



National Centre  
for the Replacement  
Refinement & Reduction  
of Animals in Research

# Research Review 2019

Pioneering Better Science

# About the NC3Rs

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is a scientific organisation that leads the discovery and application of new technologies and approaches that minimise the use of animals in research and improve animal welfare (the 3Rs).

We collaborate with scientists and organisations from across the life sciences sector, nationally and internationally, including universities, the pharmaceutical, chemical and consumer products industries, other research funders, and regulatory authorities.

We support the commitment of the scientific community to the 3Rs by funding research and early career development, facilitating open innovation and the commercialisation of 3Rs technologies, and stimulating changes in policy, regulations, and practice.

Further information can be found at [www.nc3rs.org.uk](http://www.nc3rs.org.uk)

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2019

# 1 Introduction

Funding research is a key part of the NC3Rs strategy to provide robust and reliable 3Rs models, technologies and tools that enhance scientific discovery.

We have demonstrated that research focused on the 3Rs not only leads to reductions in animal use and improvements in animal welfare, but also to wider impacts that benefit human and animal health, protect the environment, and generate commercial opportunities.

Since the NC3Rs was launched in 2004, we have committed £62.3M in grants and early career awards to advance the 3Rs. Of this, £39.4M (63%) was for research focusing on replacement, £10.1M (16%) for refinement, and £12.8M (21%) for reduction. This balance across the 'Rs' primarily reflects the focus of the applications that are submitted. Applications are reviewed by expert independent panels using rigorous criteria to assess both the quality of the scientific proposal and potential 3Rs impacts.

Based on our experience and feedback from the community, we have recently evolved the researcher-led schemes that we offer. We have introduced two new funding schemes; training fellowships to promote the development of early career scientists with less than three years' post-doctoral experience, and skills and knowledge transfer awards to help bridge the 'valley of death' that exists between the development and application of established 3Rs tools into routine practice.

With strategic grants and highlight notices, we have continued to target funding in areas where there are specific concerns about the numbers of animals used or their welfare, or where there are opportunities to build capacity or take advantage of new technologies with 3Rs potential. Over the last five years, we have made awards totalling £25M, covering a range of strategically important areas from replacing animal models of bovine TB research through to encouraging the use of human tissue.

Since the NC3Rs was launched in 2004, we have committed £62.3M in grants and early career awards to advance the 3Rs.

Working in partnership with other research funders and organisations has been critical to our success and we have secured co-funding from other public sector and charitable organisations to support our remit.

This has increased the number of awards we are able to make and extended our reach across a range of disciplines. Since 2014 we have received £2.8M for co-funding opportunities, working in partnership with the BBSRC, EPSRC and the British Heart Foundation. This year, we have new funding collaborations with Cancer Research UK for grants to facilitate the sharing of 3Rs approaches across the cancer research community, and with Unilever for PhD studentships focusing on non-animal approaches for safety testing.

63% of the awards have been for replacement, 16% for refinement and 21% for reduction.

We provide scientists across the life sciences with an opportunity to engage in 3Rs research regardless of their career stage. To date, we have made 332 awards at 80 research institutions, funding more than 560 principal and co-investigators and supporting the development of 144 PhD students and fellows. As the next generation of research

leaders, nurturing early career researchers is particularly important. We invest considerable resource in additional opportunities to engage in the 3Rs, for example through dedicated summer schools and training events, that also cater for the development of transferable skills that underpin a successful career in science.

It is essential that the 3Rs impacts of the science we support are widely disseminated and implemented. We work closely with our researchers to ensure the 3Rs advances they deliver not only benefit their own laboratories but are shared across their institute and field through publications, workshops and presentations. In some instances, maximising these benefits requires further research, scale-up or commercialisation, and access to industrial collaborators is key. We facilitate this through our CRACK IT Solutions partnering hub and seed-funding scheme to enable grant holders to work with potential end-users in the pharmaceutical, biotechnology and (agro)chemical industry sectors nationally and internationally. Recently, we have introduced the Technologies to Tools (T2T) scheme, with awards of up to £50k, to support the translation of *in vitro* models and non-animal technologies, developed with NC3Rs grant funding, into research tools that can be applied effectively in the pharmaceutical industry. The T2T scheme is in partnership with the Medicines

To date, we have made 332 awards at 80 research institutions, funding more than 560 principal and co-investigators and supporting the development of 144 PhD students and fellows.

Discovery Catapult at Alderley Park, who will match the funding we commit with in-kind contributions such as access to specialist equipment and facilities. The first awards will be made in the Spring.

Many of the publications that describe research funded by the NC3Rs report primary findings only. While this is important in showing how 3Rs models, tools and technologies can be used, helping to build confidence in new approaches, it can mean that the detailed methodology is not published. This limits the ability of others to understand or use the 3Rs model, tool or technology and can prevent the work from being reproduced. To address this, in May 2018 we launched the *NC3Rs Gateway*

in partnership with *F1000Research* to provide a platform for our grant holders to report their 3Rs model development, detail its performance characteristics and how it was validated. The gateway is open access with open peer review allowing immediate access and transparency. There are already eight papers on the gateway, which in total have been viewed 5,500 times, with 840 downloads.

In this review, we provide case studies from across our portfolio showing the breadth of the science we fund through our various schemes and the 3Rs and scientific impacts we deliver. In some cases, the grant holders we showcase have had more than one NC3Rs award, and we describe the value this has had in widening the impact and inspiring other advances.

Finally, we would like to thank the MRC and BBSRC, our core funders, for their sustained support which has allowed us to invest in amazing science and talented individuals.

**Dr Vicky Robinson CBE**  
Chief Executive

**Professor Stephen Holgate CBE**  
Chairman

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# Case Studies



# Ferrets and flu transmission studies

Professor Wendy Barclay was awarded funding to reduce the number of ferrets used to study influenza transmission.

Influenza viruses cause flu-like symptoms in a range of species, including birds, pigs, horses, dogs and humans. Each year, seasonal flu affects 10% to 15% of the UK population and is associated with 12,000 deaths. Children, the elderly and those with pre-existing medical conditions are especially vulnerable. Influenza viruses evolve rapidly giving rise to novel antigenic variants that can evade natural or vaccine-induced immune responses. Occasionally, influenza strains, which would ordinarily cause disease in one species, acquire the ability to transfer to a new host. This can enable rapid viral spread in the absence of host immunity, leading to influenza pandemics.

Ferrets are considered the 'gold standard' for modelling influenza airborne transmission

because they exhibit similar clinical signs and receptor distribution in the airways as humans. Studies in ferrets show good correlation with human transmission patterns. Animals are lightly anaesthetised and infected intranasally with the virus and daily viral titres are measured in nasal flushes or swabs. Post-infection, the ferrets (referred to as donors) are housed next to naïve sentinel animals for up to 14 days and subsequent nasal flushes of sentinels at multiple time points are used to establish whether transmission has occurred. Exposure can be by close contact where donor and sentinel animals are housed in the same cage, akin to a household setting, or to model respiratory droplet and airborne routes, animals are placed in adjacent cages separated by perforated sides.



**PI:**  
Professor  
Wendy  
Barclay



**Organisation:**  
Imperial  
College  
London



**Award type:**  
Project grant



**Start date:**  
2013



**Duration:**  
3 years



**Amount:**  
£400k

A typical experiment designed to test when during the infection transmission occurs uses four donor ferrets and depending on the number of time points up to 64 sentinel animals. The studies do not completely recapitulate what happens in humans where contact is much shorter in duration and may be over a greater distance. Better understanding of how much infectious virus a ferret exhales and its stability in airborne droplets could improve the relevance of the studies to humans and help explain why some viral strains are reported to be more transmissible than others.

