Imperial College London

ANIMAL RESEARCH

Annual Report 2016–17

TABLE OF CONTENTS

FOREWORD

Animal research at Imperial

Two years on from the publication of our first Animal Research Annual Report, we can be pleased with the significant progress we have made towards implementing our Action Plan for World Class Animal Research.

In last year's Report we focused on the transformative impact our work involving animals has on many areas of scientific research, and I am very pleased that this year's edition focuses on one of the most important areas addressed by our Action Plan: the promotion of the 3Rs – the guiding principles (reduction, refinement and replacement) that underpin the humane use of animals in scientific research.

As Establishment Licence Holder I am strongly of the view that the 3Rs should always be on our mind when we plan and undertake scientific experiments involving animals. It should not be viewed as an 'optional extra' or a methodology separate from the day-to-day scientific work we do. Indeed I would assert that it is the most important part of the culture of animal care that we are trying to create at Imperial. But culture change is not easy and requires strong and effective leadership. In that respect we are extremely fortunate to have Professor Richard Reynolds leading our efforts to promote the 3Rs.

This Report showcases some of the many innovative applications of the 3Rs from around the College, and also describes what we refer to as the fourth R – relevance – aimed at ensuring the work we do with animals directly relates to human disease and will ultimately have a positive impact on human health and well-being.

Underpinning the examples cited in this document is a wealth of activity taking place in the background to promote the 3Rs. Over the last few years Professor Reynolds has spent time engaging with every level of our animal research community on the 3Rs, and this has created a vibrant atmosphere where colleagues of all job types and career levels can contribute to our 3Rs agenda. We recognise particularly notable contributions to this via our annual Provost's Awards for Excellence in Animal Research. Congratulations to all this year's winners – you can find out who they are and how they have contributed to Imperial's research with animals on page 6.

Looking back over the past year, I am particularly pleased to be able to report on the substantial new investment being made by the College in its animal research facilities. The College is investing nearly £10 million in the facility at South Kensington, with work already started on-site in April. This investment is a substantial commitment by the College, underlining both the importance of research involving animals to the College's academic strategy, and our commitment to providing the very best facilities for our valued animal research community.

I want to end with a very big thank you to Professor Maggie Dallman, who will later this year stand down as Chair of our Central Animal Welfare and Ethical Review Body (AWERB). Her leadership of AWERB has been nothing short of transformational. Maggie's successes are far too numerous to list here. From my perspective, however, arguably the

biggest achievement of Maggie's time as Chair of AWERB has been to nurture a truly collaborative and open environment in which a broad range of interests, expertise and roles work together both to promote the welfare of animals and to consider the ethical dimension of our research. Maggie leaves AWERB in a very strong place, and we are all grateful to her for her outstanding leadership.

Professor James Stirling

Provost and Establishment Licence Holder
August 2017

DISCOVERIES

Recent findings from animal research

New sensor for improving animal welfare in dementia and brain research

Scientists have improved the way that brain activity data is collected in mice, which could advance dementia and brain research. The TaiNi sensor system is a new ultra-lightweight wireless sensor system for recording neural activity in the brains of mice. It avoids many of the welfare concerns associated with existing approaches to recording brain activity in mouse models.

TaiNi has been developed by engineers at Imperial College London in collaboration with Eli Lilly, a pharmaceutical company, with funding provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R). The sensor, which weighs just 1.5 grams, allows mice to maintain a normal repertoire of movement and behaviours in their home cages, thereby reducing stress for the animals. The battery life and signal bandwidth of the sensor will permit up to 72 hours of recording, and the monitoring of networks of individual neurons in the brain, opening up new areas of research into brain disorders.

Targeting 'broken' metabolism in immune cells reduces inflammatory disease

Scientists have found a way to 'program' immune cells to cause less damage to the body, by tapping into a 'broken' chemical pathway in inflammation.

A team led by Dr Jacques Behmoaras, of the Department of Medicine at Imperial, found that blocking a single enzyme enabled them to reprogram macrophages – the immune cells which are activated in inflammatory conditions – to calm their activity and reduce inflammation in rats and mice with human-like disease.

The team, which also comprises researchers from Queen Mary, University of London and Ergon Pharmaceuticals, hope that the approach could offer new hope in the treatment of inflammatory conditions such as arthritis, autoimmune diseases and sepsis.

New method could lead to non-invasive deep brain stimulation

Deep brain stimulation (DBS) currently involves cutting open a person's skull and inserting electrodes inside the brain to reduce physical symptoms, such as shaking. It is often the last option for people with Parkinson's disease who have very serious symptoms that cannot be controlled through medication alone.

Dr Nir Grossman, the lead author of the study, worked with a team based at the Massachusetts Institute of Technology to develop a DBS method that involves placing electrodes on the scalp, rather than inside the brain. The approach, called Temporal Interference (TI) stimulation, has been shown to activate neurons in the hippocampus – a region deep in the brain – of mouse models.

Though in its early stages, the research shows promise as a new type of non-invasive method for DBS, which could ultimately enable DBS to be used more widely.

Batrachochytrium salamandrivorans affects newts and salamanders, including the fire salamander.

Female fruit flies become more aggressive towards each other after sex

Chemicals in male fruit flies' semen cause females to become more aggressive and intolerant towards each other after mating, research has shown.

Studies in many animal species have shown a direct link between increased levels of aggression in females and reproduction, though the precise factors that cause these changes were less clearly understood. Researchers based at Imperial and the University of Oxford have determined that this change is a result of a variety of proteins found in male semen, which stimulate dramatic behavioural and physiological changes in females.

The research on fruit flies, which was undertaken by confining pairs of females to a small area with a single food source and observing levels of aggression in mated and virgin females, has implications for understanding and potentially controlling fruit fly populations.

Candidate for immunotherapy may increase tumour growth in certain cancers

Boosting a part of the immune system known to have anti-tumour properties may actually help tumours grow in cancers linked to chronic inflammation.

Cancer immunotherapies boost aspects of the body's normal immune system, to help fight tumours. There are several types of immunotherapies currently in clinical trials. A team at Imperial, working with a mouse model of liver cancer driven by inflammation, have found that one immune receptor – an attractive candidate for immunotherapy – promoted rather than delayed tumour growth.

The lead author of the research, Dr Nadia Guerra of the Department of Life Sciences, notes that though immunotherapies have shown unprecedented successes in treating cancer patients, this research shows "there are still challenges ahead to optimise therapies and reduce adverse effects. Our data unravels a shift in thinking that will inform which cancers benefit most from these new therapies, and help match the best therapy to each patient."

Deadly fungus genes identified in 'amphibian plague'

Scientists have identified the genes of a deadly fungus that is decimating salamander and newt populations in Northern Europe.

Batrachochytrium salamandrivorans (Bsal), dubbed the 'amphibian plague' affects many species of salamanders and newts, literally digesting their skin. Now, researchers from Imperial College London, Ghent University and the Broad Institute have sequenced and identified the genes responsible for Bsal from an infected salamander. Dr Rhys Farrer, co-author of the research from Imperial's School of Public Health said: "Our findings mean that policy makers and conservationists are now equipped with more knowledge on how best to curb this amphibian plague."

