PORTFOLIO COMMITTEE NO. 2 – HEALTH

INQUIRY INTO USE OF PRIMATES AND OTHER ANIMALS IN MEDICAL RESEARCH IN NEW SOUTH WALES

HEARING – 28 JUNE 2022

SUPPLEMENTARY QUESTIONS TO CODEX RESEARCH

ANSWERS BY EDWIN BRACKENREG, CEO, AND DR STEPHANIE HELDER, CSO

1. At the hearing you discussed the lack of funding opportunities for the development of alternatives to animal research in NSW. Is there a risk we will lose researchers and start-ups like yourselves to other countries if there isn't the support in place and funding opportunities?

We certainly are at risk of losing researchers who see the future of medical science research as being in human-relevant models if we don't foster the development and uptake of alternatives to animal research in NSW. We can expect such sentiment to keep gaining traction both within research and society more broadly, as witnessed by an increasing number of calls for the development of non-animal, human-relevant models in the scientific literature (outlined in the following section), as well as the legislative and policy developments in the US and Europe. As for start-ups, it is entirely possible that any good ideas born in Australia may be taken overseas to be developed in places that are more likely to have better infrastructure and financial opportunities supporting their development. This is no baseless prediction; losing promising start-ups to overseas jurisdictions has been an all-too-common occurrence in Australia.

Recent calls for the development of human-relevant models in the scientific literature:

Animal experiments: EU is pushing to find substitutes fast, Hippenstiel et al (2021), Nature Correspondence, Vol 600

The European Parliament is once again pushing to accelerate transition to a research system that does not involve testing on animals, with a timetable proposed to phase out non-essential animal experimentation as soon as possible – but this requires the scientific community to devise alternatives.

Medical regulators: look beyond animal tests, Ritskes-Hoitinga (2022), Nature, Vol 604

Introducing alternative methods could produce better medical products and reduce the time and cost to bring them to market – e.g. COVID-19 vaccines were brought to market in such a short time in part due to reducing the number of animal experiments required (and allowing a portion of them to be conducted after phase I and phase II clinical trials) and promoting the use of data from non-animal methods.

A Call to Action for New Global Approaches to Cardiovascular Disease Drug Solutions, Figtree et al (2021), Circulation 144:159-169

We need to invest in new technological approaches for drug discovery, assessment and implementation – models to study efficacy and toxicity more relevant to human disease are necessary. The cardiovascular field is dominated by therapies that show great promise in preclinical models (especially small animals) that fail to reproduce in human clinical trials because of species differences in hemodynamics, lifespan, lipid metabolism and immune function as well as the fact

that rodents are resistant to the development of atherosclerosis & unstable plaques. It is also very difficult to replicate human cardiovascular disease parameters in animal models including cardiac arrythmias (species differences in cardiac electro-mechanical coupling confound model development), heart failure with preserved ejection fraction and resistant hypertension.

In vitro ***models of neurodegenerative diseases , Slanzi et al (2020), Front. Cell Dev. Biol***

Whilst traditional cell culture and animal models have improved our understanding of human neurodegenerative diseases, models based on recent technological advances in 3D culture systems can achieve better characterisation of pathological mechanisms, also making them suitable for high-throughput drug screening. Thus *in vitro* models could accelerate drug discovery compared with current approaches in animals. However, we are still lacking a 3D model that recapitulates all the key aspects of neurodegenerative diseases – needs development. 3D models based on patient-derived cells will show how the genetic landscape of the human population contributes to the pathogenesis of neurodegenerative diseases.

Academic collaborative models fostering the translation of physiological **in vitro** ***systems from basic research into drug discovery, Silvestri et al (2021), Drug Discovery Today***

The decline in drug success and increase in development costs highlights the need for new, innovative approaches that more accurately resemble human pathophysiology. Patient-derived cellular models are more clinically relevant than cell lines, which will allow the development of disease-relevant and physiological human cellular models which if implemented in early discovery stages could help to identify compound liabilities early and enable a focus on molecules that are less likely to fail because of efficacy or safety concerns, and thereby drastically reduce the money wasted on expensive clinical studies. This paper also outlines several successful European academic and public and private sector initiatives to support growth of technologies for advancing early stage drug discovery e.g. EU-OPENSCREEN.

