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HUDSON NEWS

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HUDS ON INSTITUTE OF MEDICAL RESEARCH

Director's Message

All medical research scientists go into the laboratory each day in the hope of making the discoveries that will change, improve and ultimately save lives by easing the burden of disease.

The path for taking a discovery out of the laboratory and into a patient treatment is long, winding and complex – but ultimately rewarding.

The uncertain and competitive nature of medical research funding in Australia means that some potentially life-changing discoveries never reach patients. This is due to a lack of funds for scientists to undertake the crucial work needed to refine the data for commercial use or transition to the clinic.

The foresight of philanthropists, whether individuals or organisations, is vital to ensuring that scientific discoveries are translated to medical advances.

Supporting medical research

As the inaugural year of the Fielding Foundation's five-year program at Hudson Institute draws to a close, we are celebrating the foresight of Melbourne businessman and philanthropist, Mr Peter Fielding.

Thanks to his financial support in establishing a Fielding Fellowship and Fielding Innovation Award, two of our brightest young scientists are taking their ground-breaking discoveries beyond the laboratory and into the clinic, providing the real potential to benefit millions of people.

The Evans Family Foundation is another fantastic example of what the foresight of philanthropy can achieve. Established in memory of the late Ron Evans AM, who passed away from bowel cancer in 2007, the Evans Family Foundation's support of a cancer research fellowship enables Hudson Institute to redouble its efforts in enabling early detection and more effective therapies for patients with bowel cancer.

Both The Fielding Foundation and the Evans Family Foundation are shining examples of what can be achieved when scientists are supported to translate their discoveries to the clinic. I am extremely grateful to Mr Fielding and the Evans family for supporting our talented researchers. I hope you will enjoy reading about what their generous support has helped to achieve.

Advancing lifesaving research

Our work in cancer is being taken to the next level with the recent arrival of Dr Sefi Rosenbluh from Boston, USA to take up the new position of Head of the Centre for Functional Genomics. Dr Rosenbluh brings unique skills that will support our scientists to translate and effectively utilise the vast wealth of data produced by genome sequencing to solve some of the greatest medical challenges of our time.

Researchers in our Centre for Cancer Research and their clinical colleagues are also working to establish a service so that children with the most common form of childhood solid brain tumour, medulloblastoma, will no longer need to wait for up to three months to receive a prognosis for the disease. We are immensely grateful to the Children's Cancer Foundation. Australian Lions Childhood Cancer **Research Foundation**, Robert Connor Dawes Foundation and Bailey's Day for supporting this project. You can read more about this exciting work on page 6.

Working alongside their cancer colleagues, scientists in our Centre for Innate Immunity and Infectious



Disease scientists have made significant breakthroughs in the early diagnosis and targeted treatment of two deadly diseases – lung cancer and emphysema – that claim the lives of thousands of Australians each year. You can read about the work of the team led by Professor Brendan Jenkins on pages 8-9.

The positive intention of making a difference lies within the spirit of philanthropy. Donations, whether large or small, are key to ensuring we continue to take strides towards innovative approaches to complex diseases, tackling major health problems and saving lives.

By supporting Hudson Institute research you can be confident that your support directly reaches scientists who are dedicated to improving outcomes for human health.

We are truly grateful for your ongoing support, and we hope that you enjoy reading about the successes that you have helped to create.

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Professor Bryan Williams Director, Hudson Institute of Medical Research



Reigniting bladder cancer fighting gene

Hudson Institute researchers have discovered a way to reactivate a missing bladder cancer fighting gene, raising hope for the first new treatment for one of the most common forms of the disease in 30 years.

CEO and Director, Professor Bryan Williams and PhD student, Dr Dhanya Sooraj found that an experimental drug, pracinostat, currently in cancer clinical trials, switches on the cancersuppressing gene ATF3, which has been found to disappear in patients with advanced bladder cancer.

The study, published in the prestigious journal *Molecular Cancer Therapeutics*, showed that when the ATF3 gene is reactivated, tumours shrink and the diseased cells return to the normal state.

With pracinostat already in clinical trials for leukaemia, Professor Williams said this discovery could be a major breakthrough for the 2800 Australians diagnosed with bladder cancer each year.

"We know that as bladder cancer progresses, ATF3 switches off in patients and current treatment options, surgery and chemotherapy, offer limited benefits and a considerable reduction in the quality of life," Professor Williams said.

