Food for Thought ...

Beyond the 3Rs: Expanding the Use of Human-Relevant Replacement Methods in Biomedical Research

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Abstract

This year marks the 60th anniversary of Russell and Burch's pioneering book, *The Principles of Humane Experimental Technique*. Their 3Rs framework has helped to inspire humane and scientific progress in experimental technique. However, it is time to update its strategic application. The 21st century has already seen the development of promising, high-tech non-animal models, such as organs-on-a-chip and computational approaches that, in our view, will replace animals as the default option in biomedical experimentation. How fast this transition will take place will depend on the pace at which these new models are optimized to reflect the biology of humans, rather than that of non-human animals. While the new methods are likely to reshape all areas in which animals are currently used in science, we particularly encourage their application in biomedical research, which accounts for the bulk of animals used. We call for the pursuit of a three-prong strategy that focuses on (1) advancing non-animal methods as replacements of animal experiments, (2) applying them to biomedical research, and (3) improving their relevance to human biology. As academics and scientists, we feel that educational efforts targeted at young scientists in training will be an effective and sustainable way to advance this vision. Our strategy may not promise an imminent end to the use of animals in science, but it will bring us closer to an era in which the 3Rs are increasingly perceived as a solution to a receding problem. Russell and Burch themselves surely would have welcomed these positive changes.

1 Introduction

The Principles of Humane Experimental Technique, the landmark book that gave us the 3Rs framework of replacement, reduction, and refinement, turns 60 this year. First published in 1959, *Principles* was the outcome of a project spearheaded by the Universities Federation for Animal Welfare (UFAW), overseen by a committee that included future Nobel Prize-winning scientist Peter Medawar, and carried out by the British scientists William Russell and Rex Burch (Russell and Burch, 1959). The 3Rs framework helped to inspire and guide humane progress in experimental technique during the second half of the 20th century and beyond (Stephens and Mak, 2013; Balls et al., 2019).

The 60th anniversary of *Principles* falls in the midst of substantial developments in non-animal methods, i.e., potential replacement technology. Indeed, scientific experimentation is at the cusp of a new era of techniques hardly imagined in the mid-20th century. Relevant techniques include (among others) organs-on-a-chip (microdevices containing cells and fluids intended to simulate physiological processes in organs); organoids (three-dimensional spheroids containing multiple cell types and intended to simulate physiological processes); high-throughput systems (rapid screening of large numbers of chemicals for biological activity against panels of different cells or biomolecules); induced pluripotent stem cells (adult cells that have been genetically reprogrammed to an embryonic stem cell-like state); and computational modeling (using computation to study the behavior of complex systems).

In our view, these methods (and no doubt others in various stages of development) have the potential to replace the use of animals as the default option in both safety testing and biomedical research. That is, these methods will come to comprise the rule, with animal experiments being the exception. This is consistent with Dutch efforts to expeditiously end animal experi-

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mentation in that country, primarily through vigorous development and application of alternative methods (Netherlands National Committee for the protection of animals used for scientific purposes, 2016). The emphasis in the 21st century is likely to be on expanding and exploring the capabilities of these non-animal methods further and using them to better understand human biological pathways and the perturbations to these pathways that can cause disease.

In this perspective paper, we take the anniversary of *Principles* as an opportunity to reflect on these issues and comment on priorities that the 3Rs community and like-minded scientists should consider adopting as we move forward. We briefly describe the scale of animal use in science and comment on 21st century methods and approaches before introducing our overaching strategy and concluding with remarks regarding Russell and Burch.

2 The global scale and nature of animal experimentation

When Russell and Burch examined the "ecology of experimental animals," their focus was limited to Great Britain, where the government had long published fine-grained annual statistics on laboratory animal use. They estimated that almost 1.8 million vertebrates were used in 1955 (Table 4 in Russell and Burch, 1959). This number has increased to 5.53 million animals (Home Office, 2018). Although the latter part of the 20th century saw a sizable decline in animal use in Great Britain and elsewhere (De Greeve et al., 2004), that trend reversed when the genetic modification of animals became possible (Ormandy et al., 2009).

Only rough estimates exist for global animal use as many countries appear not to publish their animal use statistics. It was estimated that more than 127 million vertebrate animals were used worldwide for scientific purposes in 2005 (Knight, 2008) and this number has increased further since then (Taylor and Rego Alvarez, 2019). Novel, straightforward genetic modification techniques such as CRISPR will likely lead to a further increase in the number of genetically modified animals being created, bred and used, and also to genetic modifications of further species (Bailey, 2019).

In the European Union (EU), and perhaps elsewhere, basic and applied research accounts for most of the animals used in science, as indicated in the "Seventh report on the statistics on the number of animals used for experimental and other scientific purposes" released by the European Commission (EC, 2011). Other categories of use include production and quality control of devices and products used in human medicine and dentistry, and toxicological and other safety tests, among others. Between 1995 and 2011, about 65% of animals were used in basic and applied research by the 15 EU Member States (at the time) plus Switzerland (Daneshian et al., 2015). In 2016, 47% of procedures were conducted for basic research and 21% for applied research based on the national statistics of 26 reporting EU Member States (Taylor and Rego Alvarez, 2019). Directive 2010/63/EU (EU, 2010) has put in place a more comprehensive reporting framework for Member States; as a result, more precise estimates of animal use in the EU will be available in the next overall report due in late 2019.

The high numbers of animals used in biomedical research warrant additional emphasis to replace their use in these fields. Although toxicity testing accounts for less than 10% of animal use in science, much of the attention of the 3Rs to date has been focused on this area (Stephens and Mak, 2013). The reasons for this include the limited number of targets for replacement in this field, public concern over this type of animal testing, and the possibility to gain government approval for developed replacement tests. In contrast, biomedical research is a far more diverse and decentralized area, where, in principle, originality and innovation are most highly prized. Thus, this field presents a different set of challenges to replacement advocates. However, the new non-animal methods offer human-relevant insights and diverse high-tech research opportunities, which can be adapted to specific research needs. Importantly, changing to non-animal methods in this field is a matter of choice based on knowledge as well as on funding, but it is not determined by regulatory requirements.

3 The expanding toolbox of non-animal methods and approaches

In their chapter on replacement, Russell and Burch (1959) noted that "*(m)ammalian tissue cultures … have become, since the Second World War, one of the most important replacing techniques, and indeed one of the most important developments in biology*" (p. 72). This development was facilitated by the discovery of antibiotics, which suppress the growth of bacteria that often contaminate cell cultures. During the balance of the twentieth century, tissue culture began to be applied in a wide array of fields. However, this technique was largely limited to homogenous, two-dimensional arrays, short-lived *in vitro* preparations, and relatively crude reconstructed tissue.

