Magic bullet theory

CUTTING-EDGE STEM CELL RESEARCH BY AUSTRALIAN HAEMATOLOGISTS COULD HAVE FAR-REACHING IMPLICATIONS FOR A WIDE RANGE OF DEBILITATING DISEASES. BIANCA NOGRADY REPORTS.

You're mowing the lawn on a fine Sunday morning, when suddenly an invisible band wraps around your chest, squeezing tightly and painfully until you can hardly breathe. This is what patients with angina pectoris fear: the 'elephant sitting on my chest' sensation that strikes with little warning and lasts for up to 20 minutes.

Like most pain, angina is the body's warning that something is wrong. The arteries that nourish the heart muscle with oxygen-rich blood are being slowly strangled from the inside, as fatty plaques build up and constrict the blood flow.

The lack of oxygen, or ischaemia, activates pain fibres in the heart to make the body slow down and reduce the crippled heart's oxygen requirements to a manageable level.

In 2001, more than 260,000 Australians reported experiencing angina, according to a National Institutes of Health survey.

For most patients, the symptoms are relieved by medication. Surgery can open the narrowed arteries – or in more severe cases, replace the blocked section entirely. However, for a small number of patients, even the best treatments that medicine has to offer are not enough to relieve their pain and discomfort.

These are the 'no option' patients, who have tried every available medical treatment short of a total heart transplant. And they're the patients haematologist Professor David Ma and his colleagues at St Vincent's Hospital and the Victor Chang Cardiac Research Institute in Sydney are trying to help, using the 21st century's 'magic bullet' – stem cells.

Exciting possibilities

Stem cells are cells that have yet to develop into their final cell type, whether that be a skin cell, heart muscle cell, an insulin-producing cell, or any hundreds of other cell types. This makes them extremely valuable in medicine, because in theory, stem cells can be used to regenerate a huge range of damaged tissues.

Professor Ma's current research with stem cells and angina began around five years ago, when evidence began to emerge about the potential uses of adult-derived stem cells – in particular, stem cells derived from adult bone marrow.

This attracted the attention of researchers at the Victor Chang Cardiac Research Institute, who then initiated a collaborative study with researchers at St Vincent's Hospital, including Professor Ma.

"The idea of this whole study is that stem cells will open up more vessels by developing new vessels, to improve oxygen transport and blood to the heart and then improve the heart muscle's function," says Professor Ma, Professor of Haematology at the University of New South Wales and St Vincent's Hospital in Sydney.

It's a straightforward aim but a complex process, because bone-marrow derived stem cells are not easy to get to.

Patients are first given a hormone called granulocyte-colony stimulating factor (GCSF, see box page 9) that stimulates the bone marrow to produce stem cells, then pushes the stem cells out of the bone marrow and into the blood. In the first part of the study, 20 patients were simply given the GCSF to investigate the safety of the treatment. The second stage of the study involved giving patients GCSF, then extracting stem cells from their blood and reinjecting them into the coronary arteries to see what effect this would have on the health of their damaged hearts.

To collect the cells, doctors put patients' blood through a centrifugation machine that spins the blood to separate out the various cell types. This process generates around 200 mL of white blood cells, including the stem cells, which are

