To guarantee drug safety and efficacy, regulatory agencies recommend testing drugs and other chemicals in two different animal species. Testing is initially done in a rodent and then in a larger, non-rodent mammal. Most research laboratories rely on mice experiments. Mice have a similar genome to humans, are relatively cheap, have a fast reproductive rate, and a short life span. Therefore, they are considered a suitable animal model for initial trials in drug discovery.

Dogs are usually the second species selected for safety assessments of new medicines. A dog’s metabolism and response to drugs is closer to human responses.

However, what happens when a drug experiment succeeds in one animal model but fails in the other? Are animal models a fit-for-purpose strategy for advancing drug discovery? With the recent reduced success rates in drug development, how can we deal with a last-minute revelation that a particular animal was not the ideal model to study a specific drug? These are just some circumstances that make us question if our scientific process is sound.

The importance of animals in research

Animals have played a vital role in many medical and scientific advances of the past century. Due to the role of animals, insulin, penicillin, and the polio vaccine have been discovered, just to name a few examples. Scientists can reproduce human disorders in distinct animal models and reproduce manifestations, mimic pathophysiology, and use drugs to cure the condition. The use of animal models in research is a very complex and an important topic to be discussed between scientists and also with school children.

To guarantee drug safety and efficacy, regulatory agencies recommend testing drugs and other chemicals in animals and submitting a document with all the relevant information collected. The final study report must disclose all details of the study (study raw data and conclusions, name of the researchers, signatures, dates) and a summary. The document should, among other things:

- Discuss the number of animal studies conducted
- Specify the number of animals used
- Justify the rationale for the model selected
- Describe the similarities of the selected model compared to humans and the methodology used

Grant applications also require detailed disclosure of research on animals. The applicant must demonstrate that the animal facility is adequately equipped and trained staff is available. Methods and Research Design sections must discuss how the animals will be treated and justify the selected species and number of animals that will be used.

Animals have played a vital role in many medical and scientific advances of the past century.

Ethics in animal research has increased significantly over the past few years. Yet approval of new technologies for animal substitution is growing slowly. There are a lot of arguments by scientists who are familiar with traditional methods and are still not convinced by the benefits of new technologies. Scientists want — rightly — further validation to justify animal replacement. By doing research on animals, scientists avoid risking human lives in the initial phases of drug discovery. Initial experiments are invasive and could result in human death. There is a safety risk even after animal testing, but scientists argue that this risk is significantly higher if we do not test on animals first.

The European Animal Research Association describes forty reasons why animals are needed for biomedical research. Briefly, many important scientific findings, such as development of vaccines and drugs, relied on
animal data suggesting that these findings would never happen without animal research. Another reason is that animals and humans are very similar physiologically and perform tasks in a similar way.\(^5\)

An open letter from the Confederation of Spanish Scientific Societies exists detailing why animals cannot be substituted in the fabrication of antibodies.\(^6\) The authors argue that by using animals, scientists can generate antibodies with higher affinity and specificity than those generated using other methods and that substitution of traditional technologies requires further scientific validation.\(^6\)

Animal experiments can only be conducted after a harm-benefit analysis and approved by authorities. European and American committees have created guides to guarantee that the animal experiment is scientifically, technically, and humanely appropriate.\(^7\) When planning animal experiments, scientists should apply the 3Rs principle (replacement, reduction and refinement) (see Table 1).\(^8\)

### Drug development challenges

Drug development has stagnated for years, mostly because costs and time required for discovery are increasing. Pharmaceutical companies deal with many challenges during new drug identification, such as not knowing the cause and mechanism of many human disorders and the lack of good models of human disease.\(^9\),\(^10\)

Research for a new drug begins in the laboratory with *in vitro* experiments (e.g. using commercially available cell lines) and animal testing to answer basic questions and to understand diseases. However, humans are complex organisms. We differ greatly from single cells cultured in a plastic dish or mice, dogs, or any other animal used for scientific experiments.

