

Of mice and men – are mice relevant models for human disease?

Outcomes of the European Commission workshop 'Are mice relevant models for human disease?' held in London, UK, on 21 May 2010

Executive Summary

Species like yeast, flies, fish and mice have many genes in common with humans and are therefore considered 'model organisms' and are widely used in research to study human genes and human diseases.

Mouse research has led to major advances in our ability to treat a number of serious diseases and conditions. For example, work on mice resulted in successful treatments for a cancer (acute promyelocytic leukaemia, or APL) that was previously largely untreatable. The EU has long been aware of the usefulness of mice in research and since 1998 has invested EUR 550 million in over 180 projects involving mouse models.

Mice are not always reliable as preclinical models for human disease and the scientific literature is littered with examples of drugs that worked well in animals but turned out to be ineffective in clinical trials on humans. These failures cost the pharmaceutical industry millions of euros.

In May 2010, the European Commission held a one-day workshop on the usefulness of using mice in research into human health and diseases. The purpose was to identify the bottlenecks and limitations in using the mouse as a preclinical model and to find alternative ways to generate more reliable, robust and cost-effective mouse models for clinical research and drug development.

During the day, it became clear that researchers in academia and industry as well as clinicians are all using mice extensively in their work. What makes the mouse so special is how similar its genome is to the human genome (99% of human genes are conserved in the mouse), the availability of a unique battery of sophisticated molecular and genetic tools, and the animal's small size, all of which facilitate large scale/ high throughput studies and make it a cost-efficient model providing functional information on human genes in health and disease. In fact, the potential of mouse models to make medical research, and in particular drug development, more efficient could be increased by solving a series of research, intellectual property rights (IPR), communication, training and regulatory bottlenecks.

1. Introduction

Although they look very different to us, species as diverse as yeast, flies, worms, zebra fish, dogs and mice share a lot of genes and molecular pathways with humans. Therefore, by studying these so-called 'model organisms', researchers can learn a lot about human biology and human diseases and health problems.

Indeed, 99% of mouse genes have an equivalent in humans, making mice ideal for studying the function of human genes in health as well as diseases such as cancer, cardiovascular diseases and diabetes. These multifactorial diseases are in part due to mutations in our genes.

Although there are species (such as dogs, pigs and non-human primates) that are even more closely related to us than mice, working with these large animals is extremely expensive and is fraught with ethical concerns. With their small size and short generation times, breeding and keeping mice is comparatively simple and inexpensive. In addition, because they have been widely used in research for decades, researchers have built up a detailed understanding of mouse biology and genetics and developed large numbers of tools and techniques to study them. These powerful genetics tools are not yet available for larger mammals.

Recent years have seen a rise in the use of genetically engineered mice (often known as 'genetically engineered mouse models' or 'GEMMs') in research and preclinical studies. Some of these models can mimic a wide range of human diseases and health problems such as cancer and diabetes. In addition, the mouse is so far the only mammalian model in which it is technically possible to generate an organism in which a particular mouse gene has been replaced by its human counterpart. This 'humanised' mouse will produce and live with the human version of the protein. 'Humanised' mice can be created bearing a mutated version of a human gene known to be associated with a specific human disease. Such a mouse can be used to test the possible efficacy of a drug designed to bind to the relevant human protein.

2. The EU and research using mouse models

The EU has long been at the forefront of efforts to improve mouse models so that the data and knowledge they generate can be accurately applied to humans and contribute to efforts to tackle a wide range of diseases and conditions.

Since the start of the Fifth Framework Programme for Research and Technological Development (FP5) in 1998, the EU has invested over EUR 550 million in more than 180 research projects involving the use of mouse models. These have contributed to our understanding of the mechanisms behind many diseases. Some of these projects have pioneered the development and standardisation of new approaches to studying and characterising mouse models. Others have resulted in the development of new tools for high throughput research; as the term suggests, these tools allow scientists to analyse many samples extremely rapidly.

