSC would be beneficial to a particular patient ahead of treatment. While at the moment I am focused on finishing my PhD, I hope to have further opportunities to study MSC and immune cell interactions as I continue my career. MSC products have the potential to provide powerful therapeutic approaches for several conditions. I hope that I can contribute to this research in a small part; there is still so much work to do!

BRINGING MSC FROM THE LAB BENCH TO THE DOCTOR'S OFFICE

In recent years, research highlighting the potential for MSC as a powerful therapeutic has boomed. However, inconsistencies in production and quality control present a major barrier to cell based therapies entering mainstream medicine. **Professor Rhodri Ceredig** and **PhD student Andreia Ribeiro** recently developed a novel quantitative assay to test the potency of MSC strains.

Cell-based therapies: the future of medicine?

A cell-based therapy is a medical treatment during which live cells are injected into a patient to treat a disease or condition. These therapies hold the possibility of treating conditions ranging from cancer, to heart disease, to diabetes, with healing capabilities far beyond those of most traditional pharmaceuticals. Over the past decade, research in cell-based therapies has flourished, and a large number of cell-based treatments for a variety of conditions have shown great promise in clinical trials. However, very few of these potential therapies have made it through the processes of licensing and market authorisation necessary to make the leap from clinical trials to a product available to the general public. Because the product in these treatments are live cells, manufacturing is far more complex than a typical pharmaceutical, and reliable quality control measures present a formidable hurdle to widespread distribution of cell-based products. Without appropriate assays to assure the quality, safety, and potency of a cell-based treatment, these potentially powerful therapeutics may be doomed to stay in the laboratory.

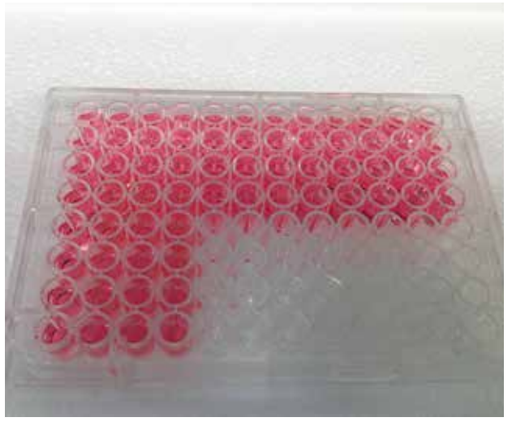
MSC: Promise & Problems

MSC (mesenchymal stromal cells) are multipotent cells that are capable of differentiating into many of the types of cells that form tissue, including bone, muscle, fat and cartilage cells. Researchers have discovered that MSC can act as powerful pharmaceuticals, working to stimulate healing in conditions that require tissue repair and as immunomodulators in inflammatory and immune conditions. These multi-talented cells have been shown to play a role in down-regulating inflammatory immune processes in autoimmune diseases, with the potential to improve acceptance of new tissue following an organ transplant. Human bone marrow-derived MSC demonstrate promise as a potential therapeutic for many diseases, and currently over 550 clinical trials involving MSC are in progress worldwide. While researchers are not yet entirely sure how MSC act to regulate immune function, these cells have shown consistent aptitude for therapeutic use in clinical trials and MSC research is booming.

Unfortunately, not all MSC are created equal in the lab. In addition to the many research institutions culturing MSC, numerous private companies have emerged with a focus on developing batches of MSC for commercial pharmaceutical purposes. There is no universal method for producing MSC, and individual facilities vary in their approaches, which may lead to differences in MSC quality and potency between sources. Even within the same facility, variation between bone marrow donors can produce widely different potencies between batches. This high potential for batch variation poses a difficult quality control problem for MSC production facilities, and is one of the greatest barriers to cell-based medical treatments making it to the general public. For MSC to advance as a viable therapeutic, a standardised potency assay that allows for the comparison of individual batches is needed. Further, as medical research advances, doctors are moving away from the 'one-size-fits-all' approach to treatment and turning to technologies that allow practitioners to take personalised medical strategies for individual patients. Because a patient may respond uniquely to MSC preparations from different sources, an assay that allowed practitioners to rapidly select the most effective MSC batch for an individual patient prior to treatment could be transformative in medicine.

Creating a novel assay

Until now, the only available procedure to test MSC potency took over 24 hours, is expensive, and requires both equipment and skilled technicians that are not common to smaller facilities and hospitals. Ceredig and Ribeiro sought to develop a practical, quantitative assay for MSC potency with potential for wide deployment across the variety of settings where MSC are used. The ideal assay should be fast, practical, inexpensive, and produce quantitative results that are comparable between assays. Additionally, an assay with the option to utilise a patient's own blood to test the effectiveness of multiple MSC batches would create opportunities for personalised medicine.