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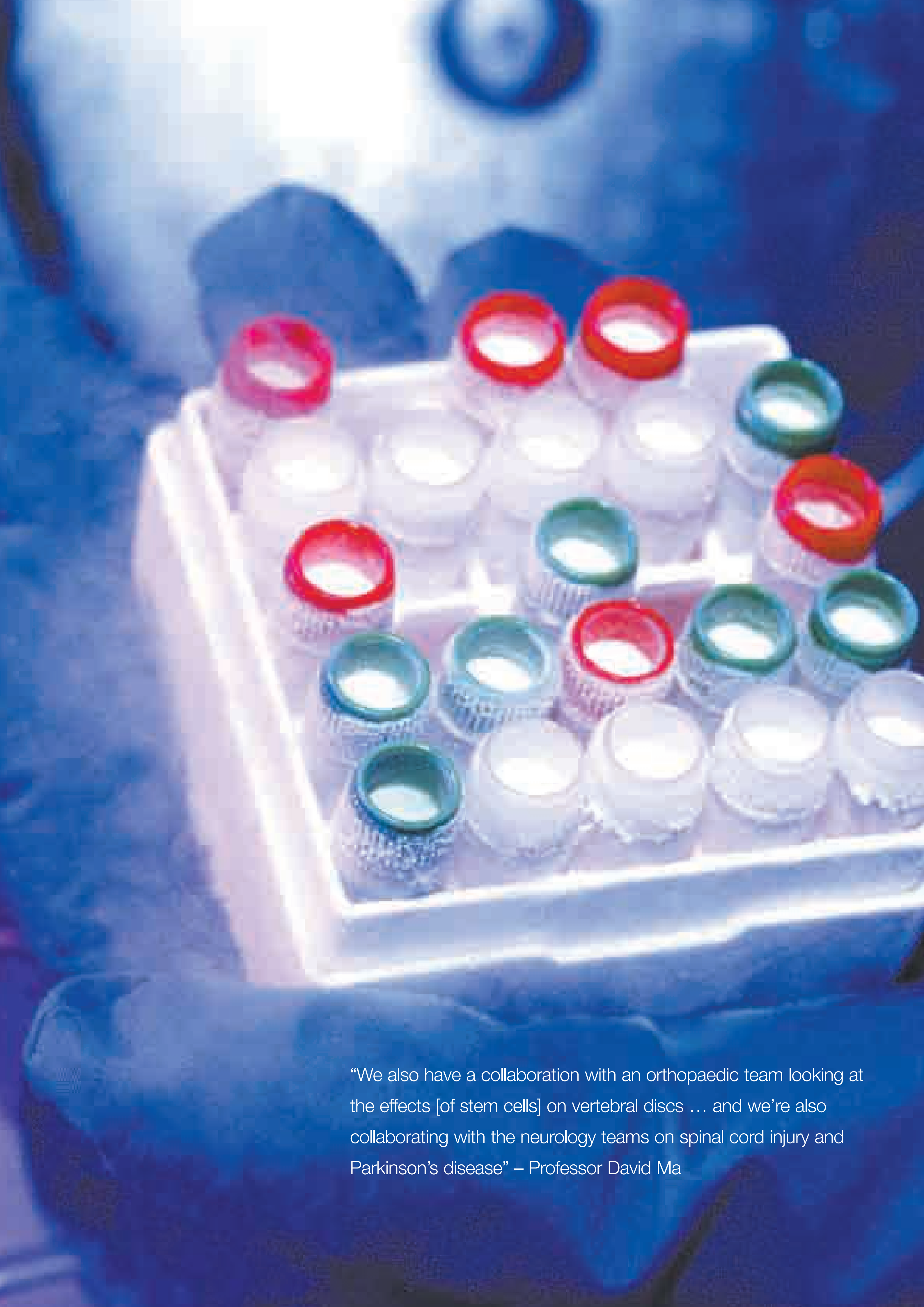