Today, the *in vitro*/cell culture toolbox is much more sophisticated and diverse. It includes organ-on-a-chip microphysiological systems (Andersen et al., 2014; Marx et al., 2016); three-dimensional organoids containing multiple cell types; robot-assisted, high-throughput systems (van Vliet et al., 2014); high-content imaging technologies; and DNA or RNA microarray screening. Also available are computational tools and approaches, machine learning and artificial intelligence (AI) to help make sense of "big data" (Hartung and Hoffman, 2009; Hartung, 2016, 2018; Luechtefeld et al., 2018).

These methods have a number of advantages over 20th century *in vitro* approaches, which can include speed, throughput, and biological relevance. Multiple cell types, 3D architecture, fluid exchange, etc. enable the models to function more like human tissues or organs and to survive in culture for longer time periods. Owing to the development of induced pluripotent stem cells, the models can now be seeded with non-cancerous, differentiated human cells and, thanks to microphysiological systems technology, different "organs" can be connected with one other.

Much has already been written about these and other 21st century tools and approaches (Langley et al., 2017; Noor, 2019; Wilkinson, 2019; Benam et al., 2019; Bowman et al., 2018; Savoji et al., 2019; Marshall and Willett, 2018; Marshall et al., 2018; Boeckmans et al., 2018; Pistollato et al., 2016; Langley et al.,

2015). Here we would like to mention several recent developments that have been key in driving non-animal methods forward: The U.S. National Research Council report on "*Toxicity Testing in the 21st Century: A Vision and a Strategy*" (NRC, 2007) and the EU legislation governing animal experimentation (Directive 2010/63/EU on the protection of animals used for scientific purposes) (EU, 2010; Hartung, 2010) created a more welcoming environment for the new methods and officially recognized their potential to replace animal use. Many of these tools and approaches are now commercially available, which has greatly increased their accessibility and diminished practical barriers to their adoption and use. Although high-throughput testing is limited to centralized laboratories owing to the sophisticated equipment required for these arrays, median-throughput versions of these assays are now more widely accessible. Also, the focus has shifted away from 1:1 replacement of animal models to developing models with human relevance, thus enhancing their translational value and their acceptance in the larger scientific community. "Human-on-a-chip" efforts have begun to interconnect numerous organ models in microphysiological arrays to approach the complexity of the human body and be able to identify organs affected by chemical substances (Marx et al., 2012). The development of these models and approaches has been facilitated by advances in stem cell technology, microengineering, microfluidics, computing power, and respective multidisciplinary cooperation.

4 A proposal to prioritize the 3Rs of replacement, research, and (human-) relevance

Not surprisingly, sixty years after Russell and Burch proposed the 3Rs, the landscape of animal experimentation and alternatives-related technology has changed. Despite successes in developing non-animal methods, especially for toxicological testing, the scale of animal use in Great Britain – home of UFAW, Russell and Burch, and the 3Rs – has more than tripled (Home Office, 2018). What must the 3Rs community change to become more effective?

We propose a strategic focus that prioritizes: (1) replacement over refinement and reduction, (2) biomedical research over safety testing, and (3) relevance to humans rather than to non-human animals. One can think of these priorities as a new set of 3Rs (replacement, research, and relevance) for the current era. These three priorities are discussed in the following subsections.

An alternatives strategy that emphasized two of the three pillars highlighted here, namely replacement over reduction and refinement, and biomedical research over safety testing has been proposed previously (Stephens, 2012). However, the importance of our third pillar – human relevance – has been underscored by recent attention to the reproducibility and translatability pitfalls surrounding animal experiments and by the power of the new animal-free methods to address human relevance in the years since then.

4.1 Prioritizing replacement

In some respects, prioritizing replacement in the era of 21st century technology is simply an extension of Russell and Burch's thinking that reduction and refinement are interim steps on the path towards replacement. They wrote:

> "*… refinement is never enough, and we should always seek further for reduction and if possible replacement. Still more generally, replacement is always a satisfactory answer….*" (p. 66) (Russell and Burch, 1959).

The motivating issue for them was the distress that animals could experience in the laboratory. They referred to this as "inhumanity," a term they sought to apply objectively without implying any ethical judgement of the experimenters or staff. "Direct inhumanity" could result from the "*unavoidable consequence of the procedure employed*," no matter how well refined. In addition, "contingent inhumanity" could result as an "*incidental and inadvertent by-product of the use of the procedure, which is not necessary for its success*" (p. 54). In their view, it was better to avoid both types of inhumanity by using replacement methods, when available.

Prioritizing replacement in the 1950s was more quixotic than practical. This was perhaps one reason for the delayed uptake of the 3Rs framework during the 1960s and 1970s (Stephens and Mak, 2013). Today, however, while much progress has been made with reduction and refinement, the replacement toolkit is more impressive and promising than ever. The new methods do not necessarily translate into direct replacements of individual animal models; combinations of methods may be needed to replace an animal model as an *in vitro* system is often still far less complex than an organism. However, suitability to answer a research question depends on whether a model or model combination includes all relevant aspects of a (human) biological system, not whether it models an organism in its entirety.

There are also societal reasons to favor replacement. A shift in focus to animal-free methods would be in line with increasing international public concerns about laboratory animal suffering as shown by the Stop Vivisection initiative in the European Union[1] and recent Ipsos MORI studies conducted by research centers such as Pew[2,3] and Gallup (Clemence and Leaman, 2016; Jones, 2017). In addition, the EU legislation governing animal experimentation not only calls for the use of non-animal methods, where available (EC, 2010) (Recital 11), but has as its long-term goal the phasing out of all animal use (EU, 2010, Recital 10). One might call this "full replacement" (Stephens, 2012).