Screening out irrelevant cell-based models of disease, Horvath et al (2016), A Guide to Drug Discovery.

Funding to support precompetitive, multidiscipinlary collaborations to develop novel preclinical models and cell-based screening technologies could have a key role in improving their clinical relevance and ultimately increase clinical success rates.

Inappropriate modeling of chronic and complex disorders: How to reconsider the approach in the context of predictive, preventative and personalized medicine, and translational medicine, Seifirad and Haghpanah (2019), EPMA Journal, 10:195-209

This article talks about the shortcomings of the drug development process for many disease models. As just one example, animal models of myocardial infarction (MI) generally do not completely mimic the MI process. Although atherosclerosis models are currently available, MI animal models are usually surgically mimicked by the clumping-reperfusion process. Hence MI is mimicked with no previous hypoxia, no hypo-perfusion etcetera, whereas in human disease - acute MI is the final sequence of atherosclerosis as a chronic process. In the case of MI modeling, the erythopoietin paradox certified inappropriate modelling of MI as a complex phenomenon - almost 500 drugs underwent trial for stroke, among them, only aspirin and early recombinant tissue plasminogen activator did not fail. Most animal models do not capture the complex character of human diseases – diabetes, atherosclerosis and dementia are all examples of chronic complex diseases that are

modeled with extremely simplified acute approaches. Finally, they make the case that there might be plenty of agents, potentially safe in humans, that were not studied in people due to observed toxicity in animals. Also there might be plenty of missed potential treatments which did not show efficacy in animals and hence never studied clinically. Therefore, we have to look back and re-evaluate hundreds of potential treatments.

2. Some researchers before this Inquiry have argued the benefits of animal research – but you state in your submission that "it is increasingly clear that animal experiments are less and less relevant". You also highlight there are many diseases we have not been able to find cures for using animal research. Can explain your concerns regarding the limitations of animal research?

Animal research is becoming less and less relevant primarily for two reasons. Firstly, we have already extracted much of the information that animal models can (somewhat) easily give us about human diseases. As the low hanging fruit is picked from decades of extensive animal research, you either have to climb higher on the current tree, or move to another tree. Climbing higher on the animal research tree means that the gains coming from this type of animal research are not obtained as readily as they were in the past, and involve much higher costs. This often means we are on ethically untenable grounds, for example, by being forced to experiment on primates.

We believe that moving to a new tree – that of *in vitro* models of human biology – will formalise new combinations of questions and methods and yield decades of novel insights into health and disease not possible in animal models.

Whilst it is true that animal models are more relevant for some diseases than others, the biggest disease burdens on society currently, such as cardiovascular and neurodegenerative diseases, are poorly recapitulated by animal models.

Cardiovascular disease is a field that is dominated by therapies that show great potential in preclinical models (especially small animals) that fail to reproduce in human clinical trials. Further, our current understanding of individual susceptibility to cardiovascular disease is particularly poor; around 25% of people that present with life threatening events from STEMI heart attacks have no known traditional risk factors. (ST-Elevation myocardial infarction, or STEMI heart attacks, are more serious and have greater risk of serious complications and death).

It is clear that to solve these types of complex problems we will require better, patient-derived and human-specific models. As a result, there are increasing calls for investing in the development of new technological approaches to replace inefficient and ineffective preclinical cardiovascular disease models (***A Call to Action for New Global Approaches to Cardiovascular Disease Drug Solutions, Figtree et al, Circulation (2021) 144:159-169***, ***Patient Endothelial Colony-Forming Cells to Model Coronary Artery Disease Susceptibility and Unravel the Role of Dysregulated Mitochondrial Redox Signalling, Besnier et al (2021), Antioxidant, 10, 1547***).

Neurodegenerative diseases are also poorly recapitulated by animal models because they don't reflect the full component of disrupted genes and therefore don't capture the variations and complexities of the disease phenotypes in humans. Further, homologous cell types between species have been shown to often have highly divergent gene expression profiles – for instance homologous neurons often have different cell receptors. As a result, there have been many drugs that have cured

mice of brain disorders such as Alzheimers, schizophrenia and autism but none of these drugs have worked in humans (***Conserved cell types with divergent features in human versus mouse cortex, Hodge et al (2019) Nature 573(772):61-68***).