To achieve these goals, Ceredig and Ribeiro chose to exploit one of the best-described immune effects of MSC treatment, reduction in pro-inflammatory products from monocytes. Monocytes are a type of immune cell that operate as part of the innate immune system, the body's first line of defence in an infection and the component of the immune system implicated in inflammation. Monocytes are widely circulating in the blood, their responses to many kinds of stimuli are rapid and well-understood, and their immune products are well-characterised. When activated by compounds associated with pathogen presence, monocytes produce many pro-inflammatory products, including TNF-***α*** (tumour necrosis factor alpha). TNF-***α*** is a cell signalling protein with known involvement in many diseases, including arthritis, inflammatory bowel disease, and asthma. When monocytes are activated by bacterial endotoxin, TNF-***α*** is produced rapidly in a dose-dependent manner, making it an excellent indicator of monocyte activity.

Given the ease of obtaining whole blood with monocytes and the quick and reliable production of TNF-***α*** in response to commonly available endotoxin, TNF-***α*** emerged as a clear target for an MSC potency assay. This choice was validated by demonstrating that TNF-***α*** expression was reduced in a dose-dependent manner when MSC were added to the whole blood mix. By comparing TNF-***α*** levels between bacterial endotoxin activated monocytes with and without a specific dose of MSC, it is possible to reliably evaluate how potent a batch of MSC is relative to other batches. Further, while different MSC batches performed similarly across volunteer blood samples, there was some variation in potency between individuals. This provides preliminary support for the use of this assay in personalised medicine, upcoming clinical trials will expand on these findings.

One of the critical components for the development of the potency assay was the availability of the BD Accuri C6™ bench-top flow cytometer with attached automatic sampler module. This two laser, four-color flow cytometer allows simultaneous detection of cell surface and intracellular proteins. In addition, the C6 sampler module allows automatic acquisition of samples from 96 well microtiter plates. Although not done routinely, the whole potency assay can be semi-automated in a 96 well format using a Perkin-Elmer Janus robot for the cell cultures and the BD Accuri C6™ cytometer for readout. In this manner, the assay has been used to screen compounds for immunosuppressive activity. The BD Accuri C6™ is an affordable, reliable, sensitive and easy to use flow cytometer that makes flow cytometry an accessible tool for any laboratory.

Why do we need an assay to quantify MSC potency?

Further, there is a glaring commercial and scientific need for a potency assay with which to test whether individual batches of MSC have equivalent immunosuppressive effects and more importantly, whether particular MSC batches will have immunosuppressive activity on a potential recipients' immune cells in a medical setting. Potency assays are available on the market (e.g. MSCGlo™-96 PRS), but these primarily deal with MSC growth and differentiation and not immunosuppressive activity.

Shaping the future of MSC in medicine

The novel MSC potency assay developed by Ceredig and Ribeiro provides a viable option for MSC quality control across research, manufacturing and clinical settings. This assay can be completed in a single day using widely available equipment and reagents, at a much lower cost than previous protocols. This assay will allow researchers to provide standardised measures of the potency of MSC lines used in their experiments. Companies producing MSC for commercial purposes will be better able to perform quality control on their MSC preparations and ensure potency of their products. From a therapeutic perspective, this assay could allow practitioners to select MSC preparations from multiple sources using a patient's own blood, opening the door to personalised medicine for a myriad of conditions that MSC show promise for treating.

The next steps in the development of this novel assay include clinical trials that correlate the in vitro results obtained in the lab to in vivo results in human patients. The assay has been patented, and if it reliably predicts how well patients will respond to MSC treatment in clinical trials, then there is a strong potential for its commercialisation and widespread distribution. This would be a major stride towards MSC therapies becoming widely available.

PROFESSOR RHODRI CEREDIG & ANDREIA RIBEIRO

Meet the researchers

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Professor Rhodri Ceredig received his PhD from the Walter and Eliza Hall Institute of Medical Research, Victoria, Australia. Through his career he has studied the immunology and development of T cells, B cells, and MSC in prestigious laboratories around the world. He joined the Regenerative Medicine Institute at the National University of Ireland, Galway, in 2008 to pursue questions about the immunological properties of MSC and train graduate students in immunology research.

Andreia Ribeiro received her Master's degree in Microbiology from the University of Aveiro, Portugal, in 2010. She went on to join the Center of Histocompatibility in Coimbra, Portugal, where she worked to characterize mesenchymal stem cells from various sources. In 2011 she joined the laboratory of Rhodri Ceredig initially as technical assistant and now as a PhD student. The primary focus of her doctoral work is on the effect of hypoxia on MSC extracellular matrix.

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