### 3Rs benefits (actual and potential)

Wendy and her team have designed, manufactured and tested a novel piece of equipment called the influenza virus transmission tunnel (IVTT) which avoids the need to use sentinel ferrets. The IVTT consists of a 100 cm long tube along which sentinel cell culture plates containing MDCK cells (an immortalised canine cell line that is highly susceptible to influenza viruses) are positioned. Infected donor ferrets are placed for a maximum of ten minutes in a chamber attached to the IVTT and viral plaque counts on the plates are used to determine the titre of infectious virus in the animal's exhaled breath. The IVTT can be attached to a nebuliser allowing fully *in vitro* experiments. It is also amenable to use in human infection studies.

### Scientific and technological benefits

Using the IVTT, the Barclay laboratory have demonstrated that the amount of virus in the ferret's nose is not a reliable indicator of the amount of viable virus in exhaled air. They showed that factors affecting virus survival in airborne droplets, such as stability of the virus particle, can be more readily studied in the IVTT than in the animal-to-animal transmission studies. Virus recovered from the MDCK cells infected by the airborne route can be easily interrogated using molecular techniques, for example, to define the genetic determinants that enhance transmission – studies which are challenging to do in the ferret as the site from which virus is transmitted is unknown or inaccessible without culling the animals.

### Added value

In 2017, Wendy presented the IVTT at the 'Transmission of Respiratory Viruses: From basic knowledge to evidence-based options for control' conference in Hong Kong. Subsequently groups in the USA and Japan have adopted a similar device, further reducing the use of ferrets. Based on the work supported by the NC3Rs funding, Wendy has secured a £1.2M Wellcome Collaborative Award to study the evolution of the influenza virus, which will exploit the use of the IVTT. There have been three papers published from the NC3Rs-funded research. Wendy has collaborated with a small company to use the IVTT to test materials suitable for use in face masks that aim to protect against respiratory virus transmission. She is currently working with a major pharmaceutical company using the IVTT to test the effects of antiviral drugs on influenza transmission.

# Fly infectivity assay for prion disease

Dr Raymond Bujdoso was awarded funding to test whether PrP transgenic *Drosophila* are a suitable invertebrate host to measure mammalian prion infectivity.

Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of fatal neurodegenerative disorders that affect both humans and animals. They include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, and scrapie in sheep. The infectious agents are misfolded versions of the PrP protein, termed prions. PrP is a cell surface glycoprotein, found throughout the brain. It is highly conserved across vertebrate species and, although relatively little is known about its normal function, evidence suggests it is involved in a diverse range of biological processes from cellular differentiation through to the maintenance of myelin.

Prion diseases can arise spontaneously or be transmitted between individuals of the same and different species. This zoonotic potential led to the BSE crisis in the UK in the 1980s and the emergence of variant CJD in humans. Much of the early research into prion infectivity, including disease transmissibility and the existence of different strains, has been dependent on the use of animals, primarily non-human primates, sheep and goats. The demonstration that scrapie could be experimentally transmissible to rodents has, however, led to the widespread use of wild type and genetically modified mice as the 'gold standard' model.

Prion infectivity is typically measured by intracerebral or peripheral inoculation of a test material such as brain homogenate into an experimental host. There is often a long incubation period and clinical signs such as weight loss, ataxia and paralysis can take months or years to appear depending on route and dose of inocula, or prion strain used. The studies require close monitoring of the animals and the judicious use of humane endpoints given the potential for animal suffering.

than the mouse. This increased sensitivity has important practical applications. For example, Raymond has shown that PrP transgenic *Drosophila* can detect prion-infected blood from asymptomatic scrapie-infected sheep, opening the possibility for a confirmatory blood test for prion diseased individuals, including humans. Importantly, assessing prion infectivity in the fly takes about six weeks to complete while testing the same inoculum in mice can take months or years.

## 3Rs benefits (actual and potential)

Raymond and colleagues generated *Drosophila* transgenic for ovine PrP to establish an invertebrate model of scrapie. Un-inoculated PrP transgenic flies show no adverse phenotypic effects. However, by feeding PrP transgenic *Drosophila* at the larvae stage with scrapie-infected sheep brain homogenate, Raymond demonstrated that the adult flies subsequently had hallmark features of mammalian prion disease, including the accumulation of infectious prions, a neurotoxic phenotype as assessed by locomotor assay, and enhanced mortality rate compared to controls.

Raymond investigated other aspects of prion transmission and infectivity in the fly model. This included studies in which head homogenate from scrapie-exposed PrP transgenic flies was inoculated into ovine PrP transgenic mice. The mice subsequently developed prion disease indicating that the fly can effectively propagate mammalian prions, providing a new model for studying prion biology. The Bujdoso laboratory have replaced the use of the rodent bioassay with the PrP fly model, saving around 200 mice a year.

## Added value

Raymond has published seven papers arising from the project grant, including in *Brain*, the *Journal of Virology* and the *Biochemical Journal*. A composite summary of the research was published on the NC3Rs gateway in 2018 – the paper has been downloaded 58 times to date.

Based on the utility of the model, Raymond has established new collaborations with research groups in Europe and North America and has generated bovine and cervid PrP flies in order to support these and replace the use of the natural hosts of prion diseases. Raymond was awarded an NC3Rs skills and knowledge transfer grant in 2017 for the validation of a fly bioassay for classical and atypical BSE prion infectivity. Working with collaborators, at the UK's Animal and Plant Health Agency, a major reference centre for TSEs, Raymond has shown that bovine PrP transgenic *Drosophila* provide a highly sensitive and rapid bioassay to assess BSE infectivity. Following further validation work, this new invertebrate prion model could replace the use of 750 mice a year at the agency.

## Scientific and technological benefits

To test the sensitivity of the PrP transgenic fly model compared to the equivalent mouse model, animals were exposed to serial dilutions of scrapie-infected sheep brain homogenate. The flies showed a significant neurotoxic response to dilutions from  $10^{-2}$  to  $10^{-10}$  of the brain homogenate while the mouse bioassay detects a  $10^{-6}$  dilution – in other words the fly bioassay is 10,000 times more sensitive



**PI:**  
Dr Raymond  
Bujdoso



**Organisation:**  
University of  
Cambridge



**Award type:**  
Project grant



**Start date:**  
2013



**Duration:**  
2 years



**Amount:**  
£262k

# Bioreactor for *Cryptosporidium* oocysts

Professor Joanne Cable was awarded funding to bring to Europe, via a transatlantic exchange, a high throughput *in vitro* culture system for the enteric protozoan parasite *Cryptosporidium*.

*Cryptosporidium* is a waterborne pathogen which poses a major threat to farm animals and humans because there is no available drug treatment and no immediate prospect of vaccine development. Together, two species of the parasite, *C. parvum* and *C. hominis*, are responsible for more than 200,000 infant deaths attributable to diarrhoea in South Asia and sub-Saharan Africa each year, but even in developed countries outbreaks can occur.

There is an urgent need to understand the host immunological response and how the parasite interacts with the host microbiome, in order to develop effective therapeutics. Until recently the generation of parasites (specifically transmissive stages, termed oocysts) for experimental purposes was dependent on the use of neonatal calf and immunocompromised

neonatal mouse infection models, because production of *in vitro* systems was difficult with poor yields and low infectivity.

In 2016, Professor Nigel Yarlett from Pace University, New York, published methodology for the continuous culture of *C. parvum* using a hollow fibre bioreactor seeded with human colon tumour-derived HCT-8 cells. The bioreactor provides an environment that mimics the gut by delivering nutrients and oxygen through the lumen of the fibres to the basal layer of host cells growing on the outside of the fibres, while allowing separate redox and nutrient control outside the fibres for parasite development. The system produces a high oocyst yield, replacing the use of animals and avoiding the suffering associated with infection (such as diarrhoea, dehydration, inappetence and lethargy leading

to severe weight loss and a high risk of death). Importantly unlike *Cryptosporidium* parasites generated through *in vivo* infection models which show batch-to-batch variation in their transcriptomic and proteomic profiles, *in vitro* production generates genetically identical parasites helping to ensure findings are reproducible from one experiment to another.