In the light of insurmountable species differences (Pound and Ritskes-Hoitinga, 2018) and new possibilities offered by the novel technologies and approaches that are human-biology based, we can and should put the most effort into replacing animal experiments with non-animal methods. Setting a priority means empha-

[1] http://ec.europa.eu/citizens-initiative/public/initiatives/successful/details/2012/000007; http://stopvivisection.eu/en/content/why-stop-vivisection

[2] http://www.pewinternet.org/2015/01/29/public-and-scientists-views-on-science-and-society/pi_2015-01-29_science-and-society-03-05/

[3] http://www.pewresearch.org/fact-tank/2018/08/16/americans-are-divided-over-the-use-of-animals-in-scientific-research/

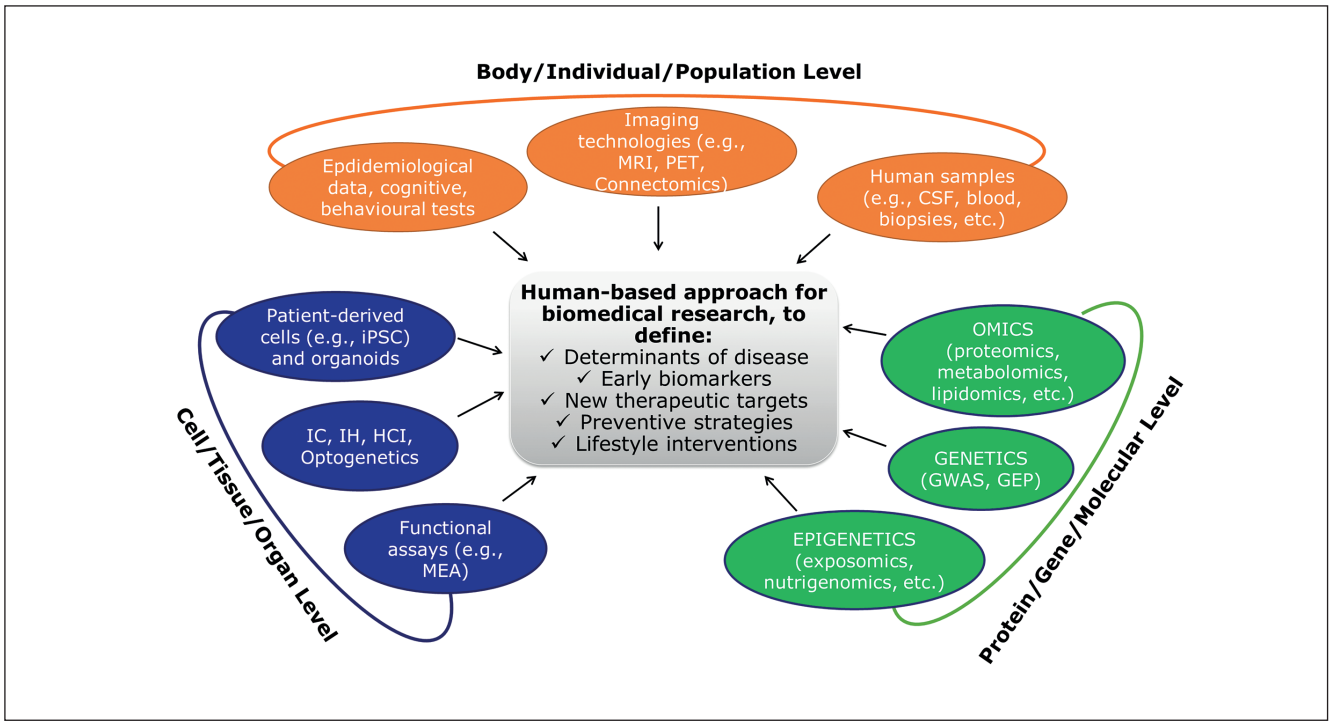

Fig. 1: Schematic view of how human-based models/tools, technological approaches and readouts can be applied in biomedical research to promote a systems biological understanding of disease etiopathology

Optogenetics involves the use of light to monitor cells in living tissue (typically neurons) that have been genetically modified to express light-sensitive ion channels. Abbreviations: CSF, cerebrospinal fluid; GEP, gene expression profiling; GWAS, genome wide association study; HCI, high content imaging; IC, immunocytochemistry; IH, immunohistochemistry; iPSC, induced pluripotent stem cells; MEA, multi-electrode array; MRI, magnetic resonance imaging; PET, positron emission tomography

sizing some things over others. Issues that are no longer a priority are de-emphasized, not necessarily abandoned. Thus, for a funding agency, prioritizing replacement could mean that more than an equal share of funds go to this topic, while some funds are nonetheless allocated to reduction and refinement.

4.2 Prioritizing biomedical research as the target of non-animal methods

The major goals of biomedical research are the advancement of human health, the discovery of effective and safe treatments for humans, and the elucidation of the role of genetic and environmental risk factors in the onset and the consolidation of human diseases. But although there is a globally recognized need to replace animal use in toxicology and regulatory testing whenever possible (Balls, 2007; Hartung, 2009; Stephens and Mak, 2013), this is not yet the case in basic and applied research, where most of the animals are used (Daneshian et al., 2015; EC, 2011). Consequently, the value of animal models for biomedial research should be critically appraised by means of systematic reviews, meta-analyses, and citation analyses (e.g., Carvalho et al., 2019; Hartung, 2013; Herrmann, 2019a; Knight, 2019; Pound et al., 2004). Also, with regards to research funding strategies and prioritization, assessing return on investment with meaningful indicators is key to enable an assessment of the impact that publicly funded animal-based research has had on citizens' health, so as to reassess how to channel funding more effectively to achieve the goals of biomedical research.

Some of the approaches (if not the tools) used in toxicology and regulatory testing could be applicable to biomedical research (Gruber and Hartung, 2004). For instance, adverse outcome pathways (AOPs) are constructed to portray existing knowledge regarding the adverse effects elicited by chemicals in an agnostic manner. AOPs identify the molecular initiating event and intermediate mechanisms (key events) underlying these effects up to an adverse health outcome, and span multiple levels of biological complexity (i.e., population, organism, organ, cell, and genetic levels). While AOPs have been conceived in the field of toxicology, their use would also be applicable to biomedical research, as a more explicit framework for mechanistic studies. For example, by using an AOP conceptual framework it could be possible to gather existing knowledge about signaling pathways that are perturbed at the onset and during the consolidation of a certain disease, and to link genetic determinants, lifestyle and environmental factors with adverse health effects (Langley, 2014; Pistollato et al., 2015).

An initiative aiming to further a human-focused, pathway-based approach to studying, preventing and treating disease is the BioMed 21 (Biomedical Research for the 21st Century) Collaboration, which is working internationally with health experts, regula-

tory and research agencies, and funding bodies to develop innovative research roadmaps that focus on understanding human disease pathophysiology.[4] BioMed 21 envisions a "*human-based pathway approach to human disease research*" applicable to a broad range of human pathologies (Langley et al., 2017). The Collaboration aims to use technological tools and biological models within an AOP framework to study human pathologies, thereby promoting the discovery of drug targets, and possibly reducing late-stage drug attrition. Information is drawn from observational, prospective, epidemiological, and interventional studies conducted in human patient cohorts. The Collaboration also promotes global efforts to improve transparency, increase reproducibility, and confirm associations described in epidemiological studies (Wang et al., 2016).