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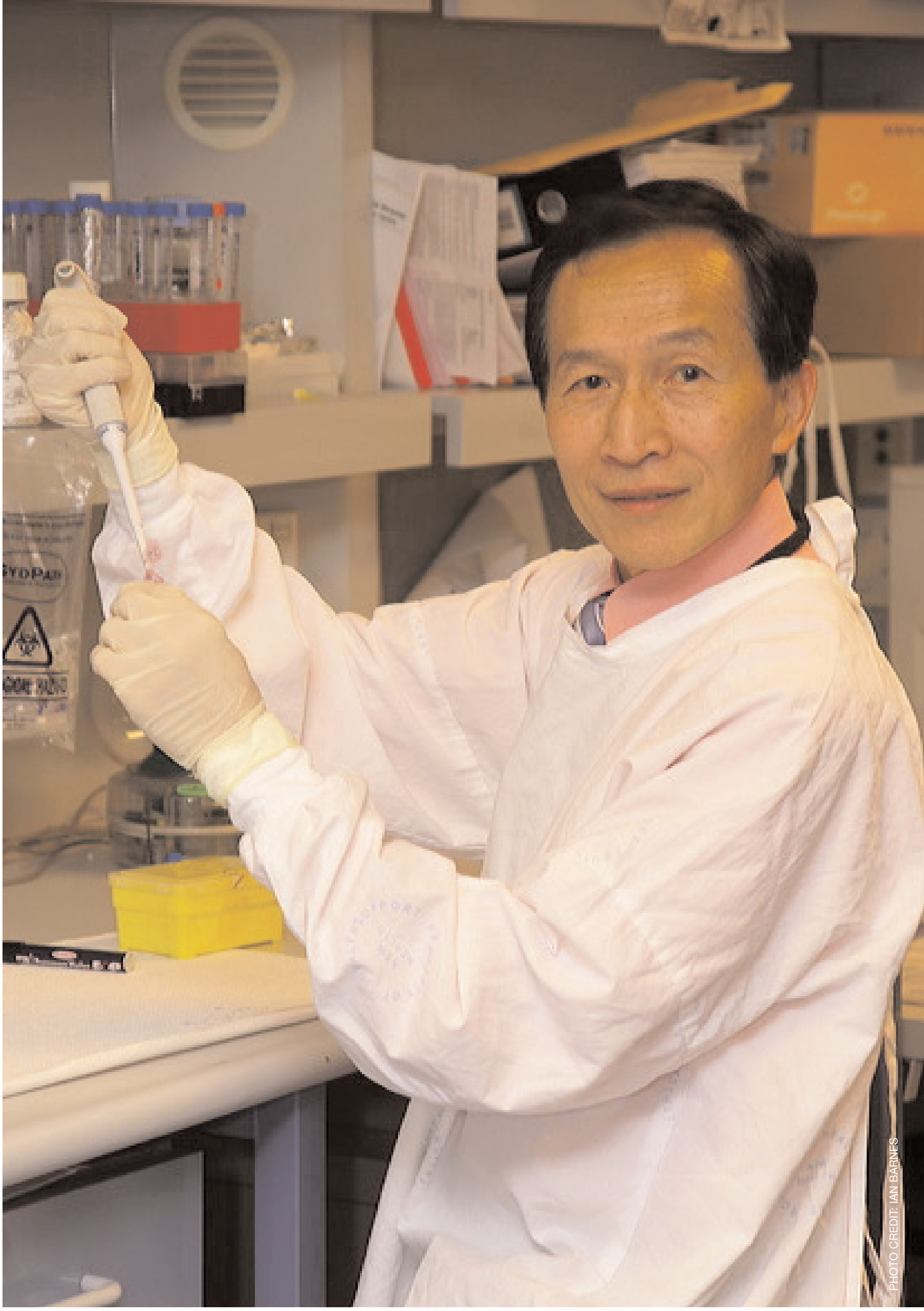

then put through another separation process to extract approximately 10 mL of stem cells.

Finally, this highly concentrated sample of stem cells is injected back into the patient's coronary artery, with the hope that the stem cells will migrate into the smaller capillaries and the heart muscle, creating new vessels and regenerating heart muscle tissue damaged by lack of oxygen. However, doctors still had to ensure the stem cells migrated to where they were needed most.

"This study is quite unique because we also make the patient do some exercise as well before we actually give the cells back," Professor Ma says.

"The reason is that we want to induce a controlled type of stress to the heart, so that allows the heart to secrete the right type of cytokines [chemical messengers] to attract the stem cells to stay in the right area."

This study, the results of which will be presented early next year at a cardiology meeting in the United States and which have been submitted for publication, was intended mainly as a safety study of the GCSF and the stem cell reinjection process. The researchers were also hoping to get some inkling of whether the treatment would be successful. The results were positive.

"Some patients noticed their medication [requirements] dropped significantly – almost a 10-fold reduction," Professor Ma says.

The study also recorded statistically significant improvements in measures such as chest pain, quality of life and also exercise stress test performance.

But then came the twist.

Hearty findings

"We found that just giving GCSF alone would achieve as much benefit as with injection of the stem cells in the coronary vessels," Professor Ma explains.

"That was a little bit unexpected."

At the time the study was launched, there were very little data about the effects of GCSF in humans in this situation, but since then, several other small-scale studies have discovered a similar pattern of GCSF benefit.