"Pracinostat could potentially be used to increase a patient's response to chemotherapy, by reducing the dose of Each year more than 2800 Australians are diagnosed with bladder cancer, the most common cancer of the urinary tract. It is the fourth most common cancer among men and the ninth most common cancer among women.

Because of high recurrence rates and continuing invasive monitoring, bladder cancer also has the highest lifetime treatment costs per patient of all cancers.

chemotherapy needed and ultimately improving survival rates. If we can restore the normal functioning of ATF3, current treatments will be more effective.

"Monitoring the level of ATF3 may also provide a more accurate way to determine how well patients will respond to chemotherapy," he said.

The team

The team that worked on the bladder cancer study included: Dr Dhanya Sooraj, Dr Dakang Xu, Dr Jason Cain, Dr Dan Gold (MEI Pharma) and Professor Bryan Williams.



Investment changes the future of health

Melbourne philanthropist, Mr Peter Fielding, recognised the funding gaps facing Australia's promising young scientists, and decided to make an investment that is changing the future of science and healthcare in Australia.



In 2014, Mr Fielding, Chairman of the Fielding Foundation, announced a pioneering \$1 million donation to establish a five-year program at Hudson Institute, designed to foster our emerging research talent.

The program is two-fold:

- The Fielding Foundation Fellowship supports Hudson Institute's most promising young medical researchers when they are most at risk of leaving research in Australia or altogether.
- The Fielding Foundation Innovation Award supports a Hudson research programme with a commercially viable discovery, to help take the work out of the laboratory to commercial reality and to benefit patients.

The inaugural recipient of the Fielding Foundation Fellowship was Dr Rebecca

Lim, and Associate Professor Marcel Nold was the inaugural recipient of the Fielding Foundation Innovation Award.

Both scientists have significantly progressed their ground-breaking

"It's safe to say that the Fielding Foundation's investment has more than paid off; it is totally gratifying to learn that Associate Professor

Nold's work is now supported by one of the world's leading pharmaceutical companies."

research, so the community will benefit from their important discoveries.

"The Fielding Foundation's investment at Hudson Institute is in health

innovation and fostering promising scientific talent in Australia, in addition to providing an exciting opportunity to play a practical role in tackling health challenges," said Fielding Foundation Executive Chairman, Mr Peter Fielding.

"It's safe to say that the Fielding Foundation's investment has more than paid off; it is totally gratifying to learn that Associate Professor Nold's work is now supported by one of the world's leading pharmaceutical companies. It's incredibly rewarding to play a small practical role in tackling the challenge of taking medical research to commercial reality, for the benefit of ours and future generations.

"Hudson Institute is overflowing with extraordinary talent. The results have far exceeded my expectations in making a difference on the frontline of medical science," he said.

2015 Fielding Foundation recipients and their pioneering achievements

2015 Fielding Foundation Fellow: Dr Rebecca Lim

The Fielding Foundation Fellowship supports Hudson Institute's most promising and brightest young scientist for one year.

In the past year, the inaugural recipient of the Fielding Foundation Fellowship, Dr Rebecca Lim, has significantly progressed her amnion cell research into safety trials for lung disease in premature babies.

Dr Lim's team has been working on amnion cells, their regenerative properties and the potential to repair damaged tissue, for 10 years.

Amnion epithelial cells are stem-like cells taken from part of the placenta, which is the baby's oxygen supply during pregnancy, but normally discarded after birth.

The cells work by attaching themselves to the damaged parts of a baby's lungs and kick-starting the repair process.

In 2015, Dr Lim worked with neonatologist Dr Atul Malhotra and Professor Euan Wallace to establish a safety trial of this ground-breaking therapy at Monash Newborn.

To date, two prematurely born babies with lung disease have been treated with amnion cells as part of the safety trial, with no adverse effects from the cells.

"Long-term, we are looking to develop a treatment for premature babies with chronic lung diseases like bronchopulmonary dysplasia that could be rolled out in hospitals around the world," Dr Lim said.

"If we can intervene in the days after their birth to successfully 'rebuild' these babies' lungs using the cells, we could increase survival rates and halt long-term complications."

Dr Lim is also working with a multidisciplinary team to commence a clinical trial at Monash Health using amnion cells to treat patients with end-stage liver cirrhosis, who have no other treatment option.