Some projects funded by the EU are part of wider international efforts in mouse research. For example, EUCOMM ('European Conditional Mouse Mutagenesis Program') represents Europe's contribution to the International Knockout Mouse Consortium (IKMC)¹. This global initiative is building a collection of mouse embryonic stem cells, each of which features a mutation in one of the mouse's 23 000 genes. Researchers can then use these cells to generate mice and study the effects of the gene that has been 'knocked out'.

EU-funded projects like EUMODIC² are also laying the foundations of the new International Mouse Phenotyping Consortium (IMPC). The IMPC is set to build on the work of the IKMC by investigating and characterising the physiology and behaviour of mice bred from cell lines created by the IKMC.

In addition, the EU also invests in research involving other model organisms, most notably zebra fish, rats and dogs.

¹ See www.knockoutmouse.org online.

² See www.eumodic.org online.

3. The event

Although mice are widely used in research, questions remain about their reliability as a model for human diseases. For example, many drugs work well in preclinical trials in mice but turn out to be ineffective when used in clinical trials on humans. Getting a potential drug to the stage where it can be tested on humans takes a long time and is extremely expensive, so substances that fail at this stage of the process cost the pharmaceutical industry a lot of money.

To tackle this and related issues, and to discuss how mouse models can be improved, the European Commission organised a workshop entitled 'Are mice relevant models for human disease?', held at the Royal Society in London, UK, on 21 May 2010. The event brought together around 100 researchers, clinicians, industry representatives, regulatory agencies and policy makers. Throughout the day, a series of presentations and discussions shed light on four key questions:

1. The mouse model is the one most commonly used for studies of human physiology and disease, but is it the best one?
2. How useful and valid are these models for mimicking human disease?
3. What characterises mouse models that have proven useful for basic science, clinical research and drug discovery?
4. What measures are needed to improve new and existing mouse models?

The outcomes of the workshop will contribute to the European Commission's thinking with regard to future calls for research proposals involving model organisms.

4. Successful use of the mouse as model of human diseases

Researchers in academia, clinicians and the pharmaceutical industry are already using mouse models extensively in a variety of ways, from very basic research on disease mechanisms right through to preclinical trials.

A number of examples presented at the EU workshop and the preceding CASIMIR workshop³ demonstrate the many ways in which the mouse is contributing to our understanding of diseases and leading to the development of new treatments.

Making an untreatable disease treatable – mice in the study of diseases and the validation of treatments

One of the most striking examples of how the use of mouse models can save human lives comes from Professor Pier Paolo Pandolfi of Harvard Medical School in the US. Early in his career he discovered the genetic mutations responsible for acute promyelocytic leukaemia (APL). APL is a cancer of the bone marrow that is most common in younger people (the majority of patients are aged between 15 and 55). Until recently, it was extremely difficult to

³ The EU-funded CASIMIR ('Coordination and sustainability of international mouse informatics resources') project held a workshop entitled 'Tools and resources for the creation and validation of mouse models' in the two days preceding the European Commission's workshop.

treat and most patients succumbed to the disease soon after diagnosis. Today, thanks to extensive research involving GEMMs, most patients are completely cured.

Professor Pandolfi's research revealed that there are in fact six types of APL, each caused by different gene fusions. He then created genetically engineered mice that mimicked the different types of APL. Using these mice, Professor Pandolfi's team was able to determine which treatment regimes were effective against the different kinds of APL. Meanwhile, he worked closely with the medical community, gaining the doctors' trust and convincing them of the reliability of the results he was generating in his GEMMs. Eventually, through co-clinical trials in mouse and human, the drug combinations and regimes that had proven successful in mice were tested then in humans. The results were clear; even patients with the deadliest form of the disease went into remission. 'Now we have three targeted therapies and we have learnt from mice how to use them,' exclaimed Professor Pandolfi. 'Now hardly anyone dies from [APL] at all!'