Certain treatments, such as monoclonal antibodies and CRISPR RNAs also don't work in animal models at all because they are so specific for human target genetic sequences. Vaccine responses are often not representative in animal models, not even in non-human primates. As a result, *in vitro* technology for viral diagnostics, vaccines, and therapeutics are in active development. For example, The FDA awarded a $5.4 million contract to The University of Liverpool and collaborators in 2020 to, in part, examine how *in vitro* models of coronavirus infection, including organ-on-a-chip technology, compare to *in vivo* responses in animal models and humans (https://www.fda.gov/emergency-preparedness-and-response/mcm-regulatory-science/fda-and-global-partners-analyze-coronavirus-samples).

Moreover, the human microbiome has been a hot topic of research in recent years due to discoveries that it has far-reaching implications for human health and disease. Animals have different microbiomes to humans, but the human microbiome can be cultured with human cells and so we are sure to learn much more about this consequential field by developing sophisticated human-based *in vitro* models.

It is unlikely that we will begin to tackle complex disease by doing more of the same. Our greatest hope in this venture is through developing sophisticated *in vitro* models based on human biology. Apart from being based on human cells, these methods have other potential benefits over animal models. Such *in vitro* systems allow researchers to decouple, and finely control, the effects of experimental parameters such as mechanical motion and fluid flow. With these devices, researchers are also able to add in relevant components one at a time over a long duration experiment.

3. Given this, why do you think some researchers are reluctant to look to new, innovative methods that do not use animals?

A career in research is dependent on publishing scientific papers and getting funding. This structure has the unintended result of often disincentivising real innovation – individual careers are more safely assured by staying close to the established norms of their field and making incremental advances as this maximises their chance of citation and therefore of promotion and funding. Scientific fields are established by a collection of problems and methods, and there is an innate assumption in any one field that these particular problems will best be solved by established methods. It takes a rare breed of researcher to have the werewithal to look outside the establishment. This inertia is only compounded by journals and reviewers often requiring animal models to publish certain results. Everyone in the ecosystem is comfortable with methods that they are familiar with and which have established and formalised research protocols and metrics. Social pressure seems to have little effect on this machinery.

As a result, it will be up to regulatory policies to overcome the inertia in the biomedical research ecosystem. Policy can push funding bodies to fund and expedite this sort of research so that those developing the technology have a market to sell into. Growing sales incentivises ongoing technological improvements, so the technology can reach the required level of fidelity and acceptance for more applications. Policy can influence publishers to find non-animal methods more enticing to publish, and influence researchers to step outside their comfort zone and adopt new, non-

animal research methodologies. In time, well implemented regulatory policies will generate a critical mass of players in the medical science ecosystem routinely using non-animal methods in their research, publishing and grant funding.

4. Can you provide some information about the device that Codex Research is developing as an alternative to animal research? How does it work and what kind of potential does it have?

A porous, biocompatible material is contained within a chamber and cell growth medium is pumped through it. Cells introduced into the system attach to the material and grow into 3D structures. Cell development is influenced by pressure, flow, fluid shear and mechanical stress provided by the system. Multiple cell types can be grown into structures resembling human tissues. Patient-derived cells can be grown under conditions matching patient parameters, providing a unique opportunity to develop methods for personalising medicine.

At Codex Research, we hope to follow the model of the digital computer industry, which successfully commercialised quite simple devices in the 1970s and 80s. By reinvesting revenues into continued technological development the computer industry has produced advances in digital computing that would have seemed like science-fiction forty years ago. We also have a clear pathway for the development of our technology. Automating the operation of the device, introducing a large range of standard protocols and developing a suite of sensing technology to monitor cell experiments in real time will lead to a similarly science-fiction outcome.

In the near term, the device will create entirely new methods for researchers to understand the intricacies of human biology. A great deal of current animal-based research could be replaced with a faster, cheaper and more reliable method. The result could be an explosion of knowledge leading to cures for many of today's diseases.

In the longer term, clinical applications are possible. Advanced versions of these devices could be used to repair damaged tissues and organs, saving the need for transplants. Beyond that, human tissues, and even whole organs, could potentially be growm from scratch using a patient's own cells. This is infinitely preferable to patients waiting on a list for a healthy, compatible donor to die then taking immunosuppresant drugs for the rest of their life.