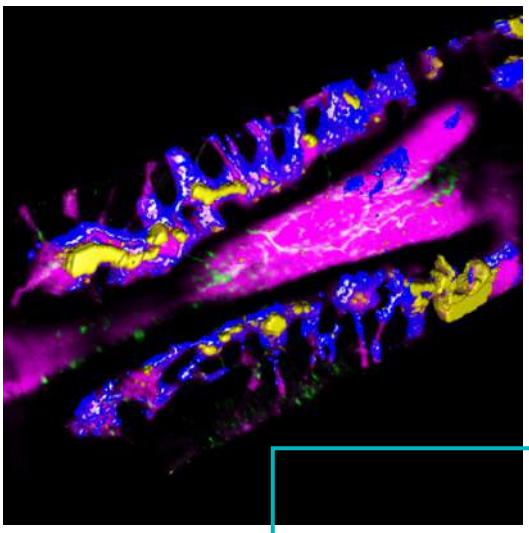
The next step for the researchers will be to sequence genes from more infected salamanders to build a bigger picture of how these genes function.

Leukaemia cell movement gives clues to tackling treatment-resistant disease

New research is shedding light on how leukaemia cells can survive cancer treatment, suggesting new possibilities for stopping them in their tracks.

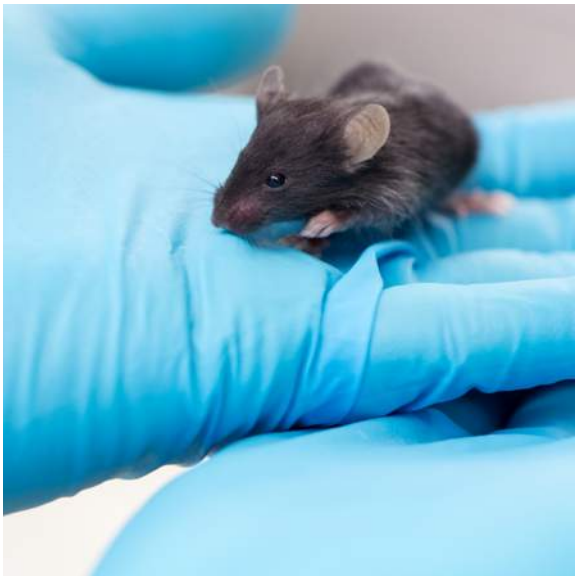
The research team led by Dr Cristina Lo Celso of the Department of Life Sciences, in collaboration with researchers at the Francis Crick Institute and University of Melbourne have found that certain leukaemia cells resistant to initial chemotherapy within the bone marrow in mice, do not sit and hide but move around rapidly throughout the bone marrow, both before and after treatment. The surviving cells moved faster than those before treatment, which suggests that the act of moving itself may help the cells survive, possibly through short interactions with our own cells.

The research, funded by the charities Bloodwise and Cancer Research UK, alongside contributions from the European Research Council, the Human Frontier Science Program and the European Hematology Association, paves the way for developing a more effective leukaemia treatment.



Environments in bone marrow (blue, purple and green) as they are invaded by leukaemia cells (yellow).

> “Although these findings are very early they suggest this gene therapy may have potential therapeutic use for patients.”
>
> – Dr Magdalena Sastre, Department of Medicine



Alzheimer’s disease could be treated with gene therapy

Researchers have prevented the development of Alzheimer’s disease in mice by using a virus to deliver a gene into the brain.

This gene, called PGC1-alpha, may prevent the formation of a protein called amyloid-beta peptide in cells – the main component of amyloid plaques, sticky clumps of protein found in the brain of Alzheimer’s sufferers. The team at Imperial College London injected a virus containing the gene into two areas of the brain in mice at early stages of Alzheimer’s disease when amyloid plaques have not developed. After treatment, mice treated with the gene had fewer plaques compared to untreated mice.

Dr Magdalena Sastre, senior author of the research funded by Alzheimer’s Research UK and the European Research Council, said, “Although these findings are very early they suggest this gene therapy may have potential therapeutic use for patients.”

PROVOST'S AWARDS

for Excellence in Animal Research: **2016–17 winners**

The Provost's Awards for Excellence in Animal Research recognise best practice and acknowledge staff who have made advances in the 3Rs, shown openness in communicating about animal research, or demonstrated outstanding collaboration between research and Central Biomedical Services (CBS) staff. Winners receive £1,000 which can be used to cover costs associated with the presentation of the award-winning work to a wider audience.

Application of the 3Rs, researchers

Dr Marie-Sophie Nguyen-Tu

Postdoctoral Research Fellow, Department of Medicine

Dr Marie-Sophie Nguyen-Tu receives the Provost's Award for leading the establishment of a new technique for researching pancreatic islets, the cell clusters that produce insulin. The technique transplants islets from the pancreas of mice to the anterior chamber of the eye, where they engraft on the iris and become fully functional. Dr Nguyen-Tu's work could greatly reduce the number of animals used in diabetes experiments, as the islets can be observed through the lifespan of the mouse. She has also led refinement initiatives, working with mechanical engineers to reduce the risk of eye damage and with an ophthalmologist to improve perioperative care.

Application of the 3Rs, CBS staff

Mr Phil Rawson

Senior Technician and Named Animal Care and Welfare Officer

Mr Phil Rawson receives the Provost's Award for leading an initiative to house post-operative guinea pigs in groups, rather than in single cages. This enrichment work, a refinement in animal care at Imperial, has led to reduced stress levels among the animals. Following discussions with the Home Office Inspector during 2015, Mr Rawson began to introduce group housing, initially with animals grouped in pairs. He worked with researchers to resolve concerns around suturing and identification. This trial was successful, and animals are now kept for up to four months, housed in groups of three or four.

Team award

Professor Wendy Barclay, Chair in Influenza Virology

Mrs Rebecca Frise, Research Technician

Ms Tess Boreham, Technician and Named Animal Care and Welfare Officer

This team receives the Provost's Award for their refinement of ferret models for research into the transmission and mutation of influenza. This research is important for predicting the next pandemics and mutations of the virus. Professor Wendy Barclay and her group worked closely with Ms Tess Boreham of Central Biomedical Services to research and embed best practices in handling ferrets and measuring responses. The ferret is widely regarded as the best model of influenza transmission, and the team has published and presented their findings in a variety of journals and forums.

Communications award

Ms Jemma Strachan, Relationship Manager, Associate Provost's Office

Ms Laura Gallagher, Head of News and Media (Research)

Mr Ryan O'Hare, Research Media Officer – Medicine

Ms Madina Wane, PhD student, Department of Life Sciences

Mr Ray Edgar, Central Biomedical Services Site Manager, Hammersmith

This team receives the Provost's Award for designing, arranging and recording a Google Expeditions Virtual Tour of Imperial's animal facilities. The Expedition is aimed at secondary level school children, and the journey takes students through our facilities for rodents, rabbits and fish. It also includes information on the legal framework regulating animal research in the UK. The team worked under significant time pressures from the production crew to create an outstanding communications tool which describes Imperial's work in clear, accessible language.

Read more about the Google Expedition on page 33.

Application of the 3Rs, researchers

Dr Charlotte Dean

Lecturer in Lung Development and Disease, National Heart & Lung Institute

Dr Charlotte Dean receives the Provost's Award for her work to introduce precision-cut lung slices (PCLS), a new technique, to her laboratory. PCLS represents an opportunity to reduce the use of mouse tissue, replacing it with specially prepared human tissue collected post-mortem. The technique has the potential to be used widely in the respiratory research community and the pharmaceutical sector. Dr Dean and her team have employed lung slices to visualise different cell types within the lungs, and as a model for research into lung injury and repair.

