

Stem cells in the clinic

While the world's attention has for some time been focused on embryonic stem cells and their new siblings, induced pluripotent stem cells, there are of course stem cells being used in clinical trials now – namely, haemopoietic and mesenchymal stem cells. Kate McDonald spoke to one of Australia's leading cell and gene therapy pioneers, Professor John Rasko.

TO SAY THAT JOHN RASKO wears a few different hats is to commit a crime against millinery. He has a personal chair in the Faculty of Medicine at the University of Sydney, directs Cell and Molecular Therapies at the Royal Prince Alfred Hospital, heads a team of two dozen research scientists in the Gene and Stem Cell Therapy program at the Centenary Institute, sits on a number of scientific advisory boards and still finds time to see patients in his role as a working haematologist. He also has an infectious enthusiasm for all that he does, no matter how exhausting it may be.

Rasko and his team are perhaps best known for their work in using recombinant adeno-associated virus (AAV) gene therapy to treat haemophilia B. While not a cell therapy – their ongoing clinical trial involves directly injecting the virus containing the gene for Factor IX into the patient's hepatic artery – a lot of the team's work is focused on improving gene therapy and gene transfer. An interesting area that this focus has led Rasko's team into is gene-directed enzyme pro-drug therapy, or GDEPT, using mesenchymal stem cells.

It is early days for this research, but Rasko's team is exploring the concept of col-

lecting autologous mesenchymal stem cells, expanding them in the lab and genetically modifying them so they are able to convert an otherwise non-toxic drug into a chemotherapeutic drug. One of the team's senior scientists, Dr Rosetta Martiniello-Wilks, is exploring how to use these modified MSCs in the context of treating prostate cancer.

For haematologists, who are obviously more interested in the blood-forming cells, mesenchymal stem cells never been high on the agenda, Rasko says. "They've always been the cells that the haematologists ignore when they're putting bone marrow into a culture dish," he says.

"They are those irritating adherent cells that people have tended to ignore. [Haematologists] are focused on the blood producing cells. But these mesenchymal so-called support cells seem to have quite extraordinary features. They seem to be able to suppress the immune system, or at least be somewhat ignored by the immune system."

A lot of work is now being done internationally using mesenchymal stem cells to treat graft-versus-host disease after bone marrow transplant, work spearheaded by Katarina Le Blanc in Sweden (see box p38).

And while he doesn't want to give too much away as the research is at a very early stage, Rasko's team is using its non-human primate model to show that mesenchymal stem cell numbers can be increased in the body. This observation arose during Dr Stephen Larsen's work on mobilising haemopoietic progenitors and stem cells into the peripheral blood.

"[MSCs] are not difficult to find when you do a bone marrow biopsy, but the really attractive thing that distinguishes them from haemopoietic stem cells is that the haemopoietic stem cells, even if you grow them for a few days outside the body, lose their ability to be stem cells," he says. "Mesenchymal stem cells may lose some features – and that's an area of active research – but in essence they appear to be readily grown outside the body without major changes in their applicability."