### 3Rs benefits (actual and potential)

Following training in the Yarlett laboratory, Dr Anna Paziewska-Harris, the post-doctoral researcher on the grant has established the bioreactor system at Cardiff University.

Calf-derived oocysts were initially required for primary seeding of the bioreactor, but subsequently oocysts produced *in vitro* have been used for experiments or for seeding of additional bioreactors. The bioreactor has allowed the Cable laboratory to replace the use of calves for parasite production – saving a minimum of six animals per year. Further savings have been made for experimental work. Because parasite viability decreases when stored outside of the host, each experiment requires a fresh batch of oocysts – without the bioreactor, a recent series of experiments by the Cable laboratory on environmental factors affecting sporozoite viability would have required four calves.

### Scientific and technological benefits

The bioreactor produces  $10^6$  oocysts per week for over six months, however, through manipulation of nutrient content in both compartments, the Cable laboratory has shown that it can be scaled-up to produce  $10^8$  oocysts per ml/week. This is similar to the commercial production in calves where the typical quantity is  $10^8$  to  $10^9$  oocysts per batch. Anna has also optimised the viability assay for oocysts and the gliding motility assay for sporozoites providing an estimation of the parasites' condition following the *in vitro* culture. Together with a newly developed 3D infectivity assay for sporozoites using HCT-8 cell spheroids, the assays constitute a quality control system for assessing the phenotype of parasites produced by the *in vitro* platform.

### Added value

Jo and Anna organised a workshop, part funded by the NC3Rs, at the 2018 British Society for Parasitology Spring meeting, to promote the use of the bioreactor for the production of parasites and other applications to advance *Cryptosporidium* research. This has led to five new collaborations, including with the NHS Cryptosporidium Reference Unit in Wales, with the Cable laboratory acting as a training hub for researchers and organisations. Wide uptake of the bioreactor system could have significant cost saving benefits. For example, more than 20 water companies in the UK each spend around £60k per annum on purchasing *Cryptosporidium* parasites for positive controls for their daily drinking water screening which is required by law – use of the bioreactor could cut the cost to around £20k per annum.

Data from studies using *Cryptosporidium* oocysts produced by the *in vitro* system have been included in successful funding applications totalling £290k to date. This includes a Marie Curie COFUND Fellowship to Anna, and a Global Challenges Research Fund project and a BBSRC-funded PhD studentship to Jo.

The project has been showcased at various public engagement events including the Welsh Eisteddfod festival in August 2018, with a 'soapbox' presentation on the 3Rs. Further-a-field, Jo has talked about the bioreactor in Namibia during a launch event for Phoenix Waters, a collaboration between the Universities of Cardiff and Namibia to understand issues relating to water security and quality in sub-Saharan Africa.



**PI:**  
Professor  
Joanne  
Cable



**Organisation:**  
Cardiff  
University



**Award type:**  
Skills and  
knowledge  
transfer  
grant



**Start date:**  
2017



**Duration:**  
2 years



**Amount:**  
£75k



# Blood flow in mouse stroke models

Professor Claire Gibson was awarded funding to improve a mouse model of ischaemic stroke to reduce inter-animal variability.

Approximately 85% of strokes in humans are ischaemic in nature. This is modelled experimentally, usually in rodents, by temporarily or permanently occluding the middle cerebral artery (MCA). Occlusion can be achieved by various methods including electrocoagulation and pharmacological intervention but mechanical obstruction by intraluminal insertion of a filament is often the method of choice, being used by almost 80% of UK stroke researchers.

The filament is inserted into the intracranial internal carotid artery through an incision usually made in the common carotid artery. The filament prevents blood flow into the MCA which, after a period of ischemia typically up to 90 minutes, is restored by removal of the filament and ligation of the common carotid artery to prevent blood loss through the incision. The surgery is technically demanding

and associated with significant animal welfare concerns in the immediate days following the induction of experimental stroke, including death, weight loss, sensorimotor deficits and seizures.

The primary outcome measure used in *in vivo* stroke research is the infarct or lesion volume. There is, however, considerable inter-animal, experiment and laboratory variation in the size of the infarct in the MCA occlusion (MCAO) model even when following the same defined protocol. In some instances, despite the animal undergoing surgery, there is no lesion. Evidence suggests that this variability can be caused by differences in the strain and age of the rodents used, cerebrovascular anatomy, and the size and type of the filament. The variability reduces the statistical power of the experiment necessitating the use of large numbers of animals to power the experiment

appropriately. Many studies are under-powered as a result – because of factors such as cost, ethical considerations, and practical issues involved with the complex surgery and subsequent intensive care of the animals required.

## 3Rs benefits (actual and potential)

During the MCAO procedure, it is typical to permanently ligate the common carotid artery and reperfusion of the MCA occurs via vessels that form the Circle of Willis. This structure is anatomically highly variable, particularly in the commonly used C57Bl/6 mouse strain, which might account for the heterogeneity seen in lesion volume. To address this, Claire investigated whether it was possible to enhance reperfusion by repairing the common carotid artery rather than relying on the Circle of Willis.

Claire used a small tissue pad coated in fibrinogen and thrombin to seal the incision following removal of the filament, rather than permanently ligating the common carotid artery. Using histological assessment and magnetic resonance imaging techniques to monitor cerebral blood flow, Claire demonstrated the repair of the vessel wall was stable and reperfusion was enhanced compared with the standard ligation model. Importantly, repairing the common carotid artery reduces lesion volume variability between animals. Based on this, Claire has shown that a study to determine treatment effect would use 40% fewer mice (35 rather than 58 per group), assuming a power of 0.8, significance level of 0.05 and a predicted 30% reduction in lesion volume between control and test animals.

To make surgical access easier, it is common practice in the MCAO model to temporarily ligate the external carotid artery prior to making an incision in the common carotid artery. However, reducing vascular supply to the external carotid artery, even temporarily, can affect the muscles involved in chewing and swallowing. Claire was also able to demonstrate with the pilot grant that it is possible to conduct the surgery without ligating the external carotid artery with no effect on lesion volume. Preliminary evidence

suggests this reduces body weight loss when compared with animals where the external carotid artery is temporarily ligated.

## Scientific and technological benefits

The improved surgical methods for the MCAO model were published in *Disease Models and Mechanisms* in 2017, with the detailed methodology reported in the *Journal of Visualized Experiments*. Claire was awarded an NC3Rs skills and knowledge transfer grant in 2017 to provide hands-on training to four major stroke research groups in Europe and North America enabling them to adopt the model and subsequently act as training hubs for others. The improved surgical approach may also have applicability in rodent models of embolic stroke.

## Added value

Claire was awarded NC3Rs funding for a PhD student in 2015. Michaela Bayliss, the PhD student, has investigated further refinements in the MCAO model in rats and mice, including whether improved environmental enrichment benefits animal welfare following stroke surgery and whether it has any impact on ischaemic damage. To date Michaela has one first author paper, published in *PLOS ONE*, which shows that in normotensive rats (unlike hypertensive rats) a pre-stroke craniotomy does not improve animal welfare in terms of decreased body weight loss, improved survival and fewer neurological deficits.



**PI:**  
Professor  
Claire  
Gibson



**Organisation:**  
University of  
Nottingham



**Award type:**  
Pilot study  
grant



**Start date:**  
2014



**Duration:**  
1 year



**Amount:**  
£75k

# Self-structuring bone *in vitro*

Professor Liam Grover was awarded funding to develop an *in vitro* model for studying osteogenesis.