FOCUS ON THE 3RS

The principles of the 3Rs, that is reduction, refinement and replacement, are at the very heart of animal research carried out at Imperial College London. At every stage of our research, from the initial planning to the experiments themselves, it is vital that we are constantly thinking about ways in which we can refine our procedures, in order to reduce the number of animals being used, to improve their welfare, and to reduce their stress and suffering.

Many of our researchers are investigating other model systems, or alternative species, that can be used to obtain the high quality data we need to answer vital questions in biomedicine. Increasing our focus on the 3Rs makes sense from both an ethical and a scientific perspective. By promoting a culture where we are constantly aware of the need to innovate to replace, refine and reduce our animal research, we will also enhance the overall quality of our research.

There is a healthy 3Rs culture at Imperial, but we don't want to be complacent. Therefore, we are constantly looking at ways in which we can educate and encourage researchers to examine their experimental design to introduce more reductions and refinements. We also employ a 3Rs Programme Manager to work with individual researchers and research groups to help them understand the importance of the 3Rs and to develop ideas specific to their area of research. This year will also see the introduction of a dedicated one day course, 'A Guide to the 3Rs and Responsible Animal Research' for new researchers at any level.

In addition to improving the application of the 3Rs in individual research projects, we aim to disseminate our knowledge and methods as widely as possible in the local, national and international research communities. At Imperial we share good practice by running 3Rs workshops in particular research disciplines, where both researchers and animal technicians can freely discuss their ideas and then plan their implementation in every day practice.

In this issue of our Animal Research Annual Report, we focus on how the 3Rs have been applied in a variety of diverse research projects across the College, including how the results of animal research have then been applied in clinical practice.

Professor Richard Reynolds
Chair of the Imperial College London
3Rs Advisory Group

REDUCTION

Each researcher who works with animals is obliged to think hard about how many they use to get the results they need. Reduction is the watchword, and researchers at Imperial have demonstrated that, with thought and effort, it is possible to use fewer animals yet still obtain the same amount of high quality scientific results.

Some of these reductions come from improving the design of experiments. Others are down to the way the research is organised, for instance with more efficient breeding programmes or the use of cryopreservation to preserve lines of genetically altered mice.

Cryopreservation to minimise breeding

Vasso Episkopou, Professor in Developmental Biology in the Department of Medicine, has employed both more efficient breeding programmes and the use of cryopreservation to reduce the number of animals used in her research.

She is interested in the way genes control embryo development, particularly the formation of the brain and the nervous system. This is basic research but it has a clear relevance for medicine. "Most of these genes also function in adult organs and are associated with diseases, therefore our studies provide the basic knowledge required to study diseases," she says.

For instance, her work has recently revealed a network of genes involved in the way motor neurons, the nerves that control all movements in the body, function. The next step is to look at how this network behaves in conditions such as motor neuron disease, and the possibility of using it to strengthen neurons that otherwise degenerate as the disease progresses.

Revealing the way genes control development relies on *in vivo* research, using live animals. "In essence our research connects genes with organs, body structures and body functions," says Professor Episkopou. "Studying the role of genes *in vivo*, in the context of the whole organism, allows us to identify how tissues and organs normally form."

Much of her research involves looking at how mouse embryos develop when certain genes, or networks of genes, are turned off only in a specific organ or tissue, and then analysing the resulting defects. The main work is therefore to produce mice carrying mutations that will turn off the expression of these genes very precisely in the specific organs or tissue in question.

This is a complex procedure, so these lines of mutated mice must be preserved for as long as researchers, both at Imperial and elsewhere, would need them. This is usually done through continued breeding, but each new generation means more mice are produced and kept in the laboratory – even if they are not subject to further research, which is not desirable.

So Professor Episkopou's group has used local facilities at Hammersmith Hospital to freeze sperm or fertilized eggs from each line of mice, which can be stored and used to recreate the line at a later date. This cryopreservation keeps mouse breeding to a minimum.

"Freezing also helps us share animals with other scientists around the world, without imposing live transport on them."

Producing the mutations is just the first step to this research, which involves observing the development of mutant mouse embryos. However, the number of mice and embryos can be reduced further by working with embryonic stem (ES) cells derived from these mutant mice. ES cells can grow in culture dishes indefinitely and have the potential to differentiate towards different tissues or organs.

"Some animal studies have already been replaced by working with ES cells, but we still need to understand how the embryo makes specific organs to be able to use similar strategies to direct ES cell differentiation in culture," says Professor Episkopou.

This shift to working with ES cells also makes it possible to study the role of gene mutations in individual cells. "This analysis cannot be done using embryos,

> "In essence our research connects genes with organs, body structures and body functions."
>
> – Professor Vasso Episkopou, Department of Medicine

Professor Vasso Episkopou,
Professor in Developmental Biology
in the Department of Medicine

> “We have both reduced the number of animals and refined their use.”
>
> – Professor George Christophides, Department of Life Sciences

as they are small and only contain small numbers of cells of each tissue type.”

These studies in mice and mouse ES cells can be then transferred to human ES cells. “Learning more about how to manipulate human ES cells will also allow us to use them in stem cell therapies.”

In the future, an even greater reduction in the number of mice used in Professor Episkopou’s research may be possible thanks to the gene editing technique called CRISPR/Cas9. At present, each mutation is produced in a single mouse line, and these mice are then bred together to produce a line of mice with a combination of the required mutations. It takes several generations to bring all the mutations together and only a subset of the offspring from each generation carry the desired combination.

CRISPR/Cas9 allows researchers to modify genes in living organisms, so it should be possible to create all the desired mutations simultaneously in one mouse, thus cutting out the requirement for breeding different mouse lines together. Professor Episkopou is eagerly awaiting the outcome of research on this approach, which is being carried out by colleagues at Columbia University in New York.

“The role of CRISPR/Cas9 in reducing the number of animals in research is a subject that is very new, but has great potential,” she says.

From top: Professor George Christophides (right) and Dr Andrew Blagborough, both of the Department of Life Sciences; Professor Vasso Episkopou of the Department of Medicine with Medicine postgraduate student Ms Peyman Ince.

Balancing reduction and refinement in tackling malaria

Researchers in Imperial's Faculty of Natural Sciences have significantly reduced the number of animals they use to study malaria.

The parasite that causes the disease is transmitted from humans when mosquitos feed on their blood. The complexity of the parasitic lifecycle means that it cannot yet be fully reproduced *in vitro*, or outside a living organism, hence the need for research using animals.

First, mice are infected with a rodent equivalent of the malaria parasite. Then mosquitos are allowed to feed on the mice, and later examined to see how many of the parasites have been successfully transmitted to the mosquito.

"We are using these animal models to understand the transmission of the disease and to identify and test the efficacy of novel interventions to reduce or block transmission altogether," explains George Christophides, Professor of Infectious Diseases and Immunity.

Work on new anti-malarial therapies also requires animal testing. "Scientifically (and legally) you traditionally have to go through animal models before you can progress to clinical trials," says Dr Andrew Blagborough, a Research Fellow working on potential new treatments.

However, it has been possible to stop using mice for routine mosquito feeding, switching instead to feeding tubes covered with a plastic membrane. "We use human blood available through the blood banks in the NHS, which would be discarded anyway," says Professor Christophides. "We also use some artificial blood as well, reconstituted from red blood cell concentrate."