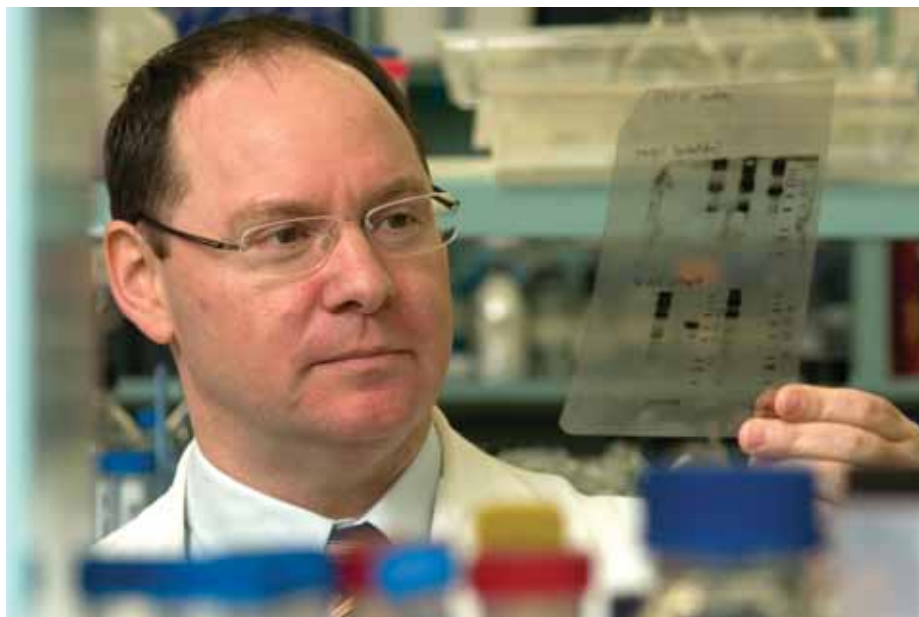
Clinical and translational research

Rasko's team's work in cell therapeutics should be boosted by another project he has long championed – the establishment of GMP cell therapy facilities at all of the major teaching hospitals in Australia. This project is at an exciting stage, with the RPA Hospital the first in NSW to receive a licence from the Therapeutic Goods Administration to deliver cell therapeutics, including stem cells, to treat leukaemias.

One of the inspirations for this new capacity at the RPA – which should be completed later this year – has been the Centre for Blood Cell Therapies (CBCT) at the Peter MacCallum Cancer Centre in Melbourne, which was pioneered by Professor Miles Prince and Dr Dominic Wall, who are close collaborators with Rasko's team.

Rasko describes the cell therapy facility being built as akin to a spaceship, with four separate laboratories, fitted with high performance filters, which will allow scientists to perform sterile procedures as well as to genetically modify cells.

Rasko chaired the committee that made a successful bid through the National Collaborative Research Infrastructure



John Rasko

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Strategy (NCRIS) for \$7.6 million – with matching state funds – to establish similar facilities in all states in Australia.

This is what Rasko calls “cell therapeutics at the clinical interface”. He is also involved in translational research, particularly for the treatment of blood disorders such as chronic myeloid leukaemia and myeloid dysplasia.

He recently penned an editorial for the journal *Pathology* to celebrate the amazing success scientists and doctors around the world have had in treating CML, particularly through tyrosine kinase inhibitors like Glivec. Rasko says Australia should be enormously proud of its reputation in this area, particularly the work of Professor Tim Hughes and his team at the Institute for Medical and Veterinary Science at the Royal Adelaide Hospital.

CML is an area of great interest for a number of reasons, not least of which is that it is one of the best examples to study cancer stem cells. CML of course has a unique molecular signature – the Philadelphia chromosome discovered back in the 1960s by Peter Nowell and David Hungerford, which was the first time that a translocation was linked to a human disease.

Rasko’s team is looking at CML in relation to the microRNAs involved in normal and malignant haemopoiesis. “We have a small group within our program who are actively exploring the role of these miRNAs both in relation to leukaemia and particularly CML, as well as normal cell development.

“That goes hand in hand of course – if you understand how the perturbation of

leukaemia alters cell development and differentiation then it also helps you understand the normal development.

“We reported in the inaugural issue of the journal *RNAi and Gene Silencing* a means of quantifying miRNA that has subsequently been used by a lot of people and has stood the test of time. Now of course there are commercial tests that you can buy but we’ve put considerable effort into it and are actively still pursuing such technologies.

“We would have love to have published a lot of our work over the last two or three years but the field is so hot that we’ve been scooped on a number of occasions and there’s no point in publishing the fact that you’ve been scooped. It’s a very competitive field.”

New directions in leukaemia

Rasko will address the New Directions in Leukaemia Research Conference, being held on the Sunshine Coast from March 30 to April 2, on another area of leukaemia research that his team is actively pursuing – the role of two zinc finger transcription factors called BORIS and CTCF and their implied role in cancer.