"My experience working with these cells is that they seem to work on



very different disease models," she said.

The next frontier of cell-based regenerative medicine

Dr Lim was one of a handful of researchers chosen from around the world to present her work to venture capitalists at Stanford University's BIO SPARK Showcase in San Francisco in October.

Back in 2012, Dr Lim noticed that the actions of the amnion cells were being carried out by their exosomes – nanosized vesicles that are released by the cells.

"I didn't expect, four years after our small five-person team made this discovery in the lab, that it would be interesting major investors," Dr Lim said.

Despite tremendous activity and investment, stem cell therapies have been slow to move to patients, due to high costs associated with manufacture, storage and delivery.

This could all be set to change. Exosomes can be produced on a large scale for a fraction of the cost of stem cells – meaning Dr Lim's work could challenge the future direction of regenerative medicine.

"We hope that our discovery will make regenerative medicine accessible to everyone, regardless of where they live," Dr Lim said.

2015 Fielding Foundation Innovation Award: Associate Professor Marcel Nold

The Fielding Foundation Innovation Award supports the commercialisation of medical research, to help ensure discoveries are translated from the laboratory into patient treatments.

The recipient of the inaugural Fielding Foundation Innovation Award, Associate Professor Marcel Nold, has significantly progressed the commercial potential of his team's research to help develop his work on small proteins called cytokines, their signalling and role in suppressing inflammation.

"The Fielding Foundation Innovation Award has greatly helped us to get an extensive amount of work done, to take the findings through to a commercial stage of developing drugs for people suffering dangerous and debilitating inflammation," Associate Professor Nold said.

"We have been able to take the research to the next level using advanced technologies and, crucially, to attract industry funding that will translate the powerful functions of this small protein into new drugs used to control or unleash the immune system.

"The discovery has wide-reaching implications on treating diseases such as stroke and heart attack, as well as autoimmune diseases such as lupus. It is because of The Fielding Foundation Innovation Award that we are hopeful our community will see the benefits of the discovery in advanced healthcare."





Australian first – crucial service to help children with brain tumours

Children with the most common type of brain cancer could be spared unnecessary radiation and chemotherapy thanks to a breakthrough new program at Hudson Institute.



Our researchers are establishing a pilot for an Australian-first service that will significantly reduce the time it takes for children with the most common type of solid brain tumour (medulloblastoma) to be diagnosed. The faster service will help doctors formulate the best treatment with the least side effects for children.

- Medulloblastoma represents 20 per cent of all childhood brain cancers.
- There are four subgroups of medulloblastoma, each with different causes and requiring different treatments.

Currently, tumour samples from Australia are sent overseas for analysis, a process that can take up to two months. The new collaborative service between Hudson Institute, Monash Health and the Monash Children's Hospital Cancer Centre, will provide the analysis and a diagnosis within three weeks.

"We are excited to be the first Australian laboratory to provide a validated local service, according to international standards," said Dr Jason Cain, Research Group Head, Developmental and Cancer Biology at Hudson Institute.

"Our service will enable doctors to provide a prognosis more quickly, and to tailor cancer treatments to fit the tumour profile with the best result for the child."

> This collaborative project is generously funded by the Australian Lions Childhood Cancer Research Foundation, Children's Cancer Foundation, Robert Connor Dawes Foundation and Bailey's Day.

New level of personalised treatment

There are four subtypes of medulloblastoma and all are treated the same, with chemotherapy drugs and radiation.

While the survival rate is around 80 per cent for average risk patients, a 'one size fits all' treatment means children can suffer permanent debilitating side effects, like intellectual disability and issues with growth, speech and hearing loss. The new local service means our researchers will also be able to provide a more in-depth diagnosis, uncovering genetic differences in tumour groups in the hope of developing personalised therapies for the children.

"It's become clear over the last five years that medulloblastoma is not a single disease, but comprised of at least these four distinct subgroups, each with different causes and prognosis," Dr Cain said.

"There is a low-risk group that doesn't need the same amount of chemotherapy or radiation.

"There are also targeted therapies in clinical trials, and they show some promising results, but are only effective in one high-risk subgroup. This is crucial information for these patients."

Taking it to the next level

Emerging research suggests there are variations within the tumour subgroups that require further subclassification. The team will analyse tumour samples stored in archived tissue banks across Australia.