While this works well for almost all mosquitos, there are some strains the researchers want to study that do not take to artificial feeding. In these cases, rats have been considered as an alternative to mice: they are bigger and react less to being bitten, meaning fewer animals need to be used. However, after careful consideration, the researchers decided to use mice rather than rats, which might otherwise suffer stress from repeated mosquito bites. This illustrates the complexities that can arise when applying the 3Rs.

"This is a refinement, because fewer animals are exposed to repeated procedures, but the number of animals used has risen comparatively," Dr Blagborough explains. The decision to favour refinement over reduction was made in consultation with regulators.

"There has been quite an in-depth discussion with the Home Office and Imperial's Animal Welfare and Ethical Review Body (AWERB), and we've hopefully found a balanced and acceptable solution."

"In terms of the licence as a whole," Professor Christophides adds, "we have both reduced the number of animals and refined their use."

"We've gained a substantial level of understanding of how these parasites infect mosquitos and then move through the mosquitos to infect humans," says Professor Christophides. This in turn will suggest new options for designing drugs and vaccines, or blocking transmission using genetic modification of mosquitos. "The results that we have produced have enabled us to attract substantial funding from the Bill & Melinda Gates Foundation to generate transgenic mosquitos that would be able to block the parasite before it is transmitted to humans."

Several new treatments are also progressing well. "We've discovered a range of new anti-malarial vaccine candidates, beginning with these animal models, and are now progressing to human studies," says Dr Blagborough.

Professor Shiranee Sriskandan,
Professor of Infectious Diseases
in the Department of Medicine

REFINEMENT

Refinement in procedures involving animals means finding ways to minimise suffering and improve welfare, while still producing the same high quality research results. The experience at Imperial suggests that improving animal welfare is in fact good for research, sharpening results and opening up new lines of enquiry.

Ingenuity and insight in *streptococcus* research

This was certainly the case for Shiranee Sriskandan, Professor of Infectious Diseases in the Department of Medicine. She is interested in group A *streptococcus* bacteria, which are responsible for a wide range of diseases, from tonsillitis to scarlet fever, from rheumatic heart disease to the flesh-eating disease necrotising fasciitis.

The development of these diseases often depends on the early stages of infection, so it is important to know how the bacteria enter and spread through the body, and how the body's immune system responds. This can suggest new treatments, or allow doctors to use existing treatments more effectively when more aggressive strains of the bacteria emerge.

The complexity of the infection process and the body's immune response means that researchers need to work *in vivo*, with animal models such as mice, to understand why infection progresses in some but not in others. But they don't necessarily need to wait until the mice fall ill.

"We realised quite early on that everything that happens in these *in vivo* models is predominantly related to the number of bacteria," Professor Sriskandan explains. "You can see which way things are going to go pretty quickly, and there is no need to wait for signs of disease. So we measure the bacteria at very early time points."

The key to this approach was finding a way to count the bacteria infecting each mouse. This was done by engineering a strain of group A *streptococcus* to give off light, which could be detected inside the living mouse, even through its skin and fur.

> "We've learned an awful lot about how quickly these bacteria can traffic around the body."
>
> – Professor Shiranee Sriskandan, Department of Medicine

"The amount of light produced is proportional to the number of bacteria," says Professor Sriskandan about the method, which the group developed with the input of Dr Siouxsie Wiles, a specialist in bioluminescence at Imperial who has since moved to the University of Auckland.

Not only did this non-invasive method allow researchers to count bacteria early in the infection, but unlike invasive methods it did not require mice to be killed every time a count was made. An infection can therefore be followed over a period of time, in the same mouse, reducing the number of animals required.

However there are limits to the sensitivity of the method, and also a concern that engineering bacteria to give off light might weaken them and therefore affect results. An alternative was found when, Mr Faz Alam, a PhD student in Professor Sriskandan's lab, who was modelling sore throat caused by *streptococcus*, decided to see if the bacteria were present in droplets in the breath of infected mice.

The naturally inquisitive animals were persuaded to sniff at agar plates, which are used to grow bacteria in the lab, and measureable quantities were detected. The same approach works if agar plates are simply placed, out of reach, in each cage.

"Now we are able to retrieve these bacteria from the mice without doing anything to them," says Professor Sriskandan.

This push to improve animal welfare has also produced scientific benefits. "We've learned an awful lot about how quickly these bacteria can traffic around the body, which we would never had known if we had not looked so early," she says.

> “The data collected to improve the welfare of the mice is also improving the research”
>
> – Dr Marcus Dorner, Department of Medicine

Dr Marcus Dorner, Department of Medicine

Welfare monitoring in infectious disease modelling

Another approach to refinement involves producing better information about the health of the animals in question, which in turn allows researchers to be more responsive to their welfare, including terminating experiments at an earlier stage. This can be seen in two different areas of research, also in Imperial’s Faculty of Medicine.

Dr Marcus Dorner, a Lecturer in Immunology, is working on animal models that can be used to study infectious diseases such as HIV and hepatitis B and C, in preference to using non-human primates such as chimps and macaques. This is challenging because these human diseases do not infect small animals such as mice, nor are there rodent equivalents.

One solution is to give the mice human characteristics, for instance replacing the mouse liver with human liver cells that can be infected with hepatitis. The infection itself does not affect the mice, but the operation to replace the liver does, hence the need to monitor their welfare.

The conventional way of doing this involves weighing the mice regularly, and stopping the experiment if their weight falls more than a set amount. Dr Dorner’s group has gone beyond this, developing a more sophisticated set of indicators that will then allow an earlier humane end point to the experiment.

“It’s a symptom guide that tells us to increase monitoring from every day to twice daily or three times a day, depending on how close we get to this cut-off point, when we need to stop the experiment,” he says.

In addition to blood monitoring, which takes place as part of the experiment, they look at the animal’s behaviour, checking to see whether it is isolated or part of a group, and whether it is active or lethargic. They also check its appearance for other signs of stress, so that a comprehensive score sheet is produced.

Observations by the researchers are backed up by the experienced eyes of animal technicians and veterinary staff from Central Biomedical Services (CBS). “It’s a combination of introducing this new kind of less subjective scoring and a close collaboration with the CBS staff that allows us to get this model going with as little stress to the animals as possible.”

Dr Dorner is using a similar monitoring approach in his HIV model, which involves making the mouse’s immune system appear human, so that it is susceptible to the virus. This humanisation process is not damaging to the mice and reducing the stress through increased monitoring both benefits the mice and reduces the variability in the data collected.

New breakthroughs for advanced malaria

For Dr Aubrey Cunnington, a Clinical Senior Lecturer and Honorary Consultant in Paediatric Infectious Diseases, the target is malaria. In particular, he is looking at severe cases of the disease, particularly in children, which do not respond to conventional anti-malarial drugs.

"When children arrive in hospital with severe malaria, just giving them drugs that are effective at killing the parasites that cause malaria is not really enough," he explains. "Quite a lot of those children will go on to die, and if we could understand the processes that are leading to death we hope we can find new types of treatment."

Improving animal welfare in research on advanced malaria is challenging because the animals need to develop a similar severe disease course to children. "We don't want mice to die in our experiments, and we go to great lengths to prevent that, but we have to allow them to become sick enough so that we can see an effect of the treatment on established disease."