Scientists are investing in improving mice models to best reflect human responses. These models are called “humanised” mice.\(^11\) Despite the capability of circulation of human-derived cells in these novel models, mice organs, central nervous system, and muscles are not altered. The question that arises is: why should we spend money and years of research improving an animal model that will never develop and respond to diseases exactly the same as humans? A missing gene, protein, or enzyme in an animal model could ruin drug discovery and innovation. While these models are useful and have contributed to a better understanding of disease mechanisms, science needs additional innovation to complement or even substitute animal models to advance drug discovery.

Agencies like the European Union Reference Laboratory for Alternatives to Animal Testing and other research laboratories and centres around the world are doing critical work showing that we have plenty of options to substitute

<table>
<thead>
<tr>
<th>3Rs</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Replacement</td>
<td>Methods that avoid or replace the use of animals</td>
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<tr>
<td>Reduction</td>
<td>Methods that minimise the number of animals used per experiment</td>
</tr>
<tr>
<td>Refinement</td>
<td>Methods that minimise animal suffering and improve welfare</td>
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Table adapted from\(^8\)
animals in research with better results when compared to animal models.\textsuperscript{12,13} We need more laboratories implementing these new techniques, to show the specificity and reproducibility of the results until these novel approaches are fully accepted by the scientific field, especially government agencies.\textsuperscript{1}

Experiment reproducibility in animal research

Concerns on the low reproducibility rate between different laboratories of pre-clinical results exist. It does not necessarily mean that the original finding was wrong but raises questions of what is correct.\textsuperscript{14,15} There are many reasons why an experiment is not reproducible, from laboratories not sharing the complete list of research materials used, to poor research design, to differences in animal experiments.\textsuperscript{15} A survey carried out by Nature in 2016 found that more than 70% of scientists have failed at reproducing other scientists’ experiments.\textsuperscript{16} When scientists around the world can obtain the same results, this gives strength to the original work. Therefore, to standardise result reports, development of principles and guidelines for reporting preclinical research has been developed by governmental agencies like the National Institutes of Health.\textsuperscript{14}

Like us, animals are directly impacted by their surroundings. Availability and type of food and habitat can impact our behaviour and response to treatments. Animals created at distinct research centres are fed and treated in different ways, which impacts how animals develop a disease and respond to treatment.\textsuperscript{17}

Mice have higher anxiety when picked up by their tails, which is the most common method of mice capture and handling. This can significantly influence experimental results. Therefore, research groups are investigating the best way to hold mice so they are not stressed during an experiment. Using acrylic tunnels to carry the mice without direct human contact or allowing the mice to freely walk on the handler’s open hand without restraining favours less stressed mice during experiments.\textsuperscript{18} This indicates that if a researcher is not careful on a particular day and holds the animals in an inappropriate manner, this may lead to different results when compared to someone that handles the animals with care.\textsuperscript{19}

Important genetic differences between humans and animals

Humans constitute the taxonomic order primates, which include lemurs, lorises, tarsiers, monkeys, and apes. Humans are well known for being social, smart, communicative, and to have a remarkable cognitive ability. We have distinct anatomy, physiology, and cognitive behaviour. Our closest living relatives are chimpanzees. The genetic difference between individual humans is around 0.1%, and when compared to chimpanzees, this difference jumps to 1%. Still, non-human primates only account for 0.28% of all laboratory animals used in research in the USA. 90% of the animals used in research are mice, rats and other rodents, from which our genetic difference can reach up to 2.5%.\textsuperscript{20}

Due to evolution, almost every gene found in humans is found in a similar format in other mammals, making them models for studying disease and researching new drugs. Other animals like fish, flies, parasites – in some respects – also have similarities to humans, allowing novel findings in science. Large variation of specific gene families can be identified between humans and other animals.\textsuperscript{21} This is natural, it is evolution, and it highlights our differences.