"Identifying key methylation differences will enable our team to catalogue medulloblastoma treatments to each tumour subgroup, minimising unnecessary toxic treatments and life-long side effects," Dr Cain said.

The team believes methylation array technology is also key to analysing other types of solid brain tumours, and may become the 'gold standard' across all extracranial solid tumours.



Personalised treatment – the new medicine frontier

Since scientists first sequenced the entire human genome during the ambitious Human Genome Project in 2001, the technique has become far more accessible to scientists and clinicians. Crucially, patients will benefit with a real understanding of how disease is affecting them and tailored treatments.



The first draft of the human genome sequence took 15 months to complete, at an estimated cost of US\$300 million in 2001. Today, scientists can sequence a human genome in a day for around \$1000, opening up the accessibility of this technology to inform patient diagnoses, treatment and care.

Scientists can now examine the entire genome to pinpoint the cause of some diseases down to a single gene mutation amongst three billion base pairs of DNA, then develop tailored drug treatments to fit the patient's genetic profile – an approach known as precision medicine.

Without a doubt, the cracking of this genetic code paved the path for significant advances in medical research and treatment. With this vast amount of genetic data at their fingertips, the question now for scientists is: how can this information be best used to inform more effective treatment for disease?

Functional genomics, including CRISPR screening, is widely considered to be the next frontier in precision medicine.

CRISPR loss of function screening is a powerful gene-editing technique that enables scientists to pinpoint the gene underlying disease and snip or 'edit' the gene responsible for causing it.

"If a sequenced genome is a library, CRISPR screening provides researchers with the tools to find any book, open it up to a specific page and edit the text," Dr Rosenbluh said.

Leading precision medicine and cancer treatment

Dr Rosenbluh has been appointed head of the newly established Centre for Functional Genomics at Hudson Institute. He arrives from the Dana-Farber Cancer Institute and the Broad Institute of Harvard and Massachusetts Institute of Technology in the United States.

Dr Rosenbluh brings expertise that will enable researchers to examine every gene in the genome and how it relates to a specific disease.

"Precision medicine is a more efficient way of treating cancer. The basic idea is to first identify genetic mutations causing cancer. Functional Genomics allow us to pinpoint these genes and find new approaches to treating complex diseases such as cancers," he said.

Dr Rosenbluh said

Hudson Institute's unique multidisciplinary environment within the Monash Health Translation Precinct was a major drawcard.

"The combination of highly trained physicians and a collaborative and enthusiastic scientific community is key for succeeding in these challenging goals," Dr Rosenbluh said.

"I truly believe that the special environment and opportunities at Hudson Institute, Monash University and Monash Health will enable many of the applications and strategies I envision to progress healthcare." Centre for Innate Immunity & Infectious Diseases

Double blow for lung cancer and emphysema offers hope to patients

Lung cancer and emphysema could be diagnosed earlier and targeted with an existing drug, vastly improving patient outcomes, researchers at Hudson Institute of Medical Research have discovered.

A team led by Professor Brendan Jenkins discovered that the drug, sgp130Fc, has the ability to halt the progression of lung cancer and emphysema. Combined these lung diseases claim the lives of 15,000 Australians each year.

The team also identified a unique signature that could be used to detect both diseases much earlier through a simple blood test. Both diseases are difficult to detect early; for instance, emphysema can currently only be diagnosed in later stages by a CT scan, which means many patients present late when treatment options are limited.

"Early detection is crucial to effective management and treatment of both diseases. A blood test has the potential to vastly improve survival rates through earlier diagnosis while sgp130Fc has the ability to halt the progression of lung cancer and emphysema," said Professor Jenkins.

The study on emphysema, a culmination of six years' work for the team, has been published in the prestigious American Journal of Respiratory and Critical Care Medicine.

It follows on from the teams' related study on lung cancer, published in the top-ranked *Cancer Research* journal earlier this year.

- Lung cancer is the most common cancer in the world, and the leading cause of cancer in Australia.
- Emphysema is the major debilitating component of the lethal chronic obstructive pulmonary disease (COPD), which is predicted by the World Health Organisation to be the third leading cause of death worldwide by 2020, behind heart disease and cancer.
- Together, lung cancer and emphysema claim around 15,000 lives in Australia each year.
- Patients with emphysema are at an increased risk (between 25 and 30 per cent) of also developing lung cancer.