“Our studies have taken us a long way towards seeing how the pathway of these two important genes relates to cell development and control of cell proliferation and differentiation. We have a small group of half a dozen who are exploring the interactome of these zinc finger transcription factors to see what these molecular machines are actually composed of so we can basically understand their mechanism of action.”

BORIS and CTCF are part of the basic research aspect of Rasko’s work. “I embrace

the idea that in order to achieve successful gene therapy you have to identify the genes involved in a given disease, otherwise you don’t make use of the molecular targeting and genes of course can’t work outside the context of cells.

“So in order to understand how to improve gene therapy, you have to not only understand the root cause of any given disease but you also have to understand the target cells and how to alter them genetically in order to achieve that therapy.”

A lot of the team’s work is focused on improving gene therapy and gene transfer into haemopoietic cells, specifically using adenovirus, adeno-associated virus, retrovirus and lentivirus vectors.

“We’ve also collaborated in delivering oncogenes and the ‘Yamanaka genes’ into stem cell types,” he says. “Our focus and our role in that has been in improving the gene delivery. Refining the viral vector technology to improve the efficiency of gene delivery rather than exploring those particular genes in of themselves.

“We have collaborations where we are helping modify embryonic stem cells to achieve the kind of results that Yamanaka has achieved and also to deliver the genes involved in CML so we can explore the biology of these diseases.”

Embracing the future

So, basic, translational and clinical research are all feathers in John Rasko’s cap. He’s a working haematologist, however, so is obviously interested in clinical applications and how we can improve treatments for patients. He’s particularly concerned at how difficult it is to run clinical trials in Australia, particularly long-term ones, and would like to urge the new Minister for Innovation, Industry, Science and Research to pay some attention to this field.

“I’d like to congratulate the minister on continuing on with the important NCRIS initiative,” he says. “That is extraordinarily important for promoting cell therapeutics in Australia. I want to encourage him to continue on that theme, to specifically invest in both the clinical as well as the basic aspects of cell therapeutics. In other words, divert specific funds to those initiatives.

“One of the difficulties in some of these areas is that because they are exclusively clinical in their applicability they often times don’t get the immediate attention of the standard grant review process. Having sat on such committees for many years, the basic research tends to succeed and

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get the grants, whereas the clinical has much more difficulty. The translational is very much third place in line, but if you are trying to establish a rationale and preliminary data to underpin a clinical trial, it is almost impossible to get funds to do that in Australia.

"For example, we started our haemophilia gene therapy trial in 2001. It was put on hold for various reasons by the regulatory authorities, but the current trial that we have now requires us to monitor our subjects for 15 years. There is no means by which that can be funded in Australia. There is no funding body that is prepared to commit to funding to follow a recipient of gene therapy for that long, but that's exactly the kind of time-frame that we need. These kinds of things are crucial, the kind of Clever Country innovations, that the minister might be looking towards.

"We have an opportunity in Australia, we have such respect internationally, but we have lost some of our key stem cell researchers, for example, and I won't hesitate to mention who they are – Paul Simmons, Martin Pera and Alan Trounson – and that haemorrhage will continue whenever we hesitate to embrace the future. We've had our Senate meetings, we've gone past the time when these things are the subject of serious controversy. Every opportunity is there for governments to really make a mark in the future." **ALS**

MSCs for graft-versus-host disease

Graft-versus-host disease is one of the more common, and significant, complications of allogeneic haemopoietic stem cell transplantation, in which T cells in the graft begin to attack several of the host's organs, especially the skin, the gut and the liver.

Treatment for the condition is usually with steroids, especially prednisone and cyclosporine, but in the more acute cases patients do not respond. Mortality for the most severe grade of GVHD is over 90 per cent.