Animals have different absorption, distribution, metabolism, and excretion (i.e. pharmacokinetics) of drugs when compared to humans.\textsuperscript{22,23} Curiously, pharmacokinetics, together with toxicology and safety are the main reasons for drugs failure in clinical trials.\textsuperscript{24} The Encyclopaedia of DNA Elements (ENCODE) Project (www.encodeproject.org) allows scientists to compare the differences and similarities between human and mouse genomes.\textsuperscript{21} By doing a direct comparison, scientists can pinpoint differences in the metabolism or immune system between species at the genome level and decide based on these differences if a murine model is indeed the best model to support their research, and this can help to reduce animals in research in a meaningful way.\textsuperscript{21}

Drug molecule and experimental animal waste

An overall estimate of global animal use in scientific procedures is around 80 million animals for 2015 alone.\textsuperscript{25} Potential drug candidates do not progress into clinical studies usually because of animal toxicity, while other approved drugs are later identified as potentially hazardous for human health, which causes drugs to be either relabelled or removed from the market. Animal studies alone can lead to loss of valuable drugs and subsequently waste of animals.\textsuperscript{23} This is one reason why we need regulatory agencies and private organisations to invest in non-animal alternatives to complement or replace animals in research.

A simple practical example is that dogs cannot eat grapes, and we still are not sure why. Grapes can cause severe reactions, lead to kidney failure, and ultimately the dog’s death. Similar danger is observed when dogs are fed chocolate. Let us pretend that chocolate is a new drug and regulatory agencies request proof that this novel compound is safe for human consumption. Scientists decide to test it on dogs as their metabolism is close to humans. After a few trials, it was identified that dogs presented panting, vomiting, tremor, hyperthermia, tachycardia, hypokalaemia, elevation of different enzymes, etc. Even after decontamination, 98% of the dogs that presented these symptoms died.\textsuperscript{26} The company ends up writing a detailed report with all the acquired data and reasons why chocolate could potentially be harmful to humans and, therefore, should not be sold. Further investigation into the chocolate chemical structure would also be considered during development of new products as a warning of possible toxicity and exclusion of new drugs with similar structures at the early stage of discovery.

The future of non-clinical testing in drug discovery

We understand more about mouse biology than our own human biology. Therefore, it is difficult to identify hidden threats and missed opportunities during research using animal models. Indeed, we cannot rely on ex-vivo experiments alone either, we need more options.

We need to generate robust data that reflects how the human body works and that can be systematically extracted, analysed, and applied in a specific field of research. Generation of in silico data combined with accurate in vitro data is one of the solutions. We should focus on improving our biotechnology devices and techniques. Dynamic culture,\textsuperscript{27} bioprinters,\textsuperscript{28} organoids,\textsuperscript{29} organ-on-a-chip,\textsuperscript{30} to name just a few. We also need to take advantage of advances in computing to upgrade in silico technology.\textsuperscript{31}

New technologies for antibody production that do not require animals have been proven to be a powerful animal substitute allowing generation of antibodies that would be extremely
difficult if using animals. The generation of antibodies that do not derive from mice has been shown to have increased therapeutic efficacy and can avoid detrimental consequences e.g. development of allergic reactions against mice generated antibodies.

Phage display technology, an in vitro antibody selection method, has been used to isolate antibody candidates to treat different diseases. Many antibodies developed by phage display technology have been recently approved by the FDA to treat different diseases like cancer. For instance, the PD-L1 inhibitor atezolizumab was approved in 2016 for bladder cancer and is currently in different clinical trials of other tumour types.

Our intention with this article is to acknowledge we still have a long way to go to completely stop using animals in research as we are still adapting. The scientific community upholds the highest scientific and ethical standards, and this article offers a perspective on that. Many countries around the globe have already established national centres dedicated to the development and validation of alternative methods, while government agencies are concurrently investing heavily in legislation and strategic roadmaps to allow drug approvals using in vitro and in silico methods. We have a tremendous amount of data from OMICS (genomics, transcriptomics, proteomics, metabolomics) and tools at our disposal to make scientific research cheaper, faster, and more relevant to human physiology, we just need more support from the scientific field.

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