Stopping deadly disease in its tracks

In both studies, the team identified that an inflammation causing molecule, Interleukin 6 (IL-6), propels both emphysema and lung cancer through a process called trans-signalling.

"Trans-signalling and IL-6 are having very important effects on lung disease - they are master regulators and can both be targeted with sgp130Fc," Professor Jenkins said.

"We were surprised to discover that lung cancer and emphysema are so closely linked when they are such different diseases. Emphysema is characterised by the loss of lung tissue and lung cancer by uncontrolled growth of tissue."

The Hudson team found that receptors which are essential for the IL-6 'transsignalling' process are present in high levels in the blood of both emphysema and lung cancer patients.



An inflammation-focused drug, repurposed

The drug sgp130Fc was developed by Professor Jenkins' German collaborator, Professor Stefan Rose-John from the University of Kiel, to specifically target the process of trans-signalling.

The drug is currently in clinical trials for other diseases which use the mechanism of trans-signalling, including inflammatory bowel disease. The team now believes the drug has clinical potential in lung cancer and emphysema.

Next steps

Professor Jenkins' team is now working with clinical colleagues at Monash Health, led by Professor Philip Bardin, to analyse the blood samples of lung cancer and emphysema patients in the hope of developing an early detection blood test for both diseases.

"By matching blood results to the severity of the disease, we will look

to confirm whether a blood test is an effective earlier test for emphysema and lung cancer," said Professor Bardin.

Professor Jenkins is cautiously optimistic about the drug's potential. Armed with both findings, his team will now also investigate the potential uses for sgp130Fc in the management of lung cancer and emphysema.



"We were surprised to discover that lung cancer and emphysema are so closely linked when they are such different diseases."

"Not every patient with emphysema or lung cancer is a candidate for this drug, but with clinical trials we can identify which patients would respond to treatment and at which stage of the disease. We believe there's a real opportunity here to target and treat patients with these lung diseases."

The team

The team that worked on the emphysema study included: Dr Saleela Ruwanpura, Ms Louise McLeod, Mr Sultan Alhayyani, Dr Michelle Tate, Dr Virginie Deswaerte, Mr Gavin Brooks, Dr Martin MacDonald, Dr Paul King, Professor Philip Bardin and Professor Brendan Jenkins.

The emphysema study is a culmination of six years' work for the team, including first author and NHMRC Biomedical Fellow, Dr Saleela Ruwanpura, and was published in the prestigious American Journal of Respiratory and Critical Care Medicine.

It follows on from a related study on lung cancer published by the team, including first author and PhD student, Gavin Brooks in the top-ranked *Cancer Research* journal.



Creatine – vital breakthrough for baby health

Boosting a pregnant woman's levels of a critical nutrient, creatine, could help prevent women from giving birth to unhealthily small babies and reduce the risk of infant brain injury and death.

One in every 16 babies is born with a low birth weight, which is regarded as weighing less than 2500 grams. In 2013, 6.4 per cent (19,597) of babies born in Australia were born with a low birth weight (Australia's mothers and babies 2013).

In Australia in 2013, seven out of every 1000 babies died either in utero, during labour or shortly after birth.

The discovery by Dr Hayley Dickinson's team in the Ritchie Centre that women with lower levels of creatine in their urine give birth to smaller babies has prompted a world-first study to determine the optimal levels of this naturally occurring molecule in pregnancy.

What is creatine?

Creatine is an amino acid derivative found in all cells in the body, but stored primarily in our muscles and brain. It helps our cells recharge their energy stores.

The human body produces 50 per cent of the creatine it requires from amino acids, while including fish, meat and dairy products in a diet meets the remaining requirements.

The world-first study of 278 pregnant women revealed those with higher

levels of creatine found in their urine were less likely to have an underweight baby.

"We found for the first time that a mother's creatine levels affect the size of her baby at birth," Dr Dickinson said.

"Most babies born small go on to live normal, healthy lives. But some are at increased risk of developmental challenges and a baby who isn't growing well in the womb is one of the leading causes of stillbirth."

Next step

Dr Dickinson's findings, published earlier this year in the British Journal of Obstetrics and Gynaecology, could be the precursor to dietary recommendations around optimum levels of creatine in pregnancy.

