National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Briefing paper

Improving translation and minimising animal use with human-relevant *in vitro* preclinical models

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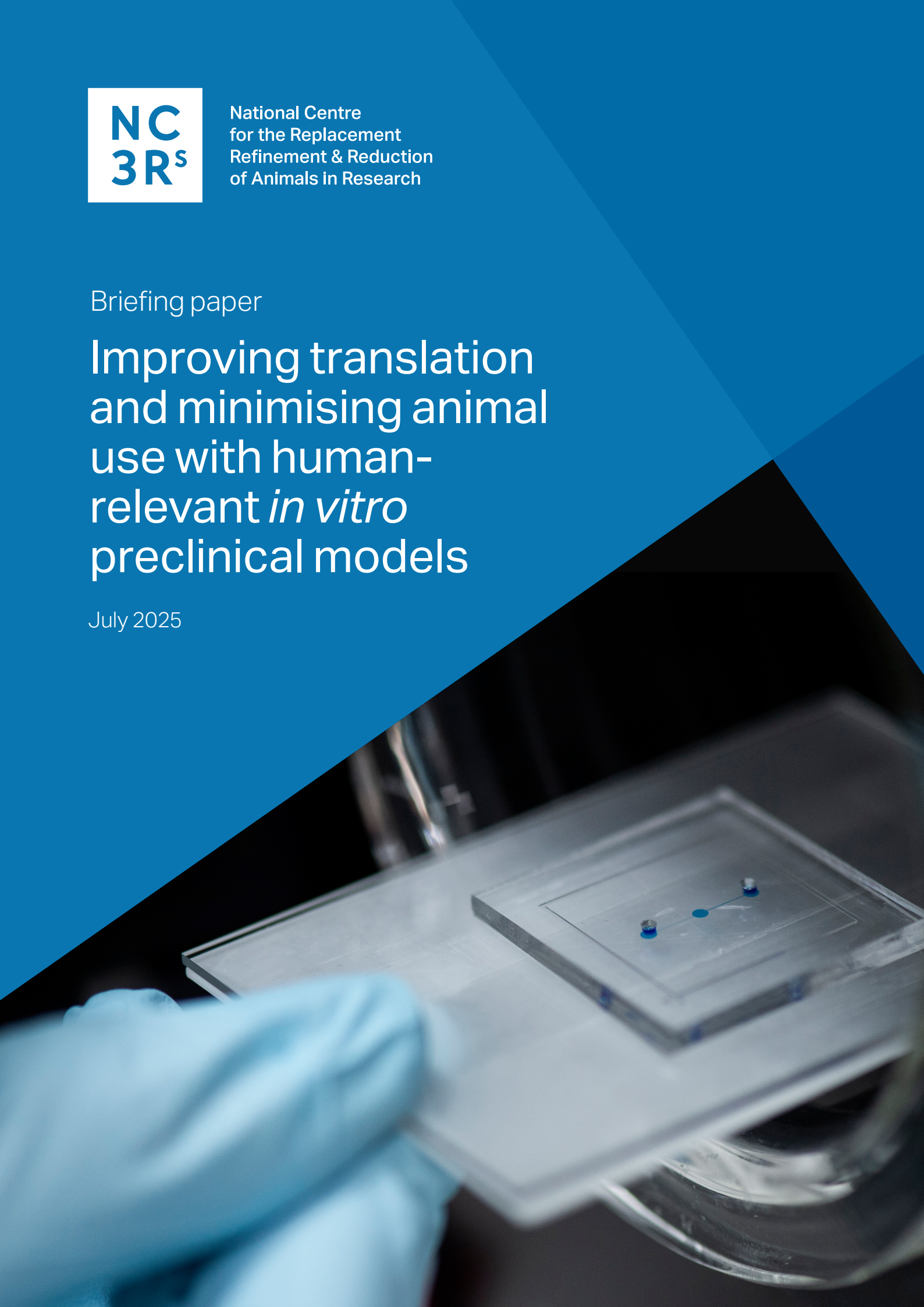