Osseous tissue forms in various physiological circumstances ranging from normal bone development and fracture repair, to pathological heterotopic bone formation in extraskelatal tissues following muscle trauma, traumatic brain or spinal cord injury, or surgical procedures of the hip and knee. Animals such as rodents, rabbits and sheep are used in research on normal and heterotopic ossification to understand the dynamic role of osteoclasts, osteoblasts and osteocytes. Many studies focus on manipulating the expression of bone morphogenetic proteins in transgenic mice or involve subcutaneous or intramuscular implantation of bone marrow-derived mesenchymal stem cells. However, for trauma models it can be necessary to cause physical damage by crushing a muscle or fracturing a bone.

The animal models are often associated with severe suffering and while they have been useful in understanding some aspects of ossification, they are not always representative of pathological states and often are too complex to investigate early phase bone formation. There are few alternative models available due to difficulties differentiating and maintaining osteocytes *in vitro* and although immortalised cell lines exist, these show reduced levels of key markers such as sclerostin, the secreted protein which inhibits osteoblast differentiation.



**PI:**  
Professor  
Liam Grover



**Organisation:**  
University of  
Birmingham



**Award type:**  
PhD  
studentship



**Start date:**  
2014



**Duration:**  
3 years



**Amount:**  
£90k

## 3Rs benefits (actual and potential)

Alexandra Iordachescu, the PhD student, has developed a self-structuring *in vitro* model for the development of mature bone, which has replaced the use of animals for screening potential demineralisation agents and studies of early bone formation in the Grover laboratory.

The model consists of a fibrin gel cast between two ceramic anchors into which osteoblastic cells, from the rat femoral periosteum, are seeded. The culture is maintained with a continuous source of calcium phosphate, supplemented with osteogenic factors. Using various imaging modalities, Alexandra has shown that the periosteal cells deposit an ordered matrix that closely resembles mature bone in terms of chemistry (e.g. the collagen/mineral ratio) and cellular composition (e.g. osteoblasts and osteocytes are present). The model remains viable in culture for over a year, recapitulating the successive phases of ossification from initiation of bone formation through to the differentiation of osteocytes from osteoblasts. Importantly, the model includes the presence of canalicular networks, essential for intracellular communication, which have not been previously observed in *in vitro* culture. Details of the model development and validation were published in *Advanced Biosystems* in 2017.

Alexandra and Liam are working with groups at the University of Oxford and Imperial College London to transfer the model into their laboratories, helping to further replace the use of animals.

## Scientific and technological benefits

Alexandra demonstrated in a pilot study that it is possible to test compounds that inhibit ossification in the *in vitro* model. The study was conducted with two compounds, one which is used to treat acquired and congenital heterotopic ossification and the other which has been shown to reduce heterotopic ossification in transgenic murine models of *Fibrodysplasia ossificans progressiva*. Both compounds led to a decrease in matrix and mineral formation. Since then, the model has been used by the Grover laboratory to identify new compounds

that block ossification. The model also has other benefits as it is possible to harvest matrix vesicles from it. These exosomes are thought to have a central role in controlling bone mineralisation but are poorly understood due to difficulties extracting them. Alexandra has developed a new protocol for harvesting the vesicles using immunoprecipitation, which should allow more detailed study of the process and regulation of mineralisation. The protocol was published in *RSC Advances* in 2018.

## Added value

Alexandra has presented the *in vitro* model at international conferences including the World Biomaterials Congress (WBC) in Montreal, where she was the recipient of a WBC Trainee Award. In 2019, Alexandra was awarded an NC3Rs training fellowship to further develop the *in vitro* model by including mechanical unloading. This will allow the model to be used to study the loss of bone in ageing and during osteoporosis, helping to replace procedures such as the hindlimb unloading model in which the rat is suspended from its tail for two to three weeks so that only the forelimbs touch the cage floor.

Liam also secured further funding from the NC3Rs in 2018 for a PhD studentship in collaboration with Dr Amy Naylor at the University of Birmingham. This also aims to further advance the model developed by Alexandra, by including human osteoblasts and osteoclasts (primary cells and mesenchymal stem cells) so that the process of bone re-modelling can be studied.

# Zebrafish and skin cancer research

Dr David Hill was awarded funding to develop a 3D *in vitro* human melanoma skin equivalent and a zebrafish xenograft model to investigate melanoma invasion, migration and metastasis.

Malignant melanoma is the most lethal form of skin cancer and the fifth most common cancer in the UK. It is a growing world health concern, with an incidence that has risen more than any other malignancy in the last 40 years. Although curable by surgical resection at early disease stages, late stage metastatic melanoma is highly invasive and currently incurable.

Melanoma research, like that for most other cancers, has a heavy reliance on mouse xenograft models for investigating tumour development and progression, and responses to therapeutics. Studies of melanoma initiation and invasion into the skin typically involve the engraftment of human skin, or skin reconstructs containing human melanoma cell lines or patient-derived melanoma cells, onto the backs of

immunodeficient mice. In some cases, transgenic mice that develop spontaneous tumours are used.

For the study of metastatic disease, melanoma cells are implanted subcutaneously and allowed to spread to distant and multiple sites. The primary engrafted tumour may be surgically removed to prevent humane endpoints based on tumour size being breached (and thus requiring animals to be killed) while the study of the metastasis is ongoing. Alternatively, melanoma cells may be injected directly into the circulation via the tail vein or the metastatic site of interest (e.g. intracranially). Depending on the cell line or patient source, metastasis to the lungs, liver, kidney or brain are common and careful monitoring of the mice is essential as the potential for pain and suffering is high.

## 3Rs benefits (actual and potential)

During his Fellowship, David focused on two approaches that have replaced the use of mice in his melanoma research, saving around 200 animals a year. David has optimised and validated a 3D *in vitro* model of human skin that recapitulates the early melanoma microenvironment. The model contains both a dermal and epidermal equivalent into which cancer cells can be introduced. These form groups or nests at the dermal/epidermal interface before breaching the basement membrane and invading the dermis. This mirrors early tumour invasion with, for example, the characteristic breakdown of type IV and VII collagens as is observed in human cutaneous tumours *in vivo*. Details of the model were published in *Molecular Cancer Therapeutics* in 2015.

David has also established a xenograft model for investigating cancer cell invasion and metastasis in zebrafish embryos which can be completed prior to five days post-fertilisation (the point when they become regulated under the Animals (Scientific Procedures) Act 1986). Fluorescently-labelled human melanoma cells injected into the yolk sac can be tracked in real time by time-lapse imaging. Using transgenic fish, for example with GFP-labelled endothelial blood vessels, David has been able to track local non-vascular spread, intravasation of metastatic cells and subsequent haematological and lymphatic metastatic dissemination. The latter requires interaction with the endothelium during entry and exit, and the metastatic melanoma cells show characteristic sticking and rolling behaviours on the surface of vascular endothelium indicative of specific interactions between human melanoma and zebrafish endothelial cells. Details of the zebrafish model were published on the *NC3Rs gateway* in 2018 – the paper has been downloaded 100 times to date.

re-growth of the tumour. Using the zebrafish embryo model, David and his colleagues have demonstrated that combined treatment with drugs that specifically block both MEK and autophagy reduced the invasion of trametinib-resistant melanoma cells – this is the first time that inhibition of autophagy has been shown to overcome resistance to targeted MEK inhibitors. The work was published in the *British Journal of Dermatology* in 2019 and could have clinical relevance. David is now using the *in vitro* human skin model to determine the effect of autophagy modulation on the invasion of melanoma cells as a potential preventative therapy for metastasis in high risk patients.

## Added value

The 3D skin model is now routinely used by Alcyomics, a Newcastle-based company which conducts preclinical skin tests on chemicals and pharmaceutical compounds to assess immune hypersensitive reactions. This has replaced the use of up to 200 rodents a year in skin contact hypersensitivity experiments.