The research has now paved the way for a study of 863 pregnant women at Monash Health, which will measure the levels of creatine in blood from the umbilical cord, where nutrients are transferred from a mother to her placenta.

The findings could revolutionise the dietary advice given to pregnant women. More than 20 years ago, it was discovered that high levels of folate can protect babies from neural tube defects, leading doctors to recommend that pregnant women, and women trying to conceive, take folic acid.

"If we can show that women with good healthy levels of creatine have better pregnancy outcomes, then we



might suggest a recommended dietary creatine intake during pregnancy," Dr Dickinson said.

"While creatine may one day become a nutritional supplement for use in pregnancy, pregnant women should not start taking supplements at this time. The safety of creatine supplements during pregnancy has not been tested yet. The important thing for women to concentrate on now is eating a



balanced diet, made up largely of fresh fruit, vegetables, whole grains, and some meat, fish and dairy products," Dr Dickinson said.

Protecting baby

Currently it is not clear whether low maternal creatine is a cause or a consequence of growth restriction.

Preclinical studies have shown that the fetus relies on the placenta to provide creatine until late pregnancy. These preclinical studies by Dr Dickinson's team showed that creatine supplementation dramatically improved birth asphyxia survival rates by protecting vital organs from the harmful impacts of oxygen deprivation.

The Team

The team that worked on the creatine research included: Dr Hayley Dickinson, Dr Miranda Davies-Tuck, Dr Stacey Ellery, Professor Euan Wallace, Professor Rod Snow (Deakin University), Dr Jessica Grieger (University of Adelaide), Associate Professor David Walker and Professor Vicki Clifton (Mater Research).



Buying time: finding a treatment window in a pandemic

Almost 100 years since the Spanish influenza pandemic, in which about 50 million people died worldwide, strategies for stopping the spread of a global pandemic are still very limited. A globalised and highly mobile population means the risk of a deadly influenza outbreak turning into a worldwide pandemic is high.



PANDEMICS

1918-1919 Spanish influenza, at least 50 million died worldwide

1957-1958 Asian influenza, about 1 million deaths

1968-1969 Hong Kong influenza, about 1 million deaths

Global health organisations are currently monitoring the progress of avian H7N9 influenza viruses, which are transmitted to humans through contact with poultry. Around 790 cases have been reported in China since March 2013, with a mortality rate of approximately 40 per cent. The potential of H7N9 to cause a pandemic has authorities worried.

CiiiD infectious diseases expert, Associate Professor Ashley Mansell said it's not a case of 'if' but 'when' a large-scale influenza or viral pandemic will hit again.

"Current strategies for dealing with a pandemic – vaccination, isolation and antiviral medications – are limited. It's vital that researchers develop new strategies for treating patients who present with a fully progressed viral infection, so that we are better prepared," Associate Professor Mansell said.



A study led by Associate Professor Mansell and Dr Michelle Tate, found that blocking the immune response provides a new window of opportunity at the height of infection in which doctors can triage the patient. Normally, once the infection has hit its peak in a patient, antivirals are no longer effective.

Staving off a patient's own immune response at the right stage of infection could literally 'buy time' during a global influenza pandemic.

The team found that blocking one part of this response (called the NLRP3 inflammasome), at the height of infection from pathogenic strains of influenza, delayed the deadly effects for up to 48 hours, potentially giving the body a chance to fight back.

"Giving the body respite from its own overstimulated immune system could offer some protection and provide enough time for the body to hopefully resolve the infection.

"The inflammasome is both 'good cop' and 'bad cop'. It is a critical early response to influenza that helps our body to mature its immune response, clear the virus and protect us from disease," Associate Professor Mansell said.

"When it is overstimulated by a deadly infectious disease, the inflammasome turns 'bad cop' and can unleash a 'cytokine storm' that makes the body burn up with inflammation, leading to organ failure and even death.

"Developing this finding could add to the war chest for fighting a pandemic, where current strategies are highly time-dependent.

"Buying vital time in a pandemic, which is a race against the clock, may allow the immune system to mature and hopefully resolve the infection."

The Team

The team that worked on the study included: Associate Professor Ashley Mansell, Dr Michelle Tate, Dr Jennifer Dowling, Mr James Ong and Professor Matthew Cooper (University of Queensland).