In 2004, Professor Katarina Le Blanc from the division of clinical immunology at the Karolinska Institute in Sweden and colleagues published a case history in *The Lancet* outlining the use of mesenchymal stem cells to treat acute or steroid-refractory graft-versus-host disease.

Since then, a number of trials have been held throughout the world to explore this novel therapy, including a small trial at the Royal Adelaide Hospital under the direction of senior consultant haematologist Dr Ian Lewis.

In addition to his work as a consultant, Lewis runs the Therapeutic Products Facility at the Institute of Medical and Veterinary Science (IMVS), a cell processing facility licensed by the TGA to produce cellular therapeutic products such as haemopoietic cells for transplantation, skin cells for burns patients and mesenchymal stem cells.

He works alongside such well-known mesenchymal stem cell researchers as Drs Stan Gronthos and Andrew Zannettino; while the latter study the potential of MSCs in tissue repair and regeneration, Lewis is looking at them for a different purpose completely.

No one is quite sure of the exact mechanism, but MSCs are thought to suppress most immune responses, Lewis says. They appear to have both immuno-modulatory and anti-inflammatory effects, but again no one is quite sure why.

"In terms of laboratory tests they have been shown to inhibit different aspects of the immune response, such as T cell responses, they inhibit cytokine production and they also inhibit dendritic cells," he says. "They seem to interact with a lot of cells in the immune system. What actually happens in humans is not known."

So far, four Adelaide patients, all with steroid-refractory GVHD, have been treated with a mesenchymal stem cell infusion and three have responded, Lewis says. It is very early days yet, but this small trial, part of the multi-institutional Le Blanc-led trial, is raising a great deal of interest.

In addition to a potential treatment, recent studies have looked at using MSCs as a prophylaxis for GVHD. Lewis says the rationale for using MSCs as a prophylactic agent is fairly sound but a large study is required to judge its efficacy.

In the meantime, US biotech Osiris Therapeutics, which is also exploring MSCs in bone and tissue repair, is carrying out a Phase III trial of its investigational therapy Prochymal for steroid-refractory GVHD. 240 patients are being enrolled in the study following very promising Phase II results, in which 94 per cent of evaluable patients had a response and 74 per cent achieved a complete response.

Not much happening upstairs

True neural stem cells, as opposed to neural precursor cells, are unfortunately quite rare, so the advice is to use your brain or lose it. Graeme O'Neill reports.

THERE IS BAD NEWS and good news for the owners of ageing human brains.

The bad news: Dr Rod Rietze's research group at the Queensland Brain Institute in Brisbane has found that the neural stem cells that renew high-maintenance regions of the brain through life are much rarer than originally thought, and their activity declines steeply with age.

The good news: Rietze and his colleagues believe that recent research showing that exercise stimulates neurogenesis in mice – an effect that almost certainly involves activation of neural stem cells – raises the exciting possibility that exercise, or drug therapy, can also make ageing human brains young again.

The new QBI findings trace to the momentous 1992 discovery by a Canadian research team that the mouse brain contains a self-renewing population of neural stem cells.

In 1887, the great Spanish neuro-anatomist and Nobel laureate Ramon y Cajal proposed, that, unlike other cells, neurons cannot regenerate, and are not replaced when they die. US researchers knew by 1990 that Cajal's conjecture was wrong, but it was a young Canadian PhD, Brent Reynolds, who discovered the elusive precursors of new neurons, in the lining of the fluid-filled ventricles towards the centre of the brain.

Isolated and grown in culture, the undif-

ferentiated cells divided and formed small ball-like clusters, or neurospheres.

The discovery yielded the neurosphere assay (NSA), which soon became standard in neural stem cell research laboratories around the world. It was crucial to researchers' ability to isolate neural stem cells and study their activity.

However, after Reynolds joined his friend and compatriot Rietze's lab at the Queensland Brain Institute in 2004, they discovered that not all neurosphere-forming cells are stem cells.

In fact, only as few as five in 100 neurosphere-forming cells are true stem cells,

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