So how is GCSF alone able to achieve the same benefit as the stem cell therapy? Cardiologist Professor Robert Graham, Executive Director of the Victor Chang Cardiac Research Institute, says GCSF does more than just get stem cells moving.

"During the time we did the first trial, what we came to know from other people's studies was that GCSF, in addition to mobilising stem cells from bone marrow, appears to directly activate GCSF receptors on heart muscle cells and protect against the effects of ischaemia," Professor Graham says.

"This activates a survival pathway and allows the cells to be more tolerant of a lack of blood supply than before."

So the heart muscle cells that previously were dying because of a lack of oxygen were now able to survive, even in their oxygen-poor situation.

The stem cells mobilised by GCSF also appear to be getting to the right place in the heart without needing to be directly injected into the area.

It may be that the oxygen-starved heart muscle is sending out strong enough chemical signals to attract and trap the stem cells.

It's an exciting finding, because GCSF is already widely used, particularly by haematologists. As well as mobilising stem cells from the bone marrow into circulation, GCSF increases production and mobilisation of white blood cells, which makes it a valuable treatment for patients whose white blood cells have been depleted by chemotherapy or disease.

GCSF is a relatively safe agent, with patients in the study experiencing no serious adverse events, despite concerns about the possible effects of mobilising white blood cells on heart function.

Upping the ante

As with all exciting and unexpected scientific findings, this one needs to be confirmed, so Professor Ma and his colleagues are now conducting a second trial to further investigate their discovery.

"So the emphasis has now shifted," he says.

"In the first study, we were actually looking at injecting the stem cells; in the second study we're saying that using GCSF, we will probably be able to mobilise just as much stem cells in the patient without having to inject it."

The second study will also be larger, involving 40 patients instead of the 20 in the first trial, and unlike the first trial, this one has a control arm.

"We have built in a placebo arm and we use the subjects as their own control," Professor Ma says.

This means the trial will have two stages – in the first stage, one group of patients will be given GCSF and the other group will be given saline fluid as a placebo. Then, after the results of that stage are recorded, all patients go through a 'washout' period to allow the effects of the treatments to pass. The two groups are then swapped over. Those who received the GCSF in the first stage will be given saline, and those given saline in the first stage will be given the GCSF.

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GCSF: an Australian pathology success story

Granulocyte-colony stimulating factor (GCSF), which Professor Ma and colleagues are using as a central plank in their work, was first cloned by a team led by RCPA Distinguished Fellow Professor Donald Metcalf.

Professor Metcalf is one of the world's pre-eminent biomedical experts, having headed the Cancer Research Unit at the Walter and Eliza Hall Institute of Medical Research in Melbourne and having been at the forefront of experimental haematology since the 1960s.

His work on the colony-stimulating factors GCSF, CSF/IL-3 and GMCSF has led to understanding of how our immune system's white blood cells get ready to fight disease, and spawned a cancer treatment that has saved millions of lives.

The results of his work are now being used successfully in clinics around the world, helping address the need to stimulate white cell production in the very young and old, and in at-risk patients with inadequate host-defence systems.

They have also given us valuable information about what goes wrong in leukaemias and lymphomas.