David has received funding for two PhD studentships from the BBSRC and EPSRC to further develop the *in vitro* skin model. The first, in collaboration with Professor Stefan Przyborski at Durham University, is to apply the model to study skin ageing to replace the use of mice, and the second in collaboration with Alcyomics focuses on bioprinting to increase the throughput of the assay. In 2015, David was awarded the British Society of Investigative Dermatology Young Investigator of the Year award.

## Scientific and technological benefits

In patients with malignant melanoma, a subset of tumour cells often become resistant to mitogen-activated protein kinase kinase enzymes (MEK) inhibiting drugs, such as trametinib. This drug resistance correlates with an increase in autophagy and leads to



**PI:**  
Dr David Hill



**Organisation:**  
Newcastle  
University



**Award type:**  
David  
Sainsbury  
fellowship



**Start date:**  
2014



**Duration:**  
3 years



**Amount:**  
£195k

# Pathways for cardiotoxicity

Dr Luigi Margiotta-Casaluci was awarded funding to curate published information on cardiotoxicity, caused by blocking L-type calcium channels, to underpin the development and use of non-animal approaches.

Over the last decade there has been considerable interest from industry and regulatory bodies in the wider use of non-animal approaches to investigate how chemicals, including pharmaceuticals, interact with biological processes and biochemical pathways to help predict whether they will be harmful to humans and/or the environment. The concept of the adverse outcome pathway (AOP) has been developed to support this by providing a framework to organise information into a description of the critical steps that occur from the initial chemical interaction (referred to as the molecular initiating event) through to molecular, cellular and tissue events that ultimately lead to adverse effects at the organ and organism-level.

In the short-term AOPs can be used to help design bespoke mechanistic *in vitro* and *in silico* models for specific toxicological endpoints and to identify knowledge gaps which need to be addressed – together supporting the long-term goal of replacing animal use in safety testing. Because of their potential benefits, the OECD has provided guidance and infrastructure to encourage the development and use of AOPs – standardising the language used and outlining a process for review and endorsement. To date, nine AOPs have been published by the OECD, including AOPs for neurological and reproductive adverse events.



**PI:**  
Dr Luigi  
Margiotta-  
Casaluci



**Organisation:**  
Brunel  
University  
London



**Award type:**  
Strategic  
grant



**Start date:**  
2017



**Duration:**  
9 months



**Amount:**  
£30k

## 3Rs benefits (actual and potential)

During this nine-month project, Luigi developed the first AOPs for cardiotoxicity. These form a mini-network of AOPs that describe three different mechanisms by which the blockade of L-type calcium channels can lead to adverse effects, including heart failure. The AOPs were developed by identifying, critiquing and collating information from over 150 primary publications on the effects of calcium channel blockers on different parts of the cardiovascular system, and publications examining the effects of genetic manipulations on different components of the L-type calcium channel.

The AOPs have been published on the AOPWiki to allow peer review as part of the process towards OECD endorsement.

## Added value

Luigi has presented the AOPs at five conferences and workshops, including the Safety Pharmacology Society 2018 Annual Meeting where he was awarded a Junior Investigator Award. As a result of the expertise developed with the NC3Rs award, Luigi is a member of a €6.7M Horizon 2020 GOLIATH consortium where he will guide the development of an AOP network for chemical-induced metabolic disruption.

## Scientific and technological benefits

Luigi curated over 1,100 *in vitro*, *ex vivo* and *in vivo* data points relevant to blockade of L-type calcium channels into a database. The database includes quantitative pharmacokinetic and pharmacodynamic data that can be used to inform and interpret *in silico* and *in vitro* models, for example, dose response data for each drug/endpoint, exposure duration and quantification method.

An innovative approach to graphically represent the strength of evidence supporting each key event relationship in the AOP was also developed by Luigi. This could be applied to other AOPs to help highlight knowledge gaps and assess the completeness of the AOP as part of the endorsement process.

# Virtual heart for drug screening

Professor Blanca Rodriguez was awarded funding to accelerate the uptake of human-based *in silico* methodologies for the evaluation of cardiac drug safety and efficacy in industry, regulatory and clinical settings.

Drug-induced cardiotoxicity is one of the leading causes for attrition during pharmaceutical development and can also result in drugs being withdrawn after market approval. International guidelines (e.g. ICH S7B) require compounds to be evaluated for their effects on ventricular repolarisation and proarrhythmic risk using *in vitro* and *in vivo* tests. Assessment is carried out during the early stages of drug development in *in vitro* assays using heart tissue and cells, typically from guinea pigs, rabbits, dogs or non-human primates. The primary function of these assays is to identify changes to electrophysiological properties (e.g. action potential duration and/or cardiac ionic currents). Later in development, cardiovascular safety including electrophysiology and mechanical read-outs of cardiac function (e.g. ECG, blood pressure

and heart rate) are assessed as part of a core battery of regulatory pharmacology tests usually performed in dogs or non-human primates. The number of animals used per drug at this stage is four or eight animals depending on whether the study is conducted in Europe or the USA respectively. In some cases, it may be necessary to conduct further animal studies to understand potency and mechanisms of action.

It is estimated that 20% to 50% of drug candidates are abandoned due to cardiotoxicity despite *in vitro* and *in vivo* testing. Worldwide there are a number of efforts to provide more predictive tools that exploit the potential of human-induced pluripotent stem cell-derived cardiomyocytes and *in silico* models in order to avoid the current reliance on animal models.

## 3Rs benefits (actual and potential)

The Computational Cardiovascular team at Oxford initially generated the *Virtual Assay* software, with EPSRC funding, to simulate the effect of drugs on the electrophysiology, and calcium dynamics linked with contractility, of populations of human cardiomyocytes. The software has been enhanced by the availability of a comprehensive database of human *in silico* models, funded by the NC3Rs, for specific disease conditions such as heart failure, myocardial ischaemia, genetic disorders and cardiomyopathies.

With NC3Rs funding, the team have gone on to compare predictions using simulations from *Virtual Assay* with data from clinical trials, animal models, and *in vitro* methods, helping to build confidence in *in silico* approaches. A publication in *Frontiers in Physiology* in 2017 describes how *Virtual Assay* simulations predict clinical risk of drug-induced Torsade de Pointes with higher accuracy than animal experiments for more than 60 reference compounds. The simulated repolarisation abnormalities were shown to be a more accurate, specific and sensitive biomarker for arrhythmia risk than action potential duration measured in animals – with the *Virtual Assay* simulations having an 89% accuracy compared to 75% in the rabbit isolated heart model. The paper has been viewed more than 10,000 times.

*Virtual Assay* is being evaluated by four major pharmaceutical companies for use in early drug development to assess arrhythmic risk, with initial estimates of a 30% to 33% reduction in animal use per year across the sector. The software is also freely available with an academic licence and is currently being used by six research groups.

## Scientific and technological benefits

Typically, computer models of heart function are based on one generic model representative of a generic cell and therefore may not be representative of the wider population where cardiac response to drugs may differ. An advantage of the *Virtual Assay* is that rather than this 'one model fits all' approach, the software generates a large population of

simulated human cardiac cells with unique profiles, allowing better modelling of disease state, drug responses and genetic variation. The population featured in the *Frontiers in Physiology* publication consisted of 1,213 unique cardiac cellular simulations, which were then used to run an *in silico* drug trial with reference compounds.

To improve *Virtual Assay's* physiological relevance the team are also adapting it to account for other cardiac comorbidities. For example, cardiotoxic adverse events are more likely to occur in patients where electromechanical function of the heart is impaired. Such comorbidities are, however, rarely reproduced in *in vivo* studies which use healthy animals. A computer simulation for *in silico* drug trials in single cell electromechanical models is currently being validated, with plans to extend the model to simulate multiple cells and eventually the whole heart.