Complex cell models, such as human induced pluripotent stem cells (hiPSCs) and their differentiated derivatives obtained from patients and healthy subjects, are suitable for adaptation for biomedical research to replace animal experiments. These models can be cultured in 3D to improve the level of physiological complexity, e.g., as organoids in microfluidic devices (Alépée et al., 2014; Park et al., 2015; Skardal et al., 2016). Stem cell discovery platforms are already yielding the first clinical candidates (Mullard, 2015; McNeish et al., 2015).

Proteins associated with signaling pathways and genetic/epigenetic factors can be studied with a wide array of omics technologies, next-generation sequencing approaches, and gene expression profiling; these, together with integrated computer modelling, are already paving the way for a systems-biological understanding of disease etiopathology (Fig. 1) without the use of non-human animals and thus the problem of interspecies differences.

Tools that are suitable to address population/organism biological complexity include human *ex vivo* material derived from healthy subjects and patients, such as blood and plasma samples, cerebrospinal fluid, post-surgical biopsies, and post-mortem tissues, which can be useful to identify early biomarkers of human diseases. Advanced imaging technologies such as imaging connectomics, which allows comprehensively mapping the neural elements and inter-connections constituting the brain, are providing new insights into brain diseases (Fornito and Bullmore, 2015). Such imaging data can be associated with cognitive test scores and omics data, allowing multi-scale data integration (Fig. 1).

4.3 Promoting human relevance in biomedical research

Prioritizing animal-free methods of high human relevance avoids the limited translational value of animal models to human biology. Russell and Burch were keenly aware of the importance of relevance in research, whether the research was aimed at understanding humans or another species. They distinguished between models that had high fidelity versus high discrimination. The former were grossly similar to what was being modeled (fidelity) but did not necessarily do a good job of predicting outcomes when the experimental parameter was varied (discrimination). Thus, the "high-fidelity fallacy" is the unfounded belief that, say, a mouse would be a good predictor of the human situation in a given context because both mice and humans are mammals. In contrast, an *in vitro* system lacks fidelity to a human situation but can nonetheless allow good discrimination, say, of which chemicals might cause skin sensitization in humans if it contains the relevant cell types that are involved in the human skin sensitization process.

Clearly, the issues of model fidelity and discrimination described in the 1950s are still relevant today. High discrimination yields high translatability, whereas the same cannot necessarily be said of high fidelity. The decades since the 1950s have given us ample evidence of the limited translatability of animal models to the human situation (e.g., Kramer and Greek, 2018; Pippin, 2012). For example, the overall likelihood of approval of a potential drug from preclinical studies, which are based largely on animal studies, is less than 10% (Hartung, 2013; Thomas et al., 2016; Meigs et al., 2018) with efficacy and safety issues accounting for the majority of failures (Harrison, 2016). In light of this, the relevance and reliability of animals for preclinical drug development have been questioned[5] (e.g., Kramer and Greek, 2018; Pound and Bracken, 2014; Pound and Ritskes-Hoitinga, 2018; van der Worp et al., 2010). Current NIH director Francis Collins, in an article discussing the translation of basic biomedical science into safe and effective clinical applications, also expressed significant reservations about animal models, stating that "*the use of small and large animals to predict safety in humans is a long-standing but not always reliable practice in translational science*" (Collins, 2011).

Some projects are still seeking to develop better animal models of diseases, e.g., the NIH-funded MODEL-AD consortium[6] is engineering mice with different genetic mutations linked with early- or late-onset Alzheimer's. However, other scientists urge caution: Bart de Strooper, a molecular biologist at the Catholic University of Leuven (KU Leuven) declared that "*the biggest mistake you can make is to think you can ever have a mouse with Alzheimer's disease*" (Reardon, 2018). Investing large amounts of money, energy and time into the 'remaking' or optimizing animal models (animal models 2.0) has not mitigated the translational failure (Sutherland et al., 2012; Leung et al., 2018). However, interviews with the animal research community on the causes of the low reproducibility and translatability of animal experiments showed no acknowledgement of limitations to extrapolating results from animals to humans (Fitzpatrick et al., 2018). A recent survey (Franco et al., 2018) amongst participants of laboratory animal science courses in four European countries (Denmark, Germany, Switzerland, and Portugal) revealed that animal experimenters considered refinement more important and more achievable than replacement. In addition, they prioritized refinement over reduction efforts, which is a reversal of the hierarchy postulated by Russell and Burch (Russell and Burch, 1959), who put replacement first and refinement last.

[4] http://biomed21.org/

[5] https://www.the-scientist.com/news-analysis/more-compounds-failing-phase-i-49707

[6] https://model-ad.org/

Both the United States National Research Council report "*Toxicity Testing in the 21st Century: A Vision and a Strategy*" (NRC, 2007) and Directive 2010/63/EU (EU, 2010) recognize the need to modernize toxicity testing and advocate an approach that emphasizes non-animal methods. Both documents highlight the need to improve human relevance, foster a paradigm shift from nearly exclusive reliance on animal experimentation to more human-based approaches, and introduce pathway-based approaches to gather a mechanistic understanding of disease etiology. These arguments are also applicable to biomedical research. Calls are becoming louder for a shift towards methods that are human biology-based and, hence, human-relevant, to avoid the inaccuracy in predicting efficacy and safety of drugs using current preclinical animal models (Archibald et al., 2018; Herrmann and Jayne, 2019; Kramer and Greek, 2018).

21st century methods allow scientists to incorporate human relevance as a primary design criterion of biomedical research models. In lung models, for example, relevant human cell types can be incorporated into a lung-on-a-chip, with mechanical forces mimicking the shear forces exerted during breathing (Huh et al., 2010; Benam et al., 2017). Human samples, large data repositories, and computational and imaging tools are also available to carry out human-relevant biomedical research.

Of course, no model is without its limitations; thus, 21st century *in vitro* systems also have limitations, such as lacking the integration and longevity of an intact organism. They are designed with the intention to simulate human biology up to a certain level of organization and complexity, and each system's relevance to study a certain scientific problem must be carefully considered and verified. Also, they must be performed by trained scientists and follow best practice guidance to ensure quality results.

It should also be mentioned that microdosing has been recently considered a promising way to assess, by means of positron emission tomography (PET) and accelerator mass spectrometry (AMS), pharmacokinetics and pharmacodynamics of tested drugs administered at non-pharmacological doses to humans. This facilitates exploratory studies directly in humans, while reducing the use of animals in preclinical toxicology (Bergstrom, 2017). The use of microdosing has been encouraged by regulatory agencies both in Europe and the United States, and a microdosing regulatory framework has been accepted by the International Conference on Harmonization (ICH, 2009).