GEMMs shedding new light on breast cancer

Jos Jonker of the Netherlands Cancer Institute has been using GEMMs to study breast cancer. He showed that GEMMs bearing the human breast cancer gene BRCA1 behave more like human cancer patients than xenograft mice, in which a sample from a human tumour is transplanted into a mouse. 'This humanised mouse model nicely mimics many features of human breast cancer,' he said. Crucially, his work with GEMMs allowed him to identify treatment regimes that delayed the onset of resistance to treatment. When these regimes were tested in human cancer patients, the results were the same as in the mice.

A possible drug for pain – using mice to find new drug targets

American biotech company Regeneron Pharmaceuticals is a pioneer in the development of technologies for generating GEMMs. Today, it has a leading role in the Knockout Mouse Project (KOMP), the US contribution to the IKMC. 'We use animals to learn about the biology and test drugs,' explains the company's Associate Director, David Friendewey. Having created a knockout mouse, Regeneron runs a series of simple tests to determine the effects of the faulty gene. The results of this primary screen are sent to Regeneron's therapeutic focus areas; if they are interested, they run more intensive tests.

The primary screens have already come up with some surprises; for example, the system uncovered a gene involved in pain that no one knew about. As a result, Regeneron quickly set up a pain focus area and is now developing drugs to treat chronic pain. The system also identified a gene involved in the development of the valves in our blood vessels that stop blood from flowing backwards.

From knockout mouse to anti-obesity drug

Knocking out the leptin gene in mice demonstrated the role this hormone has in regulating appetite and, by extension, preventing obesity. Nowadays, leptin is used as a treatment for people suffering from a certain kind of obesity.

Testing treatments – using gene therapy to treat diabetes

Cases of diabetes are on the rise worldwide, yet treating this condition remains difficult. Fatima Bosch of the Autonomous University of Barcelona in Spain developed a gene therapy treatment for type 1 diabetes in mice in which the diabetic mice were injected with viral vectors containing healthy genes. The mice were completely cured. Following the success obtained in mice, Professor Bosch went on to prove the effectiveness of this gene therapy in dogs. Clinical trials in humans are now planned. As well as paving the way for a

treatment for diabetes, this case study highlights the fact that mice can be used to develop treatments for diabetes.

Making sense of genome-wide association studies

In a genome-wide association study (GWAS) the genomes of thousands of people are scanned for genetic variations that may be linked to a certain disease or condition. However, while a GWAS will flag up the genes that are associated with a health problem, they do not provide information on their functions. Today, about 1 000 genes are known to be associated with human diseases, but for the vast majority of them the potential functional mechanisms underlying the diseases remain unknown. Studies in mice can help researchers to identify gene function(s) and determine how different mutations known to affect human health are interfering with these functions. As Stuart Cook of Imperial College London in the UK points out, GWAS studies on humans are generating reams of data. 'This will generate a lot of work for people who work with mice,' he notes, adding that mice are needed to determine gene function and identify disease mechanisms.

5. Challenges

The overwhelming message from the workshop is that mouse models have much to contribute to our understanding of health and disease and the development of new treatments and diagnostic tools.

Although mouse models are not perfect, most participants at the workshop were clear about their benefits. 'We know that the mouse is a good model – and this will show from our research,' said Dr Friendewey.

'Have GEMMs been used to guide clinical programmes? I can emphatically say yes!' added Leisa Johnson of US biotech company Genentech, which uses mice to study disease biology, identify possible drug targets and design clinical trials. Other industry representatives added that mice allow the rational design of clinical trials, they use mouse models to test the safety and efficacy of potential drugs, among other things.

Meanwhile, regulatory bodies such as the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) are increasingly interested in the results of experiments involving GEMMs.

But what improvements to mouse models are needed? While many recommendations focus on the technical aspects of research, many key outcomes of the workshop concern the way research is carried out and the links between academia, industry and clinicians.

Improving mouse models

Many speakers commented that 'the best model for human disease research is the human.' Nevertheless, as few experiments can be easily and ethically carried out on humans, mice make a very good substitute. It is generally accepted that there will never be one single, perfect mouse model for each disease. Instead, different models will be used to study different aspects of a given disease.