## Added value

In 2017, Dr Elisa Passini, a senior post-doctoral scientist in the team, was awarded the inaugural Safety Pharmacology Society Technological Innovation Award as well as the NC3Rs International Prize which is sponsored by GSK. Blanca was awarded the MPLS Impact Award for Commercial Impact in 2018 by the University of Oxford.

Blanca and the team have given presentations on *Virtual Assay* at a range of national and international scientific meetings. In 2018, with funding from the NC3Rs, they hosted a symposium titled '*In Silico Drug Safety and Efficacy*' in Oxford with 100 delegates from academia, industry and regulatory authorities. There have also been extensive outreach activities which include Elisa presenting at the Hay Festival and organising an event through The Royal Institution, where school pupils used the *Virtual Assay* in a masterclass in computational biology. An article published in *The Conversation* '*Why computer simulations should replace animal testing for heart drugs*' has been viewed more than 20,000 times.



**PI:**  
Professor  
Blanca  
Rodriguez



**Organisation:**  
University  
of Oxford



**Award type:**  
Infrastructure  
for Impact  
grant



**Start date:**  
2016



**Duration:**  
5 years



**Amount:**  
£512k

# MRI and pancreatic cancer

Dr Jane Sosabowski was awarded funding to improve non-invasive imaging of mouse models of pancreatic cancer.

There are almost 10,000 new cases of pancreatic cancer in the UK each year. While survival rates for most cancers have increased over the last 40 years, the prognosis for patients with pancreatic cancer is poor, with few surviving more than a year after diagnosis. Research on pancreatic cancer and the development of treatments predominantly uses mice. This includes genetically altered animals and xenograft models either with cell lines or patient-derived tissue.

The KPC mouse model is commonly used. This has a conditional point mutation in both the p53 and KRAS genes. It develops pancreatic ductal adenocarcinoma with similarities to human tumours as well as associated comorbidities of cachexia, jaundice and ascites. For xenograft models, cells or patient samples are either transplanted heterotopically or directly into the pancreas. Orthotopic tumours have the advantage of providing the opportunity to study tumour

development in the appropriate pancreatic microenvironment.

The mouse pancreas lies in the upper abdomen behind the stomach and is soft and diffuse in comparison with the human pancreas which makes it difficult to define and distinguish from the surrounding tissues. These factors mean that sizing orthotopic tumours and monitoring their growth over time, for example to assess treatment efficacy or for the purposes of humane endpoints, is challenging. Palpation is difficult and does not provide accurate quantitative information. Ultrasound can be used but animals have to be shaved, image acquisition is highly operator dependent, and analysis of volumetric images is time consuming. Longitudinal studies typically require mice to be culled and the pancreas removed. Consequently, studies require large numbers of mice for each time point and individual animals cannot be tracked.



**PI:**  
Dr Jane  
Sosabowski



**Organisation:**  
Queen Mary  
University  
of London



**Award type:**  
PhD  
studentship



**Start date:**  
2015



**Duration:**  
3 years



**Amount:**  
£90k

Magnetic resonance imaging (MRI) is already used in mouse studies on pancreatic cancer. It is readily applicable, even for inexperienced operators, with the fast-growing localised tumours that occur with orthotopic transplants. However, MRI image analysis is challenging with genetically altered models, such as the KPC mouse, where tumour growth is typically slower (three to six months) and where there is a gradual transformation, sometimes across the whole pancreas. Although there are commercially available tools to aid with image interpretation, these largely only work for well-defined organs such as the liver and stomach and not the pancreas.

### 3Rs benefits (actual and potential)

Joseph Brook, the PhD student, has used a low field, small animal MRI instrument to build a library of images of the healthy pancreas and pancreatic tumours in the KPC mouse. By calculating various features for each 3D pixel in the MRI images (e.g. intensity, gradient, spatial and probability features), Joseph has used machine learning to develop an autosegmentation tool that can automatically identify a tumour-positive pancreas with 95% accuracy compared to veterinary radiologists and image analysis experts. Where the model and the image analysis experts disagreed, in the vast majority of the cases, this was because the model had correctly identified a tumour earlier than the experts. This early identification can inform the use of humane endpoints and the monitoring of mice.

Joseph and Jane are working with the Boston-based company Invicro to incorporate the pancreatic segmentation tool into their existing 3D mouse atlas software. It is also being applied to other projects at the Barts Cancer Institute, Queen Mary University of London (QMUL), where there are more than 90 researchers working on pancreatic cancer, and through new collaborations with researchers in Glasgow, Cambridge and London. Research at the Institute has already shown that the use of MRI for orthotopic tumours reduces the number of mice used per study from 12 to eight animals. Wider uptake of the autosegmentation tool will

ensure the advantages of MRI imaging, such as the ability to do longitudinal imaging on the same animals, can be applied more readily to genetically altered models of pancreatic cancer.

### Scientific and technological benefits

The low field MRI instrument used by Joseph was funded through an NC3Rs Infrastructure for Impact grant in 2013 to Professor John Marshall at QMUL, with Jane as a co-investigator. The instrument has been applied to a wide range of animal studies at QMUL including cancer (e.g. pancreatic, brain and lung) and trauma injury models, demonstrating the utility of low field MRI. Impacts include developing new methods to assess lung tumours in mice to improve the humane endpoints used, and demonstrating that MRI can be applied to monitor tumours of the omentum (a fold of the peritoneum connecting the stomach and the abdominal viscera), which is adjacent to the pancreas.

John and Jane have tracked the reduction in animal use from the instrument with their '*Mouse Lives Saved*' Counter, with the number currently standing at 2,600 mice.

### Added value

Joseph has presented his machine learning tool at a number of international conferences, including the World Molecular Imaging Congress in Seattle in 2018 where he won a poster prize. He has participated in more than 50 hours of public engagement activities, including as a STEM Ambassador, to champion the 3Rs. In 2017, Jane gave a presentation on the 3Rs and imaging at an NC3Rs-hosted event in London as part of the 'Pint of Science' festival. Working with veterinary colleagues at QMUL, Jane has published papers on anaesthesia and monitoring animals during MRI and other welfare considerations.

# TB challenge *in vitro*

Dr Rachel Tanner was awarded funding to transfer the mycobacterial growth inhibition assay (MGIA) to two end-user laboratories to refine the use of monkeys in tuberculosis (TB) vaccine studies by avoiding challenge infection studies.

There are ten million new cases of TB and 1.6 million deaths worldwide each year. The disease can be cured by targeting the causative agent *Mycobacterium tuberculosis* (Mtb) with appropriate antibiotics. However, limited access to drugs in developing countries and the recent emergence of multi-drug resistant Mtb strains makes TB an increasing global health threat. The only established vaccine, the BCG vaccine, is not always protective, particularly against pulmonary disease and in areas where TB is prevalent. Consequently, there is considerable international effort from governmental and philanthropic organisations to develop new vaccines to halt the TB epidemic. Vaccine candidates are typically evaluated using mice, guinea pigs and non-human primates (NHPs), which are immunised with a test vaccine prior to challenge with Mtb.

As in humans, animals that become infected experience clinical signs that may include raised temperature, respiratory distress and weight loss, and humane endpoints are used to avoid unnecessary suffering.

### 3Rs benefits (actual and potential)

The MGIA is an *in vitro* functional assay that can be used to refine the use of animals in Mtb vaccine research by avoiding the need to undertake infection challenge studies. In the MGIA, peripheral blood mononuclear cells (PBMCs) from naïve and vaccinated animals are cultured over four days with Mtb (or other mycobacteria) and the inhibition of bacterial growth is used as an estimate of protective immunity.