The failures in drug development may be related not only to the use of animal models of questionable relevance to humans, but also to the use of these models in a hyper-reductionist approach to dissect the possible contributions of single gene(s) or protein(s) to the onset of complex, multi-factorial human pathologies. Some key issues should therefore be considered, in order to refocus current and future research strategies and priorities in biomedical research, as has already been advocated for Alzheimer's disease research (Pistollato et al., 2016). Human-based methods should be used to elucidate disease processes at multiple levels of biological complexity (Fig. 1), such as by associating novel human-based cellular and computational models with non-invasive imaging tools and epidemiological and clinical data to facilitate human-relevant data discovery. In this regard, some recent European initiatives are embracing a multidimensional and multidisciplinary perspective for the study of complex brain interactions and Alzheimer's disease research (Vaudano et al., 2015). These initiatives include the Human Brain Project[7] and the Innovative Medicines Initiative (IMI) with three complementary projects: the European Medical Information Framework (EMIF),[8] the Aetionomy project (organizing mechanistic knowledge about the biological pathways involved in the aetiology of neurodegenerative diseases, to guide the classification of disease classes and subclasses)[9] and EPAD (European Prevention of Alzheimer's Dementia Consortium).[10] Similarly, in the United States, the Accelerating Medicines Partnership (AMP) is aiming to develop new diagnostics and treatments for Alzheimer's disease, type 2 diabetes, autoimmune disorders (i.e., rheumatoid arthritis and systemic lupus erythematosus), and more recently also Parkinson's disease, by jointly identifying and validating new biological disease targets. The AMP is a public-private partnership between the National Institutes of Health (NIH), the US Food and Drug Administration (FDA), 12 biopharmaceutical and life science companies and 13 non-profit organizations.[11] Similar transdisciplinary initiatives are ongoing in other biomedical research areas and in other countries.

5 Increasing awareness, dissemination and education on non-animal approaches

We recognize that advancing human-relevant, animal-free approaches in biomedical research should be a multi-faceted effort involving, *inter alia*, funding targeted at laboratory research, graduate and post-doctoral research fellowships, and alternatives prizes. However, a key step in transforming the current animal use paradigm is increasing the awareness of currently available animal-free methods. Without knowledge of these methods, scientists cannot adopt them, funding agencies cannot create programs to fund them, ethical review committees cannot ask why they are not employed in a given protocol, and so forth. Consequently, knowledge-sharing through education and training plays a central role in achieving the move away from animal experiments and towards human-biology based research methodologies (Daneshian et al., 2011; Hartung et al., 2009; Herrmann, 2019a; Holley et al., 2016). Here we address the narrow but far-reaching issue of educating future and early-career scientists on these issues.

7 https://www.humanbrainproject.eu/en/

8 http://www.emif.eu/

9 https://www.aetionomy.eu/en/vision.html

10 http://ep-ad.org/

11 https://www.nih.gov/research-training/accelerating-medicines-partnership-amp

In Europe, the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) of the Joint Research Centre (JRC) has recently coordinated a study to review available education and training resources that support the 3Rs approach.[12] The aim of this study is "*to identify courses, modules, teaching materials, guidance, and other resources to form a snapshot view of how, where and to whom the 3Rs principles and alternative-to-animal approaches are currently being taught keeping in mind that many such initiatives might not be '3R labelled'*". In the JRC report "*Accelerating progress in the Replacement, Reduction and Refinement of animal testing through better knowledge sharing*" (Holley et al., 2016), it is acknowledged that, although there are many available 3Rs relevant knowledge sources, their impact could be increased by, e.g., (i) increasing awareness and coordination, and (ii) better curating existing knowledge sources. EURL ECVAM is currently collaborating with Directorate General Environment (DG ENV) on an initiative in which 3Rs experts design and produce eLearning modules for students and professionals involved in laboratory animal use. The modules cover the educational and training requirements under Directive 2010/63/EU with special focus on replacement methods to acclerate and aid the uptake of animal-free approaches in science.[13]

There are currently only a few courses available at the university level that are specifically teaching the 3Rs (Holley et al., 2016). In the following we give a few examples of such courses. One is run by one of the authors (KH) at Johns Hopkins University as part of the educational program offered by the Center for Alternatives to Animal Testing (CAAT). It is an 8-week course open to graduate and undergraduate students consisting of 18 lectures by and 10 interviews with international experts in their respective fields, and it is complemented by virtual tours through two laboratories that use animal-free methods. The modules cover how to fully apply the 3Rs principles and discuss the limitations of animal use in science. Other topics include how to plan, conduct, analyze and report research studies, as well as how to properly formulate a research question, to conduct comprehensive literature searches, and to critically appraise the validity of animal and non-animal models and methods in order to choose the best means for particular research questions (Herrmann, 2019b). This course will be complemented in the next academic year by courses focusing on non-animal approaches in basic and applied research, the ethics of animal use in science, and best practice approaches to reduce animal suffering and improve scientific rigor.

CAAT also offers classes on *Toxicology in the 21st Century* and *Evidence-based Toxicology*. *Toxicology in the 21st Century* addresses the current paradigm change in regulatory toxicology, the shortcomings of the current system, and the adaptation of novel technologies to overcome them. The course *Evidence-based Toxicology* familiarizes students with the concepts of evidence-based medicine and its translation to toxicology. Concepts of systematic reviews, meta-analysis, risk-of-bias, and various quality assurance schemes are introduced. Both courses are offered for free since 2018 on the online learning platform Coursera and had registered about 2500 active learners by June 2019.

To reach early career scientists, the JRC organized a Summer School on "Non-animal Approaches in Science" in 2017 and 2019[14] to share knowledge and expertise on the newest innovative animal-free methods in research and testing and to discuss the place of the 3Rs in science today. A further summer school is planned for 2020 in the US.

The University of British Columbia (UBC) will be starting a course on non-animal methods in biomedical sciences.[15] Also, the Canadian Centre for Alternatives to Animal Methods[16] and the Canadian Centre for the Validation of Alternative Methods[17] (University of Windsor) have been developing academic programs in animal replacement science. At the undergraduate level, they plan to offer a minor and a certificate program for science majors, with the intention of developing a major degree program over time. At the graduate level, they are currently developing a one-year Master's program on alternatives research – from science to ethics and hands-on training in alternative test methods – and training doctoral students in their multidisciplinary human biology-based research laboratory.[18]

We hope that targeted courses such as these will be adopted into the curricula of many additional universities internationally to inform future and early-career scientists on state-of-the-art 21st century non-animal methods sustainably.

