

Dr Smart talks to Prof Pries and Prof Dirnagl on animal experimentation in biomedical research



Moderator:
Dr Nicola Smart
University of Oxford, Department of Physiology, Anatomy & Genetics, Nucleus member of the Scientists of Tomorrow, Council on Basic Cardiovascular Science, European Society of Cardiology



Professor Axel R. Pries
Professor of Physiology and Dean, Charité University, Berlin, Germany



Professor Ulrich Dirnagl
Professor and Head, Chair for Clinical Neuroscience, Head of the Department of Experimental Neurology, Charité Universitätsmedizin, Berlin and Founding Director, Center for Transforming Biomedical Research, Berlin Institute of Health, Germany

The debate about the need to use animals in scientific experiments concerns all of us, and society as a whole. It addresses, among others, testing in the framework of consumer safety of a wide range of products, testing for drug development and use of animals for the study of disease mechanisms and discovery. The debate calls for a multidisciplinary, widespread involvement of all players and stakeholders. Researchers and academia are part of this debate and do participate actively, as e.g. in the conference organized by the European Commission' Directorate-General Environment 'Non-Animal Approaches–The Way Forward', 5–6 December 2016 (http://ec.europa.eu/environment/chemicals/lab_animals/index_en.htm, accessed on 6 April 2017).

The conference included reports and debate about the quality and contributions of animal research in disease, the strengths and limitations of non-animal alternatives and how to conduct responsible research, including the role of journals and editors.

This conference was part of a list of actions in response to a European Citizens' Initiative (ECI) in 2015, challenging the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Though this ECI did not result in repeal of the Directive, in its normal time course, the Directive will open for review this year.

Here, the ESC Scientists of Tomorrow (SoT) and CVR OnLife have taken the opportunity to talk to leading researchers engaged in the debate. Nicola Smart interviews:

- Prof. Dr. Axel R. Pries, of Charité–Universitätsmedizin Berlin, Chair of the BioMed Alliance task force for animal experiments and alternative approaches to discuss the implications of the Position Statement
- Prof. Dr. Ulrich Dirnagl, also of Charité–Universitätsmedizin Berlin, an international expert in planning and interpretation of experimental approaches in biomedical research, to discuss the ethics and quality of animal research more generally.

Background: the European Citizens' initiative

In 2015, the ECI 'Stop Vivisection' was submitted, calling on the European Commission to "consider clear ethical objections to animal experiments and solid scientific principles that invalidate the 'animal model' for

predicting human response"; they urge the European Commission to "abrogate Directive 2010/63/EU on the protection of animals used for scientific purposes and to present a new proposal that does away with animal experimentation and instead makes compulsory the use—in biomedical and toxicological research—of data directly relevant for the human species". Moreover, the ECI calls for the adoption of a new legislative framework that fully phases out animal experiments by 2020.

While the Commission agrees that the phasing out of animal testing should be an ultimate goal for EU legislation, they disagreed that scientific principles invalidate the 'animal model'; rather, these models have been "the key scientific drivers to develop almost all existing effective and safe medical treatments and prevention measures for human and animal diseases". Since the 2010/63/EU has notably enhanced animal welfare standards across the EU, in line with the concepts of replacement, reduction and refinement ('3Rs'), the ECI was rejected by the EU Commission. The Commission intend to review the Directive in 2017 and propose to further emphasize the availability of alternative approaches. The ECI and Commission's response can be accessed: <http://ec.europa.eu/citizens-initiative/public/initiatives/successful/details/2012/000007> (accessed on 6 April 2017)

In response to the ongoing debate, the Alliance for Biomedical Research in Europe (BioMed Alliance, of which the European Society of Cardiology (ESC) is a key member) issued a Position Statement in support of the EU Directive.

Nicola Smart (SoT): Professor Pries, you chaired the task force for animal experiments and alternative approaches that issued the Position Statement on behalf of the BioMed Alliance. I think it would be helpful if I could first ask you, please, to summarize, the key points of the Position Statement?

Axel Pries: Key points of the Position statement are:

- Animal experiments are still indispensable for biomedical research and clinical improvements.
- Animal experiments for the development of medical options are in line with general ethical concepts.
- Animal experiments have to be designed to maximize the benefit for patients and minimize harm for animals.
- Alternative and innovative approaches are increasingly becoming available and enable the replacement of animal experiments.