Rachel had previously undertaken optimisation of the MGIA in a number of species, including the NHP. This involved demonstrating a correlation between the *in vitro* response using PBMCs taken from macaques and *in vivo* protection in BCG and Mtb challenge studies in the same animals, as well as clinical concordance based on comparison with epidemiological data. Despite this, the MGIA has been under-utilised by the TB research community because of concerns about the reproducibility and reliability of mycobacteria-based functional assays.

With NC3Rs funding, Rachel is working with scientists at Public Health England (PHE) and the Biomedical Primate Research Centre (BPRC) in the Netherlands to build confidence and facilitate the adoption of the NHP MGIA in their TB vaccine programmes.

By using PBMCs from previous *in vivo* challenge studies in the NHP MGIA, Rachel and her collaborators Dr Sally Sharpe at PHE and Dr Frank Verreck at BPRC have shown that the assay is highly reproducible between tests, operators, labs and institutions. The MGIA is currently being used alongside the *in vivo* challenge study to provide additional validation data. As well as the animal welfare benefits of avoiding challenge studies, in the future the MGIA could lead to a combined 30% (= 45 animals) reduction in macaque use at the two institutions annually. This is because the PBMCs can be used to screen multiple clinical isolates and vaccine candidates. More broadly the work has underpinned a general shift in the acceptance of the MGIA by the TB research community and through the NC3Rs grant Rachel has helped to set up the assays for various species at 13 institutions in Europe, North America and Asia, providing protocols and technical support.

easy to manipulate individual components of the assay, it can be used to determine the immune mechanisms controlling mycobacterial growth and identify correlates of protection – studies which are difficult and costly to do *in vivo*.

### Added value

Rachel has presented the MGIA data at various international vaccine and TB conferences. This includes organising a workshop, with 25 participants from 14 different institutions, alongside the TBVAC2020 meeting in Switzerland. She was awarded the TB Vaccine Initiative Young Scientist Award in 2017 which allowed Rachel to present work arising from the NC3Rs grant at the Global Forum for TB Vaccines in Delhi; and the Collaboration for TB Vaccine Discovery Junior Investigator Award in 2018 which allowed her to present at its annual meeting in Seattle.

Rachel, who was only awarded her PhD in 2015, has recently secured a two-year Fellowship from VALIDATE (an MRC/BBSRC funded network) to continue her research on TB vaccine development and antigen discovery. Working with Professor Helen McShane at the University of Oxford and collaborators, Rachel has contributed to a publication describing a refined non-virulent NHP model of mycobacterial infection and the development of a MGIA for studying TB infection in cattle, as well as three publications on the human MGIA. She has also published a paper in *Frontiers for Young Minds*, a science journal for children and teenagers. The paper titled '*The 3Rs: What are medical scientists doing about animal testing?*' has been viewed more than 3,500 times.



**PI:**  
Dr Rachel  
Tanner



**Organisation:**  
University of  
Oxford



**Award type:**  
Skills and  
knowledge  
transfer  
grant



**Start date:**  
2017



**Duration:**  
1 year



**Amount:**  
£75k

### Scientific and technological benefits

The MGIA may be used for early vaccine evaluation as part of the 'gating' strategy that has been established by the global TB community to accelerate the development of the most promising vaccine candidates. Since the MGIA measures most aspects of the complex host immune response to Mtb and it is

# Dancing parasites and mice

Dr Joseph Turner was awarded funding to reduce the reliance on animals in filariasis research, particularly for preclinical testing of therapeutics.

Filarial diseases are caused by a group of parasitic worms, known as filariae. Infection occurs when the parasites are transmitted through the bites of flying insects, such as mosquitoes. Conditions are life-long and a major cause of disability, affecting approximately 150 million people worldwide, with a further 1.3 billion at risk of infection. The most well-known filarial diseases are elephantiasis (lymphatic filariasis) and river blindness (onchocerciasis) which are caused by *Brugia malayi* and *Onchocerca volvulus* respectively. Filarial diseases are also of veterinary importance, affecting both cats and dogs.

Mass drug administration community control programmes currently use chemotherapeutic agents, such as ivermectin, to target the transmission stage of the parasitic cycle. However, these are not effective at directly killing the adult parasites and treatment has to be continued annually for five to ten years (depending on the reproductive cycle of the parasite). There is

significant investment from governmental and private philanthropic stakeholders to develop curative drugs (macrofilaricides) that eliminate filariasis as a public health problem.

Animals are used for filarial research purposes both to grow the parasites to the life cycle stage of interest (typically the adult stage) and for the testing of potential drugs. The gerbil is the most common model. A major challenge is achieving reproducible levels of adult parasite burden in gerbils because of the high incidence of infection failures and high intra-group variation. For evaluation of the efficacy of an experimental drug, parasites are harvested from donor gerbils, counted and surgically implanted into the peritoneum of recipient animals. There are no biomarkers to assess adult parasite infection status or infectious load and consequently efficacy studies typically involve culling animals at various time points, often up to eight months post-treatment, to quantify the number of parasites.

The lack of biomarkers also means animals can develop welfare issues either due to chronic parasitism (e.g. peritoneal adhesions, ascites, adverse behavioural changes) or ageing.

## 3Rs benefits (actual and potential)

Amy Marriott, the PhD student, has validated the use of immunodeficient inbred mice instead of gerbils to provide a more consistent and reproducible filarial parasite burden for *Brugia malayi*. This has allowed a reduction in animal numbers from 14 gerbils to eight mice per compound tested (including donor animals and those excluded due to low infection rate).

In the clinic, ultrasonography is used for diagnostic purposes by detecting the random thrashing movement of parasites in the lymphatic system, referred to as the filarial dance sign (FDS). Amy has shown that the FDS can be used in the laboratory to accurately assess the presence or absence of *Brugia malayi* infection in mice and gerbils (with 100% sensitivity and specificity when more than five worms are present) and also to predict drug effectiveness in a proof-of-principle study using known macrofilaricidal and non-macrofilaricidal drugs. Tracking FDS allows longitudinal studies in the same animal avoiding the need for separate groups for experiments with multiple time points.

The use of ultrasonography has led to a further reduction from eight to five mice for drug studies in the Turner laboratory. In addition, implantation surgery can now be avoided in both mice and gerbils as it is possible to inject microscopic larvae intraperitoneally into the experimental animals and track infection by ultrasonography rather than having to take adult parasites from donors. Amy has a first author paper describing the work published in *Scientific Reports* in 2018.

## Scientific and technological benefits

The ultrasonography technique developed by Amy has been adopted in the preclinical validation testing of the candidate macrofilaricide ABBV-4083, in partnership

with AbbVie Inc. and the Drugs for Neglected Diseases initiative. The work has been accepted for publication in *Science Translational Medicine* and the drug candidate has now entered phase II clinical development.

Amy has also optimised a co-culture system of dual human lymphatic endothelial cell monolayers where adult female worms can be maintained with full viability for up to three weeks – three times the duration of commonly used *in vitro* systems. The longevity of the system allows for long-term and robust *in vitro* assessment of preclinical therapeutic candidates prior to a decision whether *in vivo* studies are justified. It has been adopted by Professor Mark Taylor at the Liverpool School of Tropical Medicine in collaboration with Professor David Sattelle at University College London to screen the potency of a new anthelmintic class of molecule against adult stage filariae. Beyond drug screening, the co-culture system can be used to study host/parasite interactions.

## Added value

The research has been presented at 12 conferences, including at the British Society for Parasitology where Amy was awarded a best poster presentation prize. Joseph is funded as part of the Bill & Melinda Gates Foundation Macrofilaricide Drug Accelerator Consortium and has been able to share the 3Rs advances with academic and industrial partners from Europe and North America. In 2018, Joseph was awarded an NC3Rs project grant of £288k to validate alternative models to cats and dogs for heartworm drug testing. He has established a new collaboration with Bayer Animal Health which will provide veterinary compounds for testing in heartworm drug screening models as part of the project.