6 Conclusions

We believe that 3Rs advocates should consider what set of priorities (informed by the 3Rs) is most appropriate to their era and is most likely to maximize return on investment. In 2019, we propose a strategy based on prioritizing Replacement, (biomedical) Research, and (human) Relevance. These three Rs are not intended to replace the original set, but to represent a strategic application of Russell and Burch's framework to an era ripe with non-animal methods, with opportunities to apply them in biomedical research, and with ways to craft these methods to reflect human biology. To achieve this, we embrace a fresh perspective on the way biomedical research is taught, planned and funded. Human-based models and methods, including complex *in vitro* systems, *in silico* tools and high-throughput approaches are now far developed and should be pursued in a multidisciplinary and collaborative effort to increase human relevance, reproducibility and, ideally, translatability of scientific data, as already envisioned in the last decades in toxicology and regulatory testing (Gibson, 2010).

12 https://ec.europa.eu/jrc/en/science-update/education-and-training-3rs

13 https://ec.europa.eu/jrc/en/science-update/calls-experts-training-tools-alternatives-animal-testing

14 https://ec.europa.eu/jrc/en/event/conference/jrc-summer-school-non-animal-approaches-science

15 Personal communication with Dr. Elisabeth Ormandy, Executive Director, Animals in Science Policy Institute and Lecturer, University of British Columbia.

16 http://www.uwindsor.ca/ccaam/

17 http://www.uwindsor.ca/ccaam/303/canadian-centre-validation-alternative-methods-cacvam

18 Personal communication with Dr. Charu Chandrasekera, Executive Director, Canadian Centre for Alternatives to Animal Methods.

We direct our proposal to scientists, advocates, funders and institutions who are interested in non-animal methods and approaches. These are the players who are motivated to accelerate progress, including through their own research, teaching, lobbying, allocation of funds, and the like.

Where does that leave Russell and Burch's analysis and the 3Rs framework in the 21st century? To a certain extent, all of the 3Rs will retain some importance as long as any animals are used in experimentation. However, if animal experiments are eventually no longer the norm, there will inevitably be less emphasis on reducing and refining the remaining animal experiments (as important as these activities still are now). Hence our call to focus energies on optimizing and implementing replacement (non-animal) methods.

Our aim is not to diminish the legacy of Russell and Burch but to mark the proper place of the 3Rs and to move forward towards a new era in the history of humane experimental technique.

References

Alépée, N., Bahinski, A., Daneshian, M. et al. (2014). t4 workshop report: State-of-the-art of 3D cultures (organs-on-a-chip) in safety testing and pathophysiology. *ALTEX 31*, 441-477. doi:10.14573/altex1406111

Andersen, M. E., Betts, K., Dragan, Y. et al. (2014). Developing microphysiological systems for use as regulatory tools – Challenges and opportunities. *ALTEX 31*, 364-367. doi:10.14573/altex.1405151

Archibald, K., Tsaioun, K., Kenna, J. G. et al. (2018). Better science for safer medicines: The human imperative. *J R Soc Med*, 141076818812783. doi:10.1177/0141076818812783

Bailey, J. (2019). Genetic modification of animals: Scientific and ethical issues. In K. Herrmann and K. Jayne (eds.), *Animal Experimentation: Working Towards a Paradigm Change* (443-479). Vol. 22. Leiden, The Netherlands: Brill. doi:10.1163/9789004391192_020

Balls, M. (2007). Alternatives to animal experiments: Time to focus on replacement. *AATEX 12*, 145-154.

Balls, M., Combes, R. and Worth, A. (2019). *The History of Alternative Test Methods in Toxicology.* Academic Press. https://bit.ly/2xHlyZq

Benam, K. H., Mazur, M., Choe, Y. et al. (2017). Human lung small airway-on-a-chip protocol. *Methods Mol Biol 1612*, 345-365. doi:10.1007/978-1-4939-7021-6_25

Benam, K. H., Gilchrist, S., Kleensang, A. et al. (2019). Exploring new technologies in biomedical research. *Drug Discov Today 24*, 1242-1247. doi:10.1016/j.drudis.2019.04.001

Bergstrom, M. (2017). The use of microdosing in the development of small organic and protein therapeutics. *J Nucl Med 58*, 1188-1195. doi:10.2967/jnumed.116.188037

Boeckmans, J., Natale, A., Buyl, K. et al. (2018). Human-based systems: Mechanistic nash modelling just around the corner? *Pharmacol Res 134*, 257-267. doi:10.1016/j.phrs.2018.06.029

Bowman, P., Flanagan, S. E. and Hattersley, A. T. (2018). Future roadmaps for precision medicine applied to diabetes: Rising to the challenge of heterogeneity. *J Diabetes Res 2018*, 3061620. doi:10.1155/2018/3061620

Carvalho, C., Alves, D., Knight, A. et al. (2019). Is animal-based biomedical research being used in its original context? In K. Herrmann and K. Jayne (eds.), *Animal Experimentation: Working Towards a Paradigm Change* (376-390). Vol. 22. Leiden, The Netherlands: Brill. doi:10.1163/9789004391192_017

Clemence, M. and Leaman, J. (2016). Public attitudes to animal research in 2016. Ipsos MORI. https://www.ipsos.com/sites/default/files/publication/1970-01/sri-public-attitudes-to-animal-research-2016.pdf

Collins, F. S. (2011). Reengineering translational science: The time is right. *Sci Transl Med 3*, 90cm17. doi:10.1126/scitranslmed.3002747

Daneshian, M., Akbarsha, M. A., Blaauboer, B. et al. (2011). A framework program for the teaching of alternative methods (replacement, reduction, refinement) to animal experimentation. *ALTEX 28*, 341-352. doi:10.14573/altex.2011.4.341

Daneshian, M., Busquet, F., Hartung, T. et al. (2015). Animal use for science in europe. *ALTEX 32*, 261-274. doi:10.14573/altex.1509081

De Greeve, P., De Leeuw, W. and van Zutphen, B. (2004). Trends in animal use and animal alternatives. *Altern Lab Anim 32, Suppl 1A*, 13-19. doi:10.1177/026119290403201s06

EC – European Commission (2011). Seventh report on the statistics on the number of animals used for experimental and other scientific purposes in the member states of the european union. 52013DC0859. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52013DC0859

EU – European Union (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJ L 276, 33-79. http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32010L0063&from=EN