This proved impossible with conventional scoring systems for assessing animal welfare, which were stopping experiments before useful data could be collected. "That defeated the object of doing the experiment in the first place."

The mouse model in question was for cerebral malaria, a neurological disease, but the scoring system was looking at more general indicators for infection, such as weight loss and the mouse's appearance. "It was clear that the mice developed a malaria-like illness, but they hadn't yet developed the neurological syndrome that we needed to see in order to determine the end point of the experiment."

So a more appropriate scoring system was devised that deals with mouse behaviour, for instance how inquisitive they are and their routine reflexes. "Essentially it combines a behavioural observation and a clinical examination of the mouse, resulting in a clear scoring system that is highly reproducible between observers."

The scoring system allows the researchers to predict with some certainty the point at which the mouse would become terminally ill. They end the experiment when this final phase is predicted, but before it begins. "Scientifically that is almost as good as allowing it to progress to the end, but much better for the mice than making them suffer once they have reached that severe stage."

The neurological examination data collected to improve the welfare of the mice is also improving the research. "It allows us to collect scores on the condition of the mice at set time points as the experiment progresses, which we can compare between groups. That means we can start to see differences even earlier on."

Preliminary results from the research are promising. "We identified a gene which was particularly strongly associated with severe malaria in children, and when we block the activity of its protein product it seems to slow the rate of progression of cerebral malaria in mice," Dr Cunnington says. "We're currently trying to optimise the dosing of the treatment to see whether the effect might be strong enough to help reverse established disease in the mice."

Dr Aubrey Cunnington, Department of Medicine

Professor Richard Reynolds,
Department of Medicine

REPLACEMENT

Replacement – the use of non-animal research techniques instead of animal models – is perhaps the most fundamental and the most challenging of the 3Rs. The experience of researchers at Imperial shows that novel approaches to replacement can yield significant breakthroughs in research.

The Multiple Sclerosis and Parkinson's Tissue Bank

In some situations, animals can be replaced in medical research by working with human tissues collected during medical procedures or after death. But obtaining these human tissues is not always straight forward.

This was the experience of Richard Reynolds, Professor of Cellular Neurobiology in the Faculty of Medicine. For many years he used animal models in his work on multiple sclerosis, trying to find out how the neurons in this degenerative condition become damaged and eventually die.

"I realised that I needed to verify some of the things I was finding in those animal models using human tissue, to make sure that they were truly representative of what was happening in the human condition," he recalls. "But when I tried to get this tissue, there weren't any good resources."

In the end, he and his colleagues set up their own tissue bank, collecting brains and spinal cords from people who had died after having multiple sclerosis. This was later extended to Parkinson's disease and for comparison they also collected brains not affected by these two conditions.

Eighteen years on, the Multiple Sclerosis and Parkinson's Tissue Bank at Hammersmith Hospital is one of the largest in the world. It holds tissue from 1,600 brains and close to 12,000 people around the UK have agreed to donate to the bank when they die. This resource is not just used by Imperial's researchers, but is shared with scientists around the world.

Having access to this material has transformed Professor Reynolds' research. First of all, it showed that the animal model he started out with was a poor fit for the research question he was working on. Then it provided an alternative to the animal model.

> "Close to 12,000 people around the UK have agreed to donate to the bank."
>
> – Professor Richard Reynolds
> Department of Medicine

"We started to spend much more time getting as much information from the human tissue as we could, to develop our hypothesis about why the cells were dying, in a very detailed way," he says.

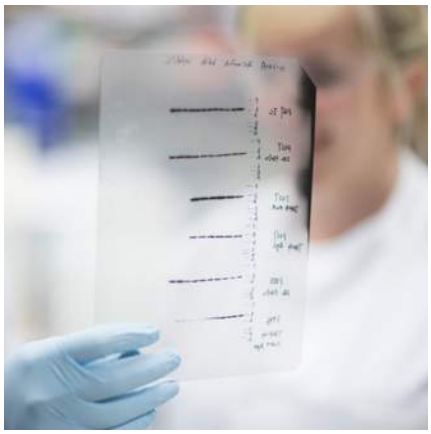
One difficulty is that each sample of human tissue represents just one moment in the development of the disease, after death, whereas animal studies allow the progress of the disease to be followed over time. That can be partly overcome if the tissue bank covers a broad range of case histories.

"Although we can't study how a single patient changed over time, we have a population who died at various stages, with various severities and length of disease. That allows us to do some sort of longitudinal study in time, effectively replacing what we might otherwise do in animals," Professor Reynolds explains. "In the end, it has replaced at least 50 per cent of our animal work."

He also thinks that the science is sounder this way. "If we are trying to develop therapies for human diseases, at least some of our studies need to involve the tissue that is affected by those diseases."

Some animal work was still necessary, however, in this case to check that the new hypothesis stood up in a living system. But thanks to the studies on human tissue, a much more effective animal model could be designed to do this, reducing the number of animals used.

"We've shown that our ideas about the mechanism probably are right, and we've identified a number of drug targets," says Professor Reynolds. The next step is to go back to the human tissue to make sure that these targets are present early enough in the development of the disease to make drugs acting on them effective in halting its progress. Once that is confirmed, the search for potential drugs begins.



Top: Mr James Bolaji, a research postgraduate in Professor Maria Belvisi's team at the National Heart & Lung Institute.

New approaches to lung disease research

Availability of human tissue is also a problem for Maria Belvisi, Professor of Respiratory Pharmacology and Head of the Respiratory Pharmacology Group at the National Heart & Lung Institute at Imperial. She works on lung diseases such as chronic obstructive pulmonary disease, asthma and chronic cough, aiming to understand their causes at the level of cells and molecules and to develop new treatments.

In this work, human lung tissue forms a bridge between animal experiments and clinical studies. "Lung tissue is important for us to see if our observations in animals are correct, and have a high chance of translation," says Professor Belvisi.

The problem is that the kind of tissue she needs, with the structure of airways and nerves intact, is hard to get in the UK. "There is no real mechanism for obtaining fresh whole lung tissue here," she says. Limited amounts can be bought in the USA and flown to the UK, but this may take too long for the tissue to be useful.

As a result, most of her group's research takes place using animal models, while the bare minimum is done with human tissues. "It's the icing on the cake, but if human tissue was available all the time we could do it the other way around," Professor Belvisi says.

She and her colleagues are exploring options for establishing a source of human lung tissue in the UK, in collaboration with the NHS Blood and Transplant Tissue and Eye Services. This will draw on post-mortem lung tissue, which is not suitable for transplantation, and where consent has been given for it to be used in research.

The first option is to see if tissue collected after death is suitable without any additional intervention. If it is not, the case will be made for ventilating and perfusing the lungs as if they were destined for transplantation.

"There are a whole number of benefits," Professor Belvisi says of the project. "The tissue would be more viable, transport costs would be cheaper and it would be more regularly available."

> "Lung tissue is important for us to see if our observations in animals are correct."
>
> – Professor Maria Belvisi, National Heart & Lung Institute

> “We’ve been able to circumvent new animal research and go directly to the human.”
>
> – Professor Graham Williams, Department of Medicine

Professor Graham Williams, Department of Medicine

Collaboration to research bone and joint diseases

Human tissue is not the only option for replacing the use of animals in research. Another is to take advantage of animal experiments that are under way for other reasons.