**PI:**  
Dr Joseph  
Turner



**Organisation:**  
Liverpool  
School of  
Tropical  
Medicine



**Award type:**  
PhD  
studentship



**Start date:**  
2015



**Duration:**  
3 years



**Amount:**  
£90k



# Flies and neuronal ageing research

Dr Alessio Vagnoni was awarded funding to build confidence in the use of *Drosophila* to study mitochondrial transport in neuronal ageing and neurodegeneration to replace some studies involving rodents.

Deficiencies in the active intracellular transport of organelles, such as mitochondria, along axons are associated with neuronal ageing and are a hallmark of many neurodegenerative diseases. Understanding the cell biology of neuronal ageing could lead to the identification of new targets for therapeutic intervention. To date axonal transport has been extensively studied in cultured primary neurons and tissue explants, however, these models do not always consistently reflect what happens *in vivo* because of the lack of other cell types (e.g. muscle cells), myelination and excitatory/inhibitory input into cell bodies.

Recent advances in intravital techniques have allowed the process to be monitored in real time *in vivo* in a range of species. Studies in mice have focused on axonal transport in the sciatic nerve and axons of the spinal cord and dorsal roots. The procedures are invasive (involving surgery), technically demanding and require the

generation of specific transgenic lines expressing fluorescent proteins to track the movement of 'cargo' along the axon. They are also limited by tissue transparency and imaging depth, and the relatively long lifespan of mice makes studying the cell biology of neuronal ageing difficult.

Working with Dr Simon Bullock at the MRC Laboratory of Molecular Biology, Alessio had previously developed non-invasive procedures for imaging axonal mitochondrial transport in the sensory neurons of the marginal nerve in the *Drosophila* wing. Since the wing is translucent and the fly has a short lifespan, axonal transport can be studied in the intact nervous system throughout ageing. This combined with the genetic tools available to manipulate the *Drosophila* genome (including the generation of neurological disease-related models) provides a powerful system for understanding neuronal ageing, with advantages over other *in vivo* approaches.

## 3Rs benefits (actual and potential)

During his Fellowship, Alessio expanded the utility of the fly wing model by developing new assays to assess interactions between the mitochondria and endoplasmic reticulum (which are often perturbed in neurodegenerative diseases) and to measure intracellular calcium levels following neuronal stimulation. To validate the model as a potential replacement for rodents, comparative mitochondrial trafficking studies were conducted in the exposed sciatic nerve of ageing *MitoMice* – transgenic mice in which mitochondrially-targeted fluorescent proteins are selectively expressed in neurons. The findings are being prepared for publication.

As an additional 3Rs advance, working with Professor Giampietro Schiavo at the Institute of Neurology, Alessio also maximised the use of the *MitoMice*. After the imaging studies, neurons from the dorsal root ganglion of young and old mice were rapidly extracted and cultured, providing an opportunity to compare the *in vivo* and *in vitro* properties of two subsets of sensory neurons derived from the same animal. This correlation between the animal and *in vitro* data could help to reduce the numbers of mice used as well as facilitating the use of functional assays that cannot be performed *in vivo*.

## Scientific and technological benefits

Previous work by Alessio and colleagues had shown that the number of actively transported mitochondria declines significantly in the wing sensory neurons during ageing. In his Fellowship, Alessio demonstrated that the cAMP/protein kinase A (PKA) pathway promotes mitochondrial transport in adult *Drosophila* wing neurons and that feeding aged flies with a small molecule agonist of PKA is sufficient to suppress the decline in mitochondrial transport, with upregulation of the motor protein, kinesin-1, an important output for PKA activation.

Evidence suggests that axonal transport of Amyloid Precursor Protein (APP), which plays a major role in the development of Alzheimer's disease, requires kinesin-1. Based on the strength of the fly wing model, Alessio has established a collaboration with Professor Chris Miller at King's College London generating mutant *Drosophila* to measure *in vivo* axonal transport of vesicles containing APP in ageing animals. This has avoided the use of approximately 160 mice. Alessio also has active collaborations to replace the use of rodents and zebrafish with flies in laboratories in the UK and Italy.

## Added value

Alessio has published two first author papers in *Nature Protocols* and *Current Biology*, highlighting key findings from the project, as well as being a co-author on a paper reviewing methodological advances in intravital imaging. He has given two public presentations on the importance of the 3Rs in research on neurodegeneration at 'Pint of Science' festivals held in Cambridge and London in 2017.

Alessio is now building an independent research career in neuronal ageing at King's College London, securing £500k to start his own laboratory. The funding includes a van Geest Fellowship and a BBSRC Collaborative Training Partnership PhD award with Ely Lilly to study the neuronal cell biology of human ageing *in vitro*. Alessio has also received a UK-Israel Science Lectureship grant from the British Council and the UK Science & Innovation Network. The grant will support a series of lectures at Israeli universities and strengthen collaborations between laboratories, allowing Alessio to further disseminate his fly model.



**PI:**  
Dr Alessio  
Vagnoni



**Organisation:**  
King's College  
London



**Award type:**  
David  
Sainsbury  
fellowship



**Start date:**  
2016



**Duration:**  
3 years



**Amount:**  
£195k

# Annexes

3



# References

## Barclay

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L-type calcium channel blockade leading to the disruption of cardiac electrophysiology  
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# NC3Rs researcher-led funding schemes

Research grants		Total awarded to date (£)
Project grants	For research projects, of up to 36 months in duration, which support the development of new 3Rs approaches and technologies. Average award value £300k.	£36M
Pilot study grants	For proof-of-principle work with awards up to £75k for 12 months.	£1.7M
Strategic grants	For research projects in areas identified as strategically important by the NC3Rs because of 3Rs potential or concerns about animal numbers/welfare.	£6.4M*
Non-research grants		
Infrastructure for Impact grants	For projects of up to 60 months duration which focus on infrastructure, networks and other resources to underpin 3Rs research.	£2.3M
Skills and knowledge transfer grants	Launched in 2016, to promote the wider adoption and use of models, tools and technologies with proven 3Rs impacts through the transfer of knowledge, skills and expertise. Awards are a maximum of £75k for up to 24 months. Joint awards with Cancer Research UK from 2019.	£0.5M
Early career awards		
PhD studentships	For the training of graduate scientists. Awards are £90k for 36 months. Joint awards with the British Heart Foundation and from 2019, Unilever.	£11.1M**
Training fellowships	Introduced in 2016, to support the development of promising early career researchers with less than three years' post-doctoral experience, focusing on developing new skills and gaining a breadth of research relevant to the 3Rs. Awards cover salary and up to £15k per annum for other directly incurred research costs and are 24 months in duration.	£1.2M
David Sainsbury fellowships	For talented, 3Rs-minded scientists with two to six years' post-doctoral experience to support the transition to an independent career. Awards cover salary and up to £30k per annum for other directly incurred research costs and are 36 months in duration.	£3.1M
Available to NC3Rs grant holders only		
Technologies to Tools	For the translation of non-animal technologies, developed with NC3Rs grant funding, into research tools that can be used in the pharmaceutical industry to support improved drug discovery. Applicants can apply for up to £50k for projects up to 24 months in duration. This is a partnership with the Medicines Discovery Catapult who will provide in-kind contributions equivalent to the funding provided by the NC3Rs.	First awards to be made in Spring 2019. Call budget is £0.2M
Public engagement grants	For outreach activities aimed at encouraging awareness of the 3Rs in the general public. Awards of up to £1,500 are available.	£12K

\*includes £1.3M and £0.5M co-funding from BBSRC and EPSRC respectively

\*\*includes £0.9M co-funding from the British Heart Foundation

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