- The Biomed Alliance urges funding agencies and policy makers to support the development and use of alternative approaches by targeted incentives.

The last point is of particular importance: The ethical basis for the acceptance of indispensable experimentation including laboratory animals requires not only high standards for animal experiments but also the active search for alternatives and a bold support for respective initiatives by the scientific community and on a political level.

Nicola Smart: What changes, if any, do you envisage, as a result of the Position Statement? Essentially, we have been working within the legislation set out under the Directive and with consideration of the 3Rs since at least 2010 and, in most EU countries, long before that—what more should be done and how will this be promoted?

Axel Pries: This is a very relevant question since, as you state, the 3Rs principles have been around for quite some time. Obviously, the implementation of these principles has been and is a continuous process. As a consequence, there have been improvements in handling of laboratory animals and also in the development of alternative approaches. However, self-stabilizing mechanisms within the scientific community may prevent implementation of new approaches with optimal speed. For example, if funding organizations and high impact journals require animal experiments for successful applications, younger researchers will comply with these conditions, in turn enforcing the expectation that animal experiments are required for high quality research. Such self-stabilizing systems often need strong external stimuli for a transition; one example was the German energy market of the 1980s: Only after energy providers guaranteed owners of wind power stations the purchase (at a fixed rate) of all the electricity that they could generate, did this component of energy production really take off. The BioMed alliance hopes that the Position Statement may trigger such a reaction within the scientific community, funding agencies, publishers and policy makers. The time would be right for a transition based both on recent technical developments for alternative approaches and a wide spread rethinking by many scientists.

Nicola Smart: And, also of great concern, is the claim of the ECI that animal research actually endangers human health by hindering the development of more reliable and relevant research models—those specifically designed for studying human systems—do you agree?

Axel Pries: New developments with respect to the use of human material from surgical procedures and cellular systems have opened new options that allow replacement of animal experiments. The BioMed alliance Position Statement wants to support the whole community in making full use of these developments. However, it is important not to use dangerous simplifications in describing the conditions for biomedical research. No single result can be carried over from any model situation to a given patient. This is true even between individual patients—otherwise there would be no need for large clinical studies. Optimal safety and reliability can only be achieved by combining a larger and well selected array of models on different levels. Animal experiments, as one component, have provided extremely relevant information and contributed centrally to many medical options made available to patients within the past decades. The BioMed alliance wants to stimulate an ever-increasing role for alternative approaches in this context, but firmly stipulates that patient safety and benefit are the ultimate criteria—requiring animal experiments especially to probe the complex interaction of many cells and organs.

Nicola Smart: Naturally, we all aspire to perform meaningful, translational research that is relevant to human patients. What alternative methods can we use to address relevant biological questions and to reliably predict how a human patient may respond to a particular therapeutic intervention?

Axel Pries: The array of alternative methods is developing very fast. We already make use of consenting patient samples to address mechanistic

questions; we can use gene editing to replicate human conditions or diseases in test cell systems; other approaches include, for example, complex multi-cellular constructs, ‘organ on a chip’ and deep tissue imaging applied to human material from surgical procedures. However, to reiterate, it is important to recognize that there is NO single model which could ‘reliably predict how a human patient may respond to a particular therapeutic intervention’.

Nicola Smart: So, as I understand, the Alliance supports the continued use of animal research, where no viable alternatives can be used to address important biomedical questions?

Axel Pries: This is exactly right: For the foreseeable future, animal experiments will be indispensable for the improvement of medical options for the population and for patient safety. But the use of animals is ethically only acceptable if alternative approaches are vigorously explored and supported.

Nicola Smart: A counter-argument, to explain the failure in translating laboratory findings to the clinic, is that preclinical studies are frequently under-powered, often deliberately to minimize animal number, and extensively rely on the use of rodents, rather than on larger animals, whose physiology more closely resembles that of humans. Should there also be a focus on improving the quality and rigour of basic research, to increase the value gained from animal usage in the laboratory?

Axel Pries: While the Status Document of the BioMed Alliance urges the consideration of alternative approaches, it also very clearly states the need to push for improvements in the value, reliability and predictive power of animal experiments. These issues are increasingly discussed by the scientific community worldwide and we need to change the way experiments are planned and performed to better comply with our ethical requirements.

Nicola Smart: One of the salient messages of the Position Statement is the call for greater cross-sectoral collaboration to develop and improve the alternative models and approaches. Please could you elaborate and tell us who you think are the key players and how you envisage that they can help promote the cause?