5. At the hearing, you mentioned the 'FDA Modernization Act', which would remove the mandatory element of doing animal studies for drug trials. Would you support similar initiatives in Australia, to phase out any mandatory requirements to test on animals? Please give details of your reasons why?

We would certainly support similar initiatives in Australia. We feel that this type of initiative is important to maintain Australia's relevance as a base of advanced medical research. There is also a significant economic advantage to be gained if Australian governments understand the opportunities to be found in the movement towards non-animal testing, and collectively implement policies to keep Australia at the forefront of this movement.

The proposed FDA Modernization Act of 2021 amends the FFDCA that broadens the scope of acceptable preclinical models for drug development, enabling researchers to test a drug's safety and efficacy using more advanced and human-relevant methods, of which we are fully supportive. We also support the removal of any mandatory requirements for animal tests in drug trials because it is clear that animal models are of limited value in determining toxicity of drugs in humans. This has

been known for some time (e.g. Aspirin was patented in 1900 however would not have passed current animal testing requirements as it causes birth defects in mice, rats, guinea pigs, rabbits, cats, dogs, sheep and monkeys). However, deaths continue to occur after drugs pass preclinical animal trials. For example, in 2019, a patient died during phase II clinical trials of inarigivir (for the treatment of hepatitis B) after trials in woodchucks, rats and mice asserted confidence in the safety and efficacy of the drug – the problem came down to an interaction with the first-line therapeutic tenofovir. Differences in genetics and physiology between species can alter biodistribution and metabolism, therefore the predictive value of toxicology testing in animals is limited. A 2014 analysis of ~2500 drugs found that the absence of toxicity in animal models provided "virtually no evidential weight that adverse drug reactions will also be absent in humans" (**An Analysis of the Use of Animal Models in Predicting Human Toxicology and Drug Safety", Bailey et al (2014) Altern Lab Anim**). The liver-chip model mentioned in my opening statement detected hepatoxicity of this drug combination in 2021 so had this model been used in preclinical studies this death could have been avoided.

Personalised medicine is where we need to head to make drug trials as predictive as possible. Even after human clinical trials, rare adverse reactions still occur due to human genetic and environmental differences and are responsible for the deaths of many people every year. Sophisticated *in vitro* technology allows for the use of patient-derived cells which can provide disease insights not possible with other models. As an example of what's possible - researchers in the Netherlands have grown mini guts from patient cells to successfully predict which cystic fibrosis patients respond to which drug (***Characterising responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis, Dekkers et al (2016), Sci Trans Med Vol 8 Issue 344)***.

Another notable FDA initiative from which we could take inspiration is the FDA Alternative Methods Working Group that was established in 2019 (https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda). This group is developing a targeted strategy for implementing *in vitro* models for regulatory testing, and last year identified funding 3Rs research as a priority for continued targeted investment.

6. At the hearing, a witness asserted that "a lot of the most successful alternatives have been taken up in the area of toxicology" (which is largely done overseas) rather than for 'basic research' (which is what is mainly done in Australia). Do you agree with this statement – and if so, how do we change this to ensure alternatives are available for the research being done in Australia?

Yes, toxicology has certainly had more success in this field in terms of uptake compared with basic research. Toxicology is highly regulated with standardised tests, and as such has been an area of early focus for the replacement of animal methods with many non-animal alternative tests gaining approval from the OECD (***Taylor, K. (2019). "Chapter 24 Recent Developments in Alternatives to Animal Testing". In Animal Experimentation: Working Towards a Paradigm Change. Leiden, The Netherlands: Brill. doi: https://doi.org/10.1163/9789004391192_025)***. This is in contrast to basic research which is not regulated through the specification of standardised tests, but rather governed by a cultural inertia among researchers, publishers and funding bodies. There are many alternative *in vitro* models that are showing a lot of promise in basic research that are still in the

proof-of-concept stage. It will take a concerted effort of more researchers using the technologies and validating and formalising their use.

To facilitate this sort of work, we suggest directly funding the development and implementation of alternative methods and looking to the U.S. and Europe to implement similar government regulatory and policy measures. It is the early stage of the development of this technology that makes this a “ground-floor” opportunity. There is an inevitable social drive towards reducing the use of animals in research. There are many solid indicators that non-animal technologies will produce better research outcomes and there is a clear economic benefit to any jurisdiction that establishes a solid industry in this field at this early stage.