Graham Williams, Professor of Endocrinology and Head of the Molecular Endocrinology Laboratory at Imperial, collaborates closely with the Wellcome Trust Sanger Institute, the International Knockout Mouse Consortium and the International Mouse Phenotyping Consortium. This extensive effort is deleting, one by one, all of the genes in the mouse and characterising the functional consequences.

“They look at developmental, metabolic, cardiovascular and nervous system parameters, for example, but it turns out the skeleton has not been investigated in any detail,” he says.

And the skeleton is exactly where his interest lies; in particular he and his colleagues are studying how bones develop, are maintained and repaired. This in turn informs their work on identifying and understanding the genetic causes of bone and joint diseases, such as osteoporosis and osteoarthritis.

“We had an idea that we would be able to look for skeletal abnormalities using tissue from knockout mice that otherwise would have been discarded,” he goes on. Bone is a very resilient material, well suited to this approach. “We can study specimens that have been preserved for many months, and they still give us excellent detailed information.”

He approached the Wellcome Trust Sanger Institute, which is generating many of the knockout mice, and was given the go-ahead to use the bone samples collected for an initial pilot study of 100 knockout mouse lines. The idea paid off. “It allowed us to discover a number of new genes that seem to be important for skeletal development and the maintenance of bone mass and strength.”

Their work has now connected up with large international genetic studies in human populations. “We’ve been able to map the genes that are identified in humans with the genes that we have shown to regulate skeletal development and bone maintenance in the mouse,” he says. “Using this collaborative approach, we’ve doubled the known genetic influence on bone density in humans and now have disease models that can be interrogated to look at the underlying mechanisms of action and hopefully identify new pathways that might be amenable to drug targeting.”

All of this has been possible without using any additional mice. “We’ve been able to circumvent new animal research and go directly to the human.”

Professor Williams and colleagues in endocrinology and rheumatology have also been working with cultured cells from donated human blood to investigate the formation of osteoclasts, the cells responsible for breaking down bone in the body.

This model can be used to look at the genes involved in osteoclast formation, which are likely to be important in diseases such as osteoporosis, inflammation and tissue repair. “We have a set of about 170 candidate genes,” he says. “This directly leads us to identify new potential osteoporosis susceptibility genes based on their functional properties in human cells. We are able to collaborate with large human genetics population studies to focus straight in on genes associated with human disease without the use of any animals.”

Dr James Gardiner,
Department of Medicine

FOCUS ON RELEVANCE

In addition to the 3Rs in animal experiments – reduction, refinement and replacement – Imperial also thinks about relevance. This fourth R means ensuring that research with animals relates to human disease and will ultimately have an impact on human health.

The long game in obesity research

The relevance of animal research is exemplified by work on conditions such as obesity. "If you are looking at food intake, you can't measure it in anything other than the whole animal," says Dr James Gardiner, Reader in Molecular Physiology in the Faculty of Medicine. "You are looking at an interaction between multiple systems, and the animal has to move to get food, which is a complex behaviour."

> "The aim of everything we do is to improve treatment."
>
> – Dr James Gardiner, Department of Medicine

Even the release of insulin, which regulates the level of blood sugar after feeding, demands a whole-animal approach. "There is an interaction between the brain, the pancreas and the gastro-intestinal tract. All of those come together, and currently there is no model system that allows us to look at all of those things *in vitro*."

Imperial's research on obesity has had significant impact, even if it takes decades to reach the clinic. It was in 1996 that its researchers, led by Professor Sir Stephen Bloom, first demonstrated that the hormone glucagon-like peptide 1 (GLP-1) plays a role in regulating eating. Subsequent work with rats showed that introducing GLP-1 directly into the brain, or blocking its effect, could influence feeding behaviour, demonstrating a physiological connection.

But GLP-1 also stimulates the release of insulin after eating, which led to it being investigated as a possible treatment for type 2 diabetes. This condition, in which insufficient insulin is produced by the body, is closely associated with obesity. GLP-1 and its analogues were found to help both insulin production and appetite, and as a result they are now widely used as a therapy.

"This is one of the few treatments that can be used in type 2 diabetes that leads to weight loss rather than weight gain," says Dr Gardiner. Meanwhile one of the analogues has recently been approved in the USA as a treatment for obesity before diabetes sets in. "You are catching the problem before it develops."

The 3Rs played an important part in the work that led to these new therapies, in particular because animal welfare has an influence on appetite and feeding. "If you have an animal that is stressed, it won't respond, so you have to ensure that the animal is not stressed, is not ill in any way, and is not suffering."

While the GLP-1 research has produced successful new therapies, this does not mean that the research is over. "In each individual person there will be differences, and while some respond well to treatments, others won't."

Dr Gardiner's most recent work focuses on thyroid hormones. These play a role in controlling appetite, but have many other functions in the body, so any drug mimicking these hormones or interfering with the receptors through which they work must be very precisely targeted if it is not to have severe side effects.

As a first step, Dr Gardiner and his colleagues succeeded in genetically modifying mice so that a particular kind of thyroid hormone receptor in their brains no longer worked. The mice were healthy, but very hungry. "When given free access to food, they would just eat and eat. We ended up with very fat mice."

This in turn opens the way for more precisely targeted drugs, although as with GLP-1 this research may take decades to come to fruition. "The work that we are doing currently will take time to be translated to the clinic, but then the aim of everything we do is to improve treatment. No-one is really interested in fat mice, they are interested in treating obesity."

Unravelling the fundamentals of cell development

Imperial's emphasis on impact does not mean that basic, curiosity-driven research is side-lined. On the contrary, some of the best examples of relevance deal with attempts to unlock the basic mechanisms of human biology.

Take the work of Professor Richard Festenstein, who is based in Imperial's Brain Sciences Division. He is interested in how genes are turned on and off during the development of different types of cells, and how these decisions are 'remembered' when cells divide.

Active genes are found in stretches of the chromosome with a loose, open structure, known as euchromatin. This contrasts with tightly packed areas of the chromosome, known as heterochromatin, which do not contain active genes.

Studies in fruit flies showed some time ago that when a gene is placed closer than usual to a region of heterochromatin, this increases the probability that it will be switched off. This is known as position effect variegation. However, it was not until 1996 that Professor Festenstein and his colleagues were able to show that a similar phenomenon could be found in mammals.

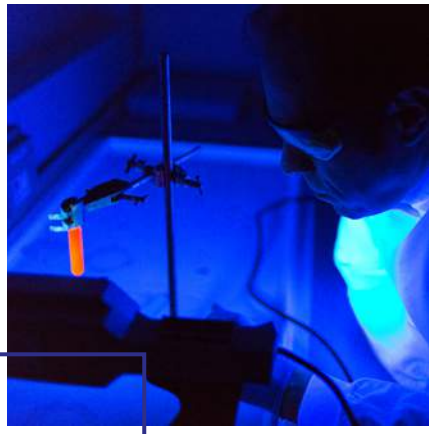
The next step was to look at how this gene silencing effect might be taking place. "There are at least 100 modifiers of the extent of this gene's silencing, and those modifiers turned out to be the molecular components of heterochromatin. So, the actual mechanism of silencing was worked out by looking at these modifiers."

These questions about the fundamentals of gene control also had a practical goal in mind. One of the characteristics of heterochromatin is that it contains repetitive DNA sequences, in particular triplets that repeat over and over again. Such repeats are also seen in abnormally high levels in a number of incurable human genetic diseases.