Axel Pries: A central prerequisite to generate a fast and significant change in biomedical research is to engage the support of the main stakeholders who shape research culture and practice. This includes individual researchers and their national and international societies (like the BioMed Alliance), the funding agencies and their financial sources, publishing houses and lastly, but very importantly, the political establishments, who can support the implementation of bolder measures to transform self-stabilizing systems. It is a good sign for the ethical basis of science that initiatives to improve the quality of experiments and the availability and use of alternative approaches were put forward by scientists. However, without the help and support of the other players, scientists will not succeed in changing the rules of the game!

Nicola Smart: And the EU would presumably have an important role to play—perhaps taking the lead on this?

Axel Pries: The EU is working directly to promote the alternative models. More than €250 million was dedicated during FP7 (2007–2013) to research into alternatives. As part of this, six large projects for a total of €140 million have been co-financed as public-private partnerships with either the cosmetics industry or the Innovative Medicines Initiative.

Nicola Smart: Finally, the ESC is a member of the BioMed Alliance; what are the other ways in which the ESC can support and promote successful basic research and its translation for patient benefit?

Axel Pries: The ESC has a broad array of activities to foster value-based science—be it basic or clinical—and translation into everyday practise. This starts with both cutting edge and educational formats at the yearly ESC congress. The large number of active participants (~30,000) provides an extremely relevant transmission belt of new insights and approaches into the cardiovascular community in Europe but increasingly also worldwide. The same is true for the ESC

educational platforms like ESCel. However, the ESC alone cannot change the boundary conditions and pressures relevant for individual scientists and clinicians. If publication frequency is more important than value and 'proof' of concepts need animal experiments, individual scientists will be needed to act accordingly or risk their career. Thus, the responsibility is upon all stakeholders of the process to initiate a fundamental change for biomedical research.

Nicola Smart: Professor Dirnagl, as we have heard, the EU commission state that the phasing out of animal testing should be a future goal for EU legislation. That's a strong statement of intent—how do you respond to that?

Ulrich Dirnagl: My view is, and I'm sure many, even most, researchers would agree, that animal experiments are still indispensable for some aspects of biomedical translation; furthermore, that animal experiments for the development of medical options are in line with general ethical concepts. However, we must recognize the scope for further refining animal experiments in order to maximize the benefit for patients and minimize harm for animals. In addition, we need to improve on the design, conduct, analysis and reporting of current research in preclinical biomedicine, not only to reduce harm, but also to improve the predictive power and value of the research.

Nicola Smart: As you know, the current EU Directive 2010/63/EU already seeks to enforce and harmonize high standards for animal experiments with the aim to both maximize beneficial outcome and minimize harm for animals. So, what more should be done and how will this be promoted?

Ulrich Dirnagl: What we need is an engagement of the stakeholders, in particular the institutions, such as the universities, the professional societies and journals, but also the funders. In particular if we want to improve the predictiveness, robustness, and value of animal experiments we need a major change of research culture. This mandates changing some of the perverse incentives in the academic reward system—which foster publication of spectacular findings with shaky evidence behind them and suppress the publication of null and negative results.

Nicola Smart: The ECI 'Stop vivisection' and groups opposing animal use in research claim that animal welfare is compromised in laboratory conditions and that experiments cause pain and suffering – is this a fair reflection of most animal research?

Ulrich Dirnagl: I personally think that there may be some 'black sheep' out there, which is regrettable, and needs to be stopped. However, I believe that most biomedical researchers take animal welfare very seriously and plan and conduct their experiments accordingly. Certainly, the strict regulations and ethics committees that govern animal welfare in EU labs, both relating to the animal housing and the actual experiments, have substantially improved mat-

ters. However, we do have a problem related to bias (lack of blinding and randomization, for example), selective analysis as well as reporting. In addition, most experimental series lack statistical power. Hence, the literature abounds with false positive results, which also explains at least partially why we are currently experiencing a 'replication crisis'.

Nicola Smart: So you believe it's more a question of increasing stringency and robustness of animal studies—not that animal models cannot accurately model human disease?