Fitzpatrick, B. G., Koustova, E. and Wang, Y. (2018). Getting personal with the "reproducibility crisis": Interviews in the animal research community. *Lab Anim (NY) 47*, 175-177. doi:10.1038/s41684-018-0088-6

Fornito, A. and Bullmore, E. T. (2015). Connectomics: A new paradigm for understanding brain disease. *Eur Neuropsychopharmacol 25*, 733-748. doi:10.1016/j.euroneuro.2014.02.011

Franco, N. H., Sandoe, P. and Olsson, I. A. S. (2018). Researchers' attitudes to the 3Rs – An upturned hierarchy? *PLoS One 13*, e0200895. doi:10.1371/journal.pone.0200895

Gibson, J. E. (2010). An integrated summary of commentary on the national academy of sciences report on "toxicity testing in the 21st century: A vision and a strategy". *Hum Exp Toxicol 29*, 33-35. doi:10.1177/0960327109354659

Gruber, F. P. and Hartung, T. (2004). Alternatives to animal experimentation in basic research. *ALTEX 21, Suppl 1*, 3-31. http://www.altex.ch/resources/altex_2004_Supp1_003_031_Gruber.pdf

Hartung, T. (2009). Toxicology for the twenty-first century. *Nature 460*, 208-212. doi:10.1038/460208a

Hartung, T. and Hoffmann, S. (2009). Food for thought ... on in silico methods in toxicology. *ALTEX 26,* 155-166. doi:10.14573/altex.2009.3.155

Hartung, T., Blaauboer, B. and Leist, M. (2009). Food for thought … on education in alternative methods in toxicology. *ALTEX 26*, 255-263. doi:10.14573/altex.2009.4.255

Hartung, T. (2010). Comparative analysis of the revised Directive 2010/6106/EU for the protection of laboratory animals with its predecessor 86/609/EEEEC – A t[4] report. *ALTEX 27*, 285-303. doi:10.14573/altex.2010.4.285

Hartung, T. (2013). Food for thought … Look back in anger – What clinical studies tell us about preclinical work. *ALTEX 30*, 275-291. doi:10.14573/altex.2013.3.275

Hartung, T. (2016). Making big sense from big data in toxicology by read-across. *ALTEX 33*, 83-93. doi:10.14573/altex.1603091

Hartung, T. (2018). Making big sense from big data. *Frontiers In Big Data 1*, 5. doi:10.3389/fdata.2018.00005

Harrison, R. K. (2016). Phase II and phase III failures: 2013-2015. *Nat Rev Drug Discov 15*, 817-818. doi:10.1038/nrd.2016.184

Herrmann, K. (2019a). Refinement on the way towards replacement: Are we doing what we can? In K. Herrmann and K. Jayne (eds.), *Animal Experimentation: Working Towards a Paradigm Change* (3-64). Vol. 22. Leiden, The Netherlands: Brill. doi:10.1163/9789004391192_002

Herrmann, K. (2019b). Teaching animal-free approaches in basic and applied biomedical research. Poster Presentation at JRC Summer School on Non-Animal Approaches in Science. 21-14 May 2019, Ispra, Italy.

Herrmann, K. and Jayne, K. (2019). *Animal Experimentation: Working Towards a Paradigm Change.* Vol. 22. Leiden, The Netherlands: Brill. doi:10.1163/9789004391192

Holley, T., Bowe, G., Campia, I. et al. (2016). Accelerating progress in the replacement, reduction and refinement of animal testing through better knowledge sharing. *Publications Office of the European Union*. doi:10.2788/934083

Home Office (2018). Additional statistics on breeding and genotyping of animals for scientific procedures, Great Britain 2017 Home Office. https://bit.ly/2PGvzSf

Huh, D., Matthews, B. D., Mammoto, A. et al. (2010). Reconstituting organ-level lung functions on a chip. *Science 328*, 1662-1668. doi:10.1126/science.1188302

ICH (2009). Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. M3(R2) May 27, 2019. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf

Jones, J. M. (2017). Americans hold record liberal views on most moral issues. *Gallup Poll Social Series*. https://bit.ly/32iYlLe

Knight, A. (2008). 127 million non-human vertebrates used worldwide for scientific purposes in 2005. *Altern Lab Anim 36*, 494-496.

Knight, A. (2019). Critically evaluating animal research. In K. Herrmann and K. Jayne (eds.), *Animal Experimentation: Working Towards a Paradigm Change* (321-340). Vol. 22. Leiden, The Netherlands: Brill. doi:10.1163/9789004391192_015

Kramer, L. A. and Greek, R. (2018). Human stakeholders and the use of animals in drug development. *Business and Society Review 123*, 3-58.

Langley, G. R. (2014). Considering a new paradigm for Alzheimer's disease research. *Drug Discov Today 19*, 1114-1124. doi:10.1016/j.drudis.2014.03.013

Langley, G., Austin, C. P., Balapure, A. K. et al. (2015). Lessons from toxicology: Developing a 21st-century paradigm for medical research. *Environ Health Perspect 123*, A268-272. doi:10.1289/ehp.1510345

Langley, G. R., Adcock, I. M., Busquet, F. et al. (2017). Towards a 21st-century roadmap for biomedical research and drug discovery: Consensus report and recommendations. *Drug Discov Today 22*, 327-339. doi:10.1016/j.drudis.2016.10.011

Leung, V., Rousseau-Blass, F., Beauchamp, G. et al. (2018). ARRIVE has not arrived: Support for the ARRIVE (animal research: Reporting of in vivo experiments) guidelines does not improve the reporting quality of papers in animal welfare, analgesia or anesthesia. *PLoS One 13*, e0197882. doi:10.1371/journal.pone.0197882

Luechtefeld, T., Rowlands, C. and Hartung, T. (2018). Big-data and machine learning to revamp computational toxicology and its use in risk assessment. *Toxicol Res 7*, 732-744. doi:10.1039/C8TX00051D

Marshall, L. J. and Willett, C. (2018). Parkinson's disease research: Adopting a more human perspective to accelerate advances. *Drug Discov Today 23*, 1950-1961. doi:10.1016/j.drudis.2018.09.010

Marshall, L. J., Austin, C. P., Casey, W. et al. (2018). Recommendations toward a human pathway-based approach to disease research. *Drug Discov Today 23*, 1824-1832. doi:10.1016/j.drudis.2018.05.038

Marx, U., Walles, H., Hoffmann, S. et al. (2012). 'Human-on-a-chip' developments: A translational cutting-edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man? *Altern Lab Anim 40*, 235-257. doi:10.1177/026119291204000504