"We went on to see whether repeats that expand in human diseases could also induce this kind of gene silencing," says Professor Festenstein.

This was done by producing transgenic mice with varying numbers of triplet repeats in the heterochromatin. These were linked with a gene whose activity could be easily detected – a 'reporter gene' – that would show that silencing was taking place, without creating any disease or discomfort in the animals.

"We found that the repeats induced a very similar type of silencing to that seen in humans, and this was also sensitive to the genetic modifiers that modified position effect variegation."



> "We are now preparing to do a clinical trial to see whether this is an effective therapy."
>
> – Professor Richard Festenstein, Department of Medicine

Connecting triplet repeats in heterochromatin with gene silencing opened up the possibility that targeting heterochromatin might be a way of turning silenced genes back on again. The test case was Friedreich's ataxia, a degenerative disease in which nerves controlling movement are progressively damaged.

This is a rare disease, affecting 1 in 30,000 people, for which there is no existing therapy. It normally occurs in childhood, with individuals developing clumsiness, slurred speech, and impaired hearing. An associated form of heart disease is frequently fatal.

"We went on to work on a mouse model for Friedreich's ataxia, as well as looking in patients' cells at various known inhibitors of the enzymes that are involved in the silencing mechanism," Professor Festenstein explains.

They found that high doses of vitamin B3 could switch the gene back on, by modifying the heterochromatin. "In mice, and in cells from people, we have shown that we can restore expression of the gene that is aberrantly silenced in the disease. So this is potentially a radical therapy for this disease."

This result was reached without subjecting mice to the full-blown disease. Even with a mild form, it was clear when the gene had been turned back on.

A subsequent study with Friedreich's ataxia patients showed that high doses of vitamin B3 were able to turn on the gene that is turned off in the condition. "So, we are now preparing to do a clinical trial to see whether this is an effective therapy."

Professor Festenstein thinks that Friedreich's ataxia is just the beginning. "We see this as a potential proof of principle, that other diseases caused by a similar mechanism might also be amenable to this kind of treatment," he says.

"It has taken quite a while to get to this point, but it is looking very promising in terms of being able to break into disease areas that previously have been completely intractable to therapies. And the use of the mice was crucial in this process."

Professor Richard Festenstein,
Department of Medicine

A still image from a Google Expedition around Imperial's animal research facilities. The Expedition will be used by teachers and school pupils in classrooms around the world to learn more about scientific research with animals.

SUPPORTING RESEARCH WITH ANIMALS

Central Biomedical Services

Central Biomedical Services (CBS) provides the infrastructure and support for animal research at Imperial. Its staff are responsible for the day-to-day care of animals, and the training of its own animal technicians and researchers in the most appropriate ways to work with animals.

Facility refurbishment

For CBS staff this year, the animals in their care and the researchers they support have been shaped by refurbishment works at the Hammersmith and South Kensington facilities. At Hammersmith, mid-2016 saw the completion of a suite of seven new rooms, including accommodation for small animals, a new operating theatre and pre-operating room.

Staff at South Kensington, meanwhile, spent the early part of 2017 focused on a complex 'decant' of animals, equipment and staff, in preparation for the extensive refurbishment of the CBS facility that began in April. While some space remains operational in South Kensington, many staff and animals have moved to facilities at other Imperial campuses.

When complete in early 2018, the new facilities at South Kensington will feature state-of-the-art washing and sterilisation automation as well as specifically designed research space to accommodate the increasing complexity of the projects undertaken. Animal holding areas will be upgraded to the latest design and décor to provide a comfortable and pleasing environment for both animals and staff, complete with new caging and associated support equipment.

Environmental Enrichment Committee

In 2015, CBS technicians established the Environmental Enrichment Committee with a goal of standardising and improving initiatives to maximise the quality of life of the animals in their care. Such initiatives have included improved housing (e.g. nesting materials and co-habitants); food; and devices intended to stimulate natural behaviour.

During this year, the Committee has focused on establishing clear processes for evaluating enrichment initiatives, to ensure that animals across all CBS sites can benefit from enrichment approaches that have been researched and trialled by CBS staff.

Using these improved processes, the Committee is currently working on a trial to utilise the mouse enrichment tube as an aid for handling and examining mice rather than using conventional methods. Recent evidence has shown that this can greatly reduce stress levels potentially caused by mouse handling. The Committee will also be reviewing enrichment options for zebrafish.

Mr Phil Rawson, Senior Technician and Named Animal Care and Welfare Officer, was awarded a Provost's Award for Excellence in Animal Research for his environmental enrichment work, as detailed on page 7.

> "The group discussed environmental enrichment, animal research governance and ethics, and the College's work on the 3Rs."

Student visit

In May 2017, Professor Maggie Dallman, Chair of the College's Central Animal Welfare and Ethical Review Body, welcomed a group of students from the Animal Protection and Education Society (APES) to the CBS facility at Hammersmith. APES, a society affiliated with the Imperial College Students' Union, promotes compassion, respect and justice for animals through educating, advocating and fundraising work.

Accompanied by the Site Manager, Senior Technician and CBS Director Mandy Thorpe, the students and Professor Dallman viewed and discussed facilities, including cages and floor pens for rats and rabbits respectively and the cage wash automation system. The group discussed environmental enrichment, animal research governance and ethics, and the College's work on the 3Rs.

During the same month, the CBS team manned a stand depicting the work they do to thousands of visitors at the Imperial Festival. Turn to page 31 for more information.

Welcoming a new NTCO

The Named Training and Competency Officer (NTCO) has a critical role in any organisation undertaking research with animals. He or she is responsible for ensuring that all those dealing with animals are appropriately educated, trained and supervised until they are competent and that they continue to undertake appropriate further training to maintain their expertise.

In April 2017, Mark Freeman joined Imperial as its new NTCO, with responsibility for these areas across all CBS sites. Mark has worked for over 25 years as an *in vivo* scientist in academia and industry; he has worked in the fields of oncology and respiratory disease, and particularly in inhalation toxicology and safety pharmacology.

Of his new role, Mark says: "I have always had a passion for training and developing staff, so the role of NTCO, where I can combine this with my experience, was always my ideal job."

COMMUNICATING

Telling the stories of research with animals

In May 2017, the signatories of the Concordat on Openness on Animal Research in the UK, which guides how Imperial communicates about its research with animals, celebrated its 3rd anniversary.

Imperial was a founding signatory of the Concordat, and this year the College has continued to build its communication about animal research and its impact, while exploring new and innovative approaches to sharing stories of our research and animal care with the public.

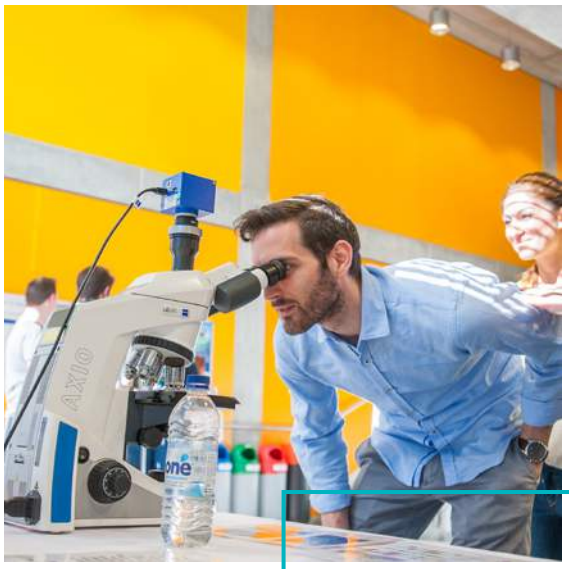
As the features in this Report illustrate, some of the most impactful research taking place at Imperial, with the power to transform lives, is made possible by working with animals. In reporting on this research in news stories and press releases, we are clear where, how and why animal studies have been involved, and we also emphasise where our researchers are developing techniques to reduce or replace the need for animals, or refine techniques to improve welfare of the animals.