Headline impacts from the NC3Rs human-based *in vitro* models research and innovation portfolio

£60.1M invested in research and innovation awards	**£18.9M** co-funding leveraged by the NC3Rs	**100,000+** animal replacement potential
551 academic groups supported	**75** PhD students trained	**116** overseas partners
£1 → £9.52 NC3Rs grant holders secured on average £9.52 in follow-on funding per £1 invested by the NC3Rs	**859** grant holder publications leading to 44,188 citations FCR 4.12, RCR 2.22*	
£30M+ venture capital investment secured	**£450k** in-kind contributions provided by major companies for CRACK IT Challenges	
77 consortia fostered through CRACK IT Challenges	**46** SMEs supported including four SMEs formed through NC3Rs investment	

*Dimensions software was used to calculate the Relative Citation Ratio (RCR) and the Field Citation Ratio (FCR) of NC3Rs grant holder publications. The RCR provides the relative impact of a publication compared to National Institues of Health-funded publications in the same research area and the FCR provides the relative impact of a paper within its own research field. Both ratios are normalised to one, with a value of more than one indicating that the paper has had an above average number of citations for its research area or field, and publishing year.

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Introduction

Investing in predictive and robust human-based *in vitro* models for preclinical research is key to the NC3Rs mission to replace the use of animals. Since the NC3Rs was launched in 2004 we have committed £60.1M for research and innovation to develop human-based *in vitro* models, focusing on replacing studies that involve large numbers of animals, high levels of animal suffering or are poorly predictive in terms of their translatability or reproducibility (and therefore waste animals). Our long-term commitment to supporting this area has shaped the field, not only fulfilling obligations set out under Section 20b of the Animals (Scientific Procedures) Act 1986[1] but also helping to embed human *in vitro* models in biomedical research. The impacts we deliver go beyond the NC3Rs mission and include helping to realise the substantial market and growth potential for *in vitro* models, which the UK is well placed to deliver. Our work is essential for making research and development more efficient and effective, helping to reduce drug attrition caused by poor translation of data from animals to humans and lowering the time and cost of bringing new drugs to market. We have played a pivotal role in the increasing focus on human-relevant approaches, laying the foundation for major new investments in this area.

Our approach

We fund researchers to develop *in vitro* models that better recapitulate human physiology and disease than existing animal models, facilitating their use for various purposes including for disease modelling, the identification of therapeutic targets and safety and toxicity testing. Our starting point is addressing the scientific limitations of the *in vivo* models and then building on this to optimise and assess the utility and comparative performance of *in vitro* alternatives so that they are fit-for purpose for replacing animal use. Alongside this, we tackle the barriers to uptake and how these can be resolved, including engaging with researchers in industry, academia and regulatory bodies nationally and internationally. Here we describe our approach that includes developing the next generation of preclinical models, supporting model qualification and uptake, bridging the translational gap through collaboration with industry, and providing the underpinning infrastructure and networks required to accelerate wide adoption.

[1] Section 20b relates to support for the development and validation of alternative strategies.

Developing the next generation of preclinical models

Model development is our forte. We have made 185 awards for academic scientists and early career researchers to develop human-based *in vitro* models for a wide range of applications. These models range from "simple" *in vitro* models that can deliver highly predictive platforms to the more "complex" organ-on-a-chip approaches that enable the study of intricate physiological mechanisms.

We have supported the development of human-based 2D *in vitro* models for toxin testing. For example, we funded Professor Andrew Peden at the University of Sheffield to develop an *in vitro* assay to replace the *in vivo* mouse lethality bioassay for batch release potency testing of Botulinum B neurotoxin products and for the quality control testing of Botulinum antitoxin – tests that involve significant levels of animal suffering. In 2023 in Great Britain 48,526 mice were used for Botulinum testing, while globally the figure is likely to be close to a million. The *in vitro* assay, which uses a human neuroblastoma cell line, has