Marx, U., Andersson, T. B., Bahinski, A. et al. (2016). Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing using animals. *ALTEX 33*, 272-321. doi:10.14573/altex.1603161

McNeish, J., Gardner, J. P., Wainger, B. J. et al. (2015). From dish to bedside: Lessons learned while translating findings from a stem cell model of disease to a clinical trial. *Cell Stem Cell 17*, 8-10. doi:10.1016/j.stem.2015.06.013

Meigs, L., Smirnova, L., Rovida, C. et al. (2018). Animal testing and its alternatives – The most important omics is economics. *ALTEX 35*, 275-305. doi:10.14573/altex.1807041

Mullard, A. (2015). Stem-cell discovery platforms yield first clinical candidates. *Nat Rev Drug Discov 14*, 589-591. doi:10.1038/nrd4708

Netherlands National Committee for the protection of animals used for scientific purposes (NCad) (2016). Transition to non-animal research. The Hague. https://www.ncadierproevenbeleid.nl/documenten/rapport/2016/12/15/ncad-opinion-transition-to-non-animal-research

Noor, F. (2019). The changing paradigm in preclinical toxicology: In vitro and in silico methods in liver toxicity evaluations. In K. Herrmann and K. Jayne (eds.), *Animal Experimentation: Working Towards a Paradigm Change* (610-638). Vol. 22. Leiden, The Netherlands: Brill. doi:10.1163/9789004391192_026

NRC (2007). *Toxicity Testing in the 21st Century. A Vision and a Strategy*. Washington, DC, USA: The National Academies Press. doi:10.17226/11970

Ormandy, E. H., Schuppli, C. A. and Weary, D. M. (2009). Worldwide

trends in the use of animals in research: The contribution of genetically-modified animal models. *Altern Lab Anim 37*, 63-68. doi: 10.1177/026119290903700109

Park, J., Lee, B. K., Jeong, G. S. et al. (2015). Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an in vitro model of Alzheimer's disease. *Lab Chip 15*, 141-150. doi:10.1039/c4lc00962b

Pippin, J. J. (2012). Animal research in medical sciences: Seeking a convergence of science, medicine, and animal law. *South Texas Law Review 54*,469-511.http://faculty.smu.edu/jkazez/ar13/pippin.pdf

Pistollato, F., Cavanaugh, S. E. and Chandrasekera, P. C. (2015). A human-based integrated framework for Alzheimer's disease research. *J Alzheimers Dis 47*, 857-868. doi:10.3233/JAD-150281

Pistollato, F., Ohayon, E. L., Lam, A. et al. (2016). Alzheimer disease research in the 21st century: Past and current failures, new perspectives and funding priorities. *Oncotarget 7*, 38999-39016. doi:10.18632/oncotarget.9175

Pound, P., Ebrahim, S., Sandercock, P. et al. (2004). Where is the evidence that animal research benefits humans? *BMJ 328*, 514-517. doi:10.1136/bmj.328.7438.514

Pound, P. and Bracken, M. B. (2014). Is animal research sufficiently evidence based to be a cornerstone of biomedical research? *BMJ 348*, g3387.doi:10.1136/bmj.g3387

Pound, P. and Ritskes-Hoitinga, M. (2018). Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *J Transl Med 16*, 304. doi:10.1186/s12967-018-1678-1

Reardon, S. (2018). Alzheimer's researchers seek better mice. *Nature 563*, 611-612. doi:10.1038/d41586-018-07484-w

Russell, W. M. S. and Burch, R. L. (1959). *The Principles of Humane Experimental Technique*. https://books.google.it/books/about/The_principles_of_humane_experimental_te.html?id=j75qAAAAMAAJ&redir_esc=y

Savoji, H., Mohammadi, M. H., Rafatian, N. et al. (2019). Cardiovascular disease models: A game changing paradigm in drug discovery and screening. *Biomaterials 198*, 3-26. doi:10.1016/j.biomaterials.2018.09.036

Skardal, A., Shupe, T. and Atala, A. (2016). Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug Discov Today 21*, 1399-1411. doi:10.1016/j.drudis.2016.07.003

Stephens, M. L. (2012). Pursuing Medawar's challenge for full replacement. *ALTEX Proc 1*, 23-26. http://www.altex.ch/resources/023026_Stephens131.pdf

Stephens, M. and Mak, N. (2013). History of the 3Rs in toxicity testing: From Russell and Burch to 21st century toxicology. In D. G. Allen and M. D. Waters (eds.), *Reducing, Refining and Replacing the Use of Animals in Toxicity Testing*. The Royal Society of Chemistry. https://pubs.rsc.org/en/content/chapter/bk9781849736527-00001/978-1-84973-652-7

Sutherland, B. A., Minnerup, J., Balami, J. S. et al. (2012). Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. *Int J Stroke 7*, 407-418. doi:10.1111/j.1747-4949.2012.00770.x

Taylor, K. and Rego Alvarez, L. (2019). A summary of eu national statistical reports of animal experiments in 2014-2016. *ALTEX 36*, 314-319. doi:10.14573/altex.1812211

Thomas, D. W., Burns, J., Audette, J. et al. (2016). Clinical development success rates 2006-2015. https://www.bio.org/sites/default/files/Clinical%20Development%20Success%20Rates%202006-2015%20-%20BIO,%20Biomedtracker,%20Amplion%202016.pdf

van Vliet, E., Daneshian, M., Beilmann et al. (2014). Current approaches and future role of high content imaging in safety sciences and drug discovery. *ALTEX 31*, 479-493. doi:10.14573/altex.1405271

van der Worp, H. B., Howells, D. W., Sena, E. S. et al. (2010). Can animal models of disease reliably inform human studies? *PLoS Med 7*, e1000245. doi:10.1371/journal.pmed.1000245

Vaudano, E., Vannieuwenhuyse, B., Van Der Geyten, S. et al. (2015). Boosting translational research on Alzheimer's disease in Europe: The innovative medicine initiative ad research platform. *Alzheimers Dement 11*, 1121-1122. doi:10.1016/j.jalz.2015.02.002

Wang, S. V., Verpillat, P., Rassen, J. A. et al. (2016). Transparency and reproducibility of observational cohort studies using large healthcare databases. *Clin Pharmacol Ther 99*, 325-332. doi:10.1002/cpt.329

Wilkinson, M. (2019). The potential of organ on chip technology for replacing animal testing. In K. Herrmann and K. Jayne (eds.), *Animal Experimentation: Working Towards a Paradigm Change* (639-653). Vol. 22. Leiden, The Netherlands: Brill. doi: 10.1163/9789004391192_027

Conflict of interest

The authors declare that they have no conflicts of interest.