As Dr Friendewey commented, 'the mouse is the best model system we have for developing an understanding of human biology, but we should not oversell it.'

During the panel discussions, industry participants were clear about what they expected from mouse models produced by academia. 'We want deep, rich, reliable, high quality data,'

said Dr Friendewey. 'We need a basic understanding of mammalian biology to guide our decisions.' According to Steve Wedge of Astra-Zeneca in the UK, industry needs models that are 'reproducible and robust,' further adding that 'we also need models that are informative about disease biology and maybe reveal new targets.'

Moreover, large-scale research studies should also take into account ageing, environmental factors and genetic variability.

Most mice used in research are rather young, yet many of the diseases that are of greatest interest to researchers (such as cancer and heart disease) are most common among the elderly. This raises financial issues as keeping mice until they reach old age costs money. Nevertheless, ageing is an increasingly important political issue, as governments face up to the challenge of caring for an ageing population.

Questions were also raised regarding the environment in which mice are raised. An individual's appearance and behaviour are determined by both genes and the environment. Researchers investigating the genetic aspects of disease need to take environmental factors (e.g. nutrition, infectious diseases, stress and exercise levels) into account. As Eero Vuorio of the University of Turku in Finland pointed out, the lifestyles of laboratory mice are far from natural. 'We are studying mice that are stationary or living in a little cage,' he commented. 'If a mouse has a running wheel, it runs 5 km every night!'

The issue of genetic variability was also debated. 'One of the biggest challenges we are facing is how to apply information from one organism to a diverse human population,' said Igor Pogribny of the FDA. At the same time, researchers need to bear in mind that genetic variations among mice can affect results, and whilst genetic background can be very informative about modifier genes.

The need to facilitate the dynamic and effective exchange of information between human clinical trials and mouse trials was one of the key subjects of the debate. Ideally, there should be a constant flow of information from the mouse model to human patients, and back again. Professor Pandolfi enthusiastically set out the idea of 'co-clinical trials', in which mice suffering from a disease are treated in a 'mouse clinical space' in the same way as human patients are treated in an ordinary hospital. Mice and humans alike would follow the same treatment protocols, and the outcomes in both clinics would shed light on the progress of the disease and its response to the treatments. Although it takes time to create the right genetically engineered mice, Professor Pandolfi pointed out that 'the lag is still faster than any clinical trial.'

Concerns were also expressed regarding the extent to which GEMMs mimic human diseases. Many human cancer patients are on lots of different drugs and have tumours that have become resistant to certain medicines. 'In our phase I trials, we go into relapsed, refractory and resistant disease and models don't reflect these patients,' explained Dr Johnson. Mouse models may probably be more reliable when addressing early stages of the diseases which are rarely included into clinical trials. Furthermore, Dr. Johnson added that there are question marks over the doses given to mice. 'We often see publications where drug exposures are so far off what we would use in the clinic that it's not translatable,' she said, adding that 'mice can help us understand these things.' Furthermore, many mouse models used in research do not have a fully functioning immune system, which is far from the situation found in human patients.

There was also support for using mouse models in new areas, such as the development of therapies for mental disorders or in toxicological studies. On this last point, rats are currently preferred for toxicology tests for the simple reason that they are larger animals and have a greater volume of blood on which tests can be performed. Investing in research to

miniaturise toxicology tools and tests would allow for greater use of mice in toxicology research.

Mice should also be used more extensively to validate GWAS, particularly those in neglected areas such as mental illness. For example, addiction to smoking and alcohol is a major cause of disease worldwide. GWAS findings could shed new light on this important area. Mental illnesses such as schizophrenia and depression are strongly influenced by our genes; developing mouse models for these complex conditions would be far from easy, yet there is an urgent need for new treatments in this area.

Facilitating the use of GEMMs

Intellectual property rights (IPR), communication and training were seen as major topics that need to be urgently addressed in order to build bridges between mouse researchers and the end-users of mouse models.