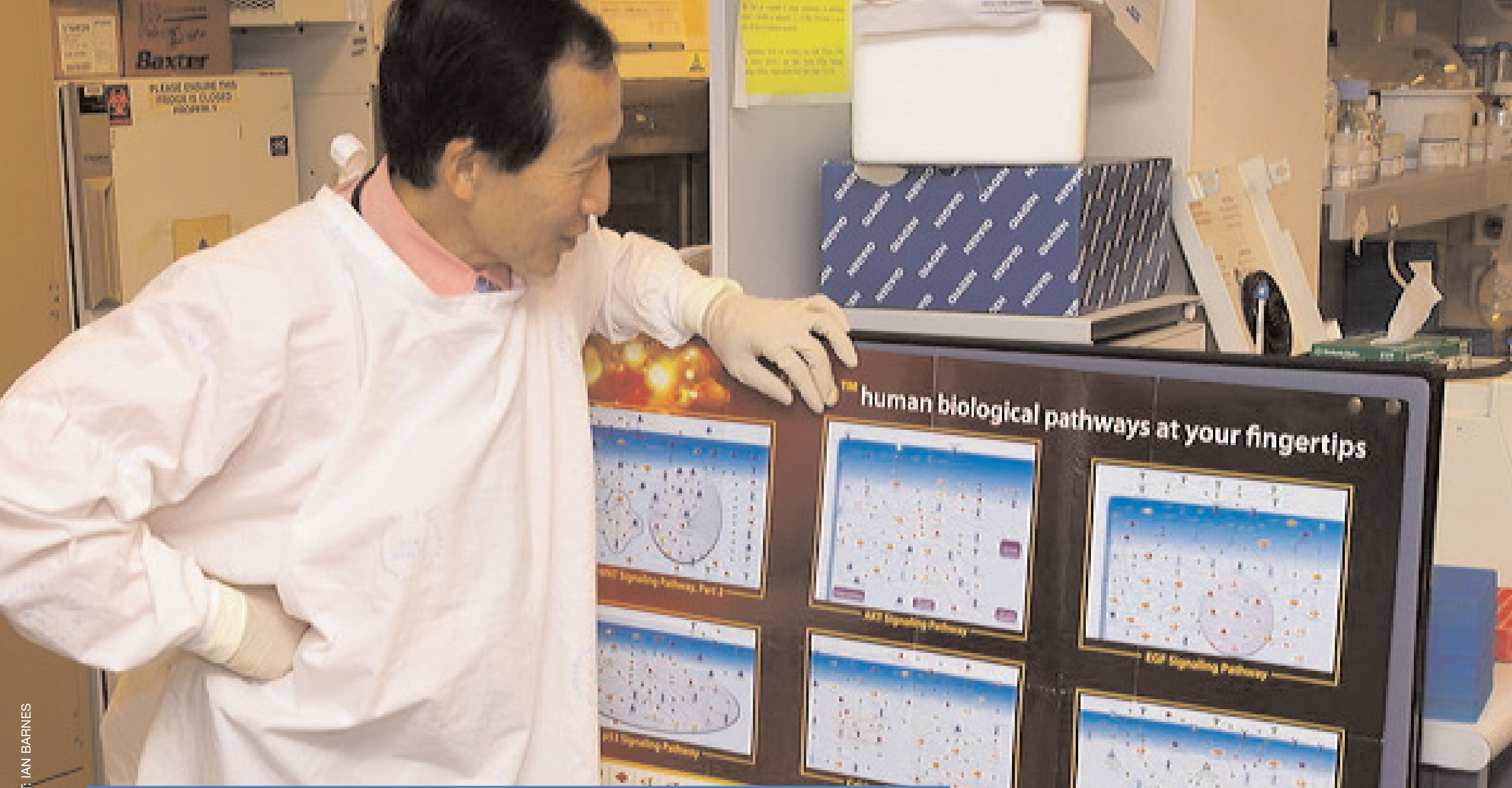

PHOTO CREDIT: IAN BARNES

Pathologists at the cutting edge

Australian pathologists are very much at the forefront when it comes to stem cells, both in the research field and the clinical field, according to haematologist Professor John Rasko, Director of Cell and Molecular Therapies at the Sydney Cancer Centre, Royal Prince Alfred Hospital.

"They span the gamut from basic research all the way to clinical trials involving stem cells, and I mean that in the rainbow of colours for all different stem cells," Professor Rasko says.

While embryonic stem cell research is still mired in controversy, there are few such problems in the field of adult stem cells.

"There has been such an explosion of excitement internationally and within Australia in terms of the relatively recent realisation that there are stem cells in many organs of the body that were previously unrecognised, and a number of these are approaching reality in terms of clinical applications," Professor Rasko says.

For example, haematologists play a life-saving role in delivering bone marrow stem cell therapies to patients with blood cancers and some inherited conditions. Pathologists are also the key figures in the well-established field of pre-implantation diagnosis of genetic disease, in the context of in vitro fertilisation.

Less established but equally exciting are mesenchymal stem cells – stem cells that can differentiate into anything from fat cells to bone cells. These have shown great promise in treating degenerative joint diseases, as well as graft versus host disease – a devastating disease that results when a bone marrow transplant attacks the recipient's body. Several pathologists in Australia are researching the potential of mesenchymal stem cells.