This year, our researchers and senior staff have participated in the public conversation on research with animals in several ways. The College joined other institutions in proactively releasing data about the number of animals used in 2016; we have hosted visits of media organisations to our mosquito labs to help tell the story of our malaria research; and our academics have been interviewed by media organisations about their research and use of animals. In addition, we have hosted colleagues from Brazil and the Netherlands who are engaged in animal research and its governance.

Outreach and public engagement

Once again in 2017, staff from Central Biomedical Services (CBS) demonstrated the work that they do at the Imperial Festival, by showcasing typical animal care equipment and environmental enrichment products. Visitors to the stand, located in the Superbug Zone, had the opportunity to talk to CBS staff about Imperial's work with animals and see some of the features of our animal facilities.

Alongside the Festival, the College has also focused on expanding outreach activities around animal research to schools. Year 12 students from schools which are engaged with the College have attended Understanding Animal Research Days, involving visits to animal research facilities, workshops and talks with researchers about research with animals.



> "The College joined other institutions in releasing data about the number of animals used in 2016."

A unique collaboration with Google

The expansion of our activity with school students at our campuses during this year inspired the College to collaborate with Google on an Expedition around Imperial's animal research facilities, giving curious school pupils and teachers across the world the chance to find out more about our work.

Imperial suggested to Google that we could develop a Google Expedition around Imperial's animal research facilities, as a way of giving virtual access to these facilities to curious school pupils and teachers across the world. Google Expeditions is a collection of linked virtual reality content and supporting materials that can be used alongside an existing school curriculum. These trips comprise virtual reality panoramas and 3D images annotated with details, points of interest, and questions that make them easy to integrate into the curriculum already used in schools. The expeditions are used by around one million students globally and the number of users is growing.

Imperial's animal research expedition enables students and their teachers to explore six different animal research labs at Imperial and learn more about the work that goes on in each. Along the way, it gives information about why scientists work with animals, what the benefits of this work can be, and the importance of providing the highest standards of care for the animals.

In addition to visiting the six animal research labs, students can also go to Imperial's outreach lab to watch other school pupils learning about animal research, through an experiment involving water fleas. They can also visit an Imperial scientist, Professor Sian Harding, working with a patient with a heart condition who advocates for the potential benefits of animal research for improving human health.

The Concordat on Openness on Animal Research

By signing the Concordat on Openness on Animal Research, Imperial has made the following commitments:

Commitment 1

We will be clear about when, how and why we use animals in research

Commitment 2

We will enhance our communications with the media and the public about our research using animals

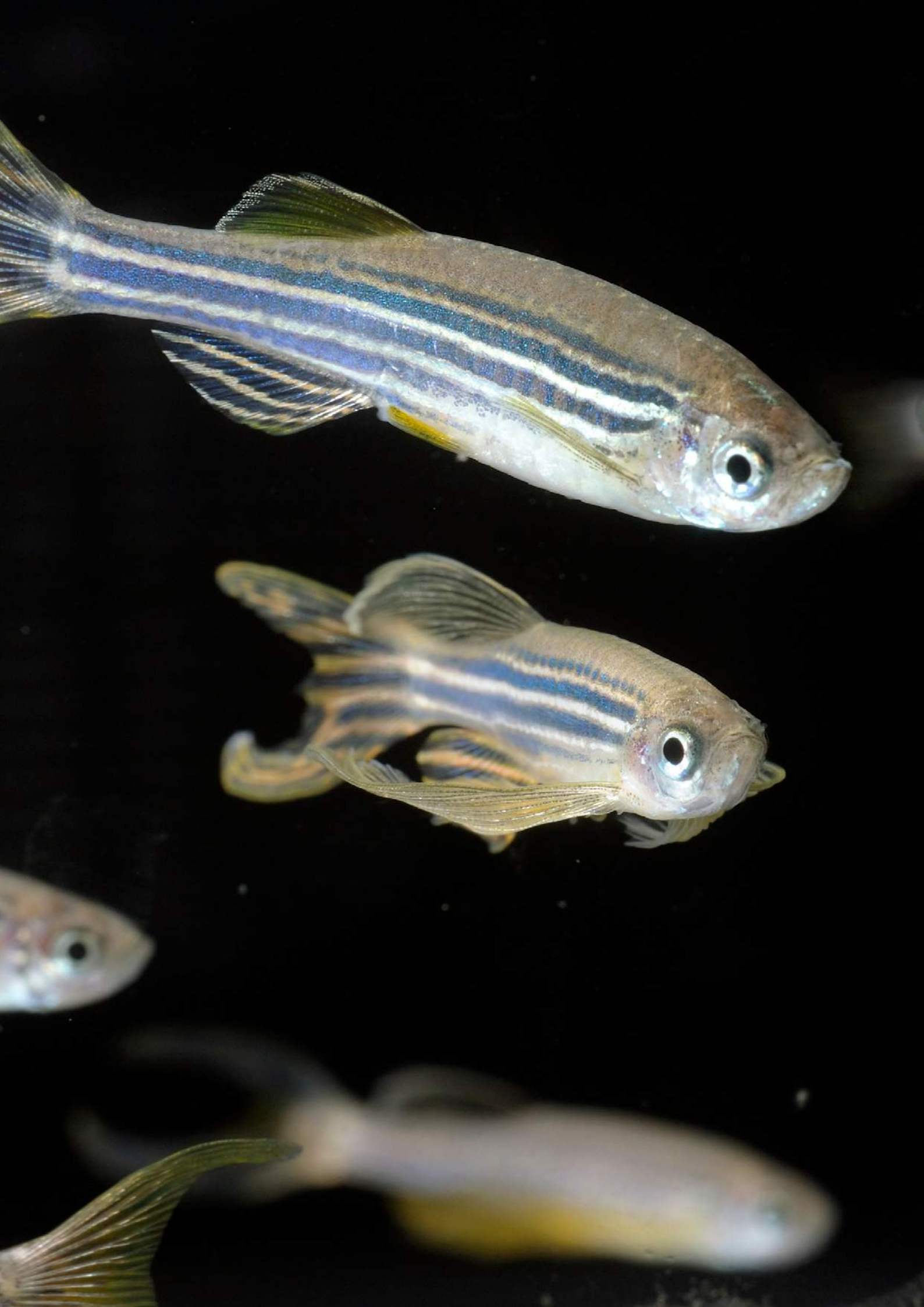
Commitment 3

We will be proactive in providing opportunities for the public to find out about research using animals

Commitment 4

We will report on progress annually and share our experiences

Editor: Joanna McGarry
Writers: Ian Mundell, Kerry Noble
Photography: Jason Alden, Science Photo Library
Design: Communications and Public Affairs, Imperial College London



www.imperial.ac.uk/animal-research