Many industry representatives complained about the high fees charged by academics for their mice. 'Pharma is no longer a bottomless pit of money,' said one meeting attendee, warning that if costs continued to rise, industry would simply find another solution, such as making their own GEMMs for example. Tania Bubela, an intellectual property (IP) lawyer and researcher at the University of Alberta in Canada, recommended that researchers resist the urge to patent mouse models and consider simpler, more effective ways of partnering with industry. 'Don't patent research tools and methods, such as mice and methods for creating GEMMs, generated from public funds,' she advised. For example, a 1999 NIH policy discourages the filing of patents on mice as research tools generated from intramural research programs.

Research institutions, funding agencies and journals should carefully consider implementing the Rome Agenda stemming from a CASIMIR lead meeting and published in *Nature* in 2009. That document made concrete recommendations for mouse research on post-publication sharing of data and bioresources, including mouse models. Wherever possible, mouse models should be shared under the least restrictive terms possible. For example, the Jackson laboratory facilitates the distribution of mice to researchers with a simple notification that the mice are to be used solely for research purposes and are not to be sold or transferred to third parties without permission.

If necessary, IP agreements should be negotiated up front and contributions need to be realistically assessed, she said, cautioning that neither academia nor industry should overestimate their respective contributions to a project. She pointed out that a major problem is the conflict between the incentive structures in academia and industry, adding that funders can do a lot to ensure that incentive structures are aligned around the common goal of drug discovery

The problem of poor communication among researchers in academia, industry and clinics came up time after time during the workshop. John Hickman of the Institut de Recherche Servier in France is involved in the Innovative Medicines Initiative (IMI), which brings together researchers from industry and academia. 'When the academics met the two of us from industry, they seemed to think we were pressing buttons and doing screening, and were surprised we were doing interesting biology,' he remembered. 'So I hope [the IMI] will have a cultural impact as well.' He also mentioned that mouse research should be put right at the very beginning of the drug discovery process, where it has not been previously - before drugs have been made - in studies to 'validate' potential drug targets. Tumours from mice that molecularly and phenotypically resemble patient cohorts could be used to provide material for complex in vitro platforms, such as tissue slices, thus allowing researchers to

perform studies of tumour biology and biochemistry under conditions which better reflect the complexity of a human tumour.

The importance of appropriate training for clinicians and researchers working with human and mouse tissue samples was also emphasised. As Alexander Nikitin of Cornell University in the US explained, all too often pathologists with different backgrounds classify the same tumours differently. 'Their findings are not always consistent,' he pointed out, citing the example of bronchioloalveolar carcinoma, which looks different in mice and humans. 'It is dangerous because people can come to the wrong conclusions. The shortage of pathologists with experience in mouse pathology is an important problem. Human pathologists don't seem to realise that organs in mice are not the same as in humans!'

According to one participant, many problems with mouse models in the past can be attributed to the fact that the models were poorly characterised and researchers were looking at mice with the wrong disease. He is trying to redress this imbalance by running courses in mouse pathology. Another participant reported on an initiative to standardise classifications between mouse and human pathology. Although this experience was 'very rewarding,' some issues could not be resolved and some words remain different.

Nadia Rosenthal of the European Molecular Biology Laboratory (EMBL) told attendees about her organisation's training programmes. 'We need to be able to collaborate across communities,' she said. 'The training of clinicians has had a great effect and we're proud of how many people are using mice that otherwise wouldn't be.'

'To my way of thinking there is only one answer and that is we need to get greater proximity between clinical medicine and other specialities,' commented Sir Magdi Yacoub of Imperial College London in the UK, offering a clinician's point of view. 'The time has come for a unified approach to the problem.'

The IMPC – getting to grips with a mammalian genome

It is clear that mice are invaluable in allowing researchers to probe the precise function of individual mammalian genes, including those that have been linked with disease. A major development in this respect is the establishment of the International Mouse Phenotyping Consortium (IMPC), which plans to elucidate the function of every single gene in the mouse genome. Under the IMPC, scientists will take mice in which one gene has been deactivated and run a battery of tests to identify physical, biochemical and behavioural differences caused by the faulty gene. Information and data arising from the IMPC will be made freely available to other researchers.