Ulrich Dirnagl: We know that animal experiments can be highly predictive, and are mandatory to inform on the safety and efficacy of novel treatment strategies. But we have to concede that rather a lot of previous animal research produced results that were non informative, in some cases even misleading. Again, I strongly believe that a large proportion of the translational failures are grounded in low internal and external validity of some of the experimental studies on which the clinical trials were based, as well as their exceedingly low statistical power. In combination with a strong bias towards the publication of positive findings, it is perhaps not surprising that we see a low reproducibility and minimal predictive power for human relevance. This problem, by the way, also affects the literature which utilizes the so-called 'alternative methods', where the same shortcomings (low internal validity and power) are combined with potentially contaminated or non-validated biologicals and cell lines. In sum, we need to improve preclinical research in general.

Nicola Smart: You have underlined a rather urgent need to bring about change. What can we, the scientific community, do to improve the reproducibility and applicability of our findings? We strive to demonstrate impact in our research but are we in danger of losing sight of what is most important—relevance?

Ulrich Dirnagl: Clearly, what's needed is a change in culture. This includes not only modifications in the reward and incentive system of academic medicine, but also education and training, as well as measures, such as the use of electronic laboratory notebooks, replication studies and multicenter randomized preclinical trials, study preregistration and publication of negative results, among many others. I think we know what to do, over the last few years there have been many symposia and learned papers with a lot of good intention and excellent suggestions. Now it's time to act.

Nicola Smart: Indeed. On that note, I would like to thank both our interviewees, Professors Pries and Dirnagl, once again, for sharing with us their views and the value of their experience. A timely dialogue with the scientific community on maximizing the value and rigour of animal experimentation, alongside the drive to identify and consider alternative approaches, may promote the drive towards improving the translation of basic discoveries and putative therapies from the laboratory to the clinic.

**Professor Axel R. Pries****Professor of Physiology and Dean, Charité - Universitätsmedizin Berlin**

Axel Radlach Pries studied medicine at the University of Cologne and defended his doctoral thesis in 1980 with 'summa cum laude'. He worked as postdoctoral fellow in Cologne and Berlin University and 1997-1998 at the Institute of Anaesthesiology of the German Heart Center Berlin. 1998 he became full professor at the Department of Physiology, Free University Berlin and 2001 head of the Charité Institute for Physiology. His scientific interests include microcirculation, tumour vasculature, blood rheology, vascular adaptation, angiogenesis, and the endothelial surface layer. He was general secretary of the ESM (European Society for Microcirculation) and is chair of the International Liaison Committee for Microcirculation. In the European Society of Cardiology (ESC), he was chair of the Working Group for Coronary Pathophysiology and Microcirculation and of the Council for Basic Cardiovascular Science (CBCS). His awards include the Abbott Microcirculation Award, the Malpighi Award of the ESM, Lafon Hemorheology-Microcirculation Award of the International Society for Clinical Haemorheology and the Silver Medal of the ESC. Since 2015, he is dean of the Charité University Medicine Berlin.

**Professor Ulrich Dirnagl****Professor and Head, Chair for Clinical Neuroscience, Head of the Department of Experimental Neurology, Charité - Universitätsmedizin Berlin and Founding Director, Center for Transforming Biomedical Research at the Berlin Institute of Health**

The research of Ulrich Dirnagl is focused on stroke, cerebral blood flow regulation, and brain imaging. In preclinical models as well as clinical trials he and his coworkers and collaborators explore mechanisms by which brain ischemia leads to cell death, and develops novel methods to intercept mechanisms of damage in acute brain damage, as well as to foster regeneration and repair of the lesions. He is particularly interested in how the brain protects itself ('endogenous neuroprotection'), and how the brain interacts with other systems of the body after it has been injured. Closely linked to his interest in stroke pathophysiology is his interest in the coupling of regional blood flow to neuronal activity, the mechanism underlying functional brain imaging with MR and PET. Beyond imaging structure and function of the CNS he and his team are developing, validating and using techniques that allow the non-invasive imaging of brain biochemistry and molecular signaling. To this end they use optical, MR, and nuclear medicine approaches in mouse and man. To improve the predictiveness of preclinical translational research he is actively promoting the introduction of quality standards for experimental design and reporting, as well as international collaboration in large, phase III-type preclinical trials. At the Charité Universitätsmedizin Berlin Ulrich Dirnagl serves as Director of the Department of Experimental Neurology. Since 2017 he is also the founding director of the Center for Transforming Biomedical Research (CTBR) at the Berlin Institute of Health. CTBR aims at overcoming the roadblocks in translational medicine by increasing the value and impact of biomedical research through maximizing the quality, reproducibility, generalizability, and validity of research.