Pathologists are not only involved in the bench-to-bedside aspect of stem cells, but are also helping to shape policy and practice for the collection, handling, transport and manipulation of stem cells, Professor Rasko says. In Australia, pathologists have spearheaded an initiative to establish specially designed labs to handle stem cells.

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The doctors administering the treatments and recording the results are blinded to the treatment type, so they won't know which treatment their patients are receiving. Only at the end of the trial will the results be 'unblinded' to decipher the effect.

The trial, and his work as a haematologist, has provided Professor Ma with the opportunity to be involved 'from bench to bedside'.

"Although I'm a practising haematologist, I do devote significant effort into basic and translational research," he says.

"The unique aspect of haematology is that we do have contact with the patient as well."

Professor Ma says he was attracted to pathology because it covered such a broad scientific foundation.

"Pathology provides the scientific platform for understanding the cause and processes of human diseases.

"In my view, the training to become a pathologist is unique among medical specialties as it provides a sound scientific foundation for laboratory-based translational research."

Professor Ma began his pathology career as a specialist in haematology at Sydney's St Vincent's Hospital – a field he chose because it allowed him to be directly involved in not only identifying the cause of disease, but also care of the patient.

He then spent several years in various overseas institutions exploring the rapidly growing field of stem cell research – an area he had always been attracted to.

"I would say stem cell research is the focus of this century – it's a huge area," Professor Ma says.

He is now in the process of setting up a quality assurance program under the auspices of the RCPA to standardise measurement of haemopoietic stem cells – stem cells that give rise to the various types of cells in the blood, including white blood cells.

"The aim is to make sure that measurement of blood stem cells is well standardised because the number of stem cells in the blood is so small that the error is quite high."

Stem cells not only have benefits for patients – they are also having the bonus effect of bringing together a range of different medical disciplines, Professor Ma says.

"We have really broadened our research collaboration, which is also exciting because it allows networking and cross-fertilisation of information and knowledge."

For example, this angina study involves the haematology and cardiology departments of St Vincent's Hospital, as well as cardiologists from the Victor Chang Cardiac Research Institute and a radiologist to monitor the effects of the treatment using magnetic resonance imaging.

"We also have a collaboration with an orthopaedic team looking at the effects [of stem cells] on vertebral discs … and we're also collaborating with the neurology teams on spinal cord injury and Parkinson's disease," Professor Ma says.

Astonishing potential

Even within the study currently being conducted, there is also the possibility that the GCSF treatment may have benefits for patients with other conditions beyond angina. For example, patients with peripheral vascular disease are at risk of limb damage and loss due to poor circulation in their extremities. In theory, GCSF could benefit these patients by encouraging the growth of new blood vessels and improving the state of cells in oxygen-deprived tissues.

A similar principle may apply in patients with stroke and possibly even heart failure, Professor Graham says.

"Even people with cardiomyopathies, which are not traditionally due to lack of blood supply, may benefit because there is an ischaemic component to those as well," he says.

And finally, research with stem cells is also leading to the development of new techniques and technologies that are likely to have wide-ranging applications.

For example, the technique used in the first study to separate the stem cells from the rest of the white blood cell population is very new, and at the time of the study, was only being used in a few centres around the world for research, not clinical purposes.

The study is also breaking new ground in developing a protocol for using GCSF in a cardiology setting rather than the haematology setting, according to Professor Graham.

So far, GCSF has been largely used by haematologists in larger doses, with the aim of significantly increasing the number of white blood cells, he says.

However, in the cardiology setting, a careful balance has to be struck between the number of stem cells and the number of white cells.

"You will increase the viscosity of the blood by increasing the number of white blood cells, and an early concern was that this may induce an infarct by causing sludging of cells in the blood vessel," Professor Graham says.

Instead, the researchers used a lower dose of GCSF over a longer period of time.

But in the end, those who stand to gain the most from this research are the patients whose angina has made their life a misery, and this is foremost in the mind of researchers like Professor Ma.

"Hopefully, we get something that will help patients – that's the ultimate drive."

GPs NOTE: This article is available for patients at http://pathway.rcpa.edu.au