EU-funded projects such as EUMODIC are laying the groundwork for the IMPC by assessing the feasibility of different screening procedures. EUMODIC has highlighted the importance of standardising the way tests are carried out so that results from different laboratories can be easily compared. Initial data from EUMODIC suggests that the tests used so far are effective at revealing gene function.

Several questions still remain to be answered concerning the IMPC's research strategy and goals. For example, should the project first focus on a smaller number of knockout mouse strains to obtain more in-depth information? How much effort should be spent on analysing ageing mouse colonies? What challenges (such as infectious agents) should the mice be exposed to? How should the impacts of the animals' environment be studied?

Ultimately, researchers and research funders will have to decide together on the battery of tests that will offer the greatest benefits for our understanding of gene function in both health and disease. Several representatives of the pharmaceutical industry (Pfizer, Servier,

Genetech and Regeneron) expressed their support for the IMPC and registered their interest in participating in the debate concerning the tests to be included in it.

The US National Institutes of Health (NIH) announced at the meeting that it planned to commit USD 110 million over the next 5 years for projects in line with the objectives of the IMPC.

6. Key outcomes

- The mouse is the most common model organism for preclinical studies even though it has not proven particularly reliable at predicting the outcome of studies in humans.
- Mice have many advantages over other model organisms: their genome is similar to the human genome (99%), a good genetic/ molecular toolbox is available and the animal's small size facilitates large scale/ high throughput studies making it a cost-efficient model. Therefore, its potential for making medical research and in particular drug development more efficient could be increased by solving a range of identified bottlenecks.
- Mouse models have been successfully used to validate drug targets and to determine efficacious and safe dosage schemes for combination treatments in humans. These cases have one factor in common: they do not aim to fully model a disease or disease mechanisms, but rather set out to obtain specific functional information.
- Genetically modified mice need to be validated, reproducible, robust and cost-effective to be considered optimal by the pharmaceutical industry. Those who succeed in producing such models can make good money out of it, Regeneron being a good example.
- Transgenic humanised mice could provide good preclinical screening and safety testing models for use in lead identification and optimisation.
- The large-scale phenotyping of genetically engineered mice can provide valuable information on gene function which is relevant for human health and disease. It is clear that the newly launched IMPC will play an important role in driving this work forward. Meanwhile the industry, which until now has not participated in these kinds of initiatives, appears to be interested and may become involved in the future. However, the IMPC should also include challenges (e.g. infectious agents, nutrition), ageing mouse colonies and/or embryonic developmental studies.
- The use of mice in clinical studies has proven effective in a number of cases. Dosage regimes of new treatments or treatment combinations can rapidly be optimised by co-clinical trials with mice, allowing fast application in humans with greater patient safety. This provides increasing opportunities in particular for the rapid development of treatments for very rare diseases where low patient numbers otherwise hamper the creation of clinical trials.
- One of the key bottlenecks that needs to be solved concerns the aggressive patenting strategy (including the patenting of mouse genes and broad based methods patents) and overly restrictive licensing terms in Material Transfer Agreements which hamper the construction, sharing, use and proper exploitation of mouse models.
- The efficient development of mouse models should involve collaborations among academia, clinicians and industry. The IMI was presented as a good model for this kind

of collaboration, which would ensure that end users' needs and constraints are taken into account in the early development of the models.

- There is a huge need for specialist training programmes for mouse pathologists and also for human pathologists and clinicians to increase their understanding of the opportunities and limitations of mouse models.
- Representatives from the EMA and the FDA confirmed that data stemming from mouse models will be taken into account wherever the relevance has been clearly proven within the given context.
- A final discussion point was the issue of standardisation of phenotype descriptions, databases and data sharing between academia and industry.

The workshop was considered one of the few meetings where mouse researchers and end users from industry and clinics had the opportunity to exchange their respective expectations and experiences on mouse models. As a result, the different communities have a better understanding of each other's needs and a greater willingness to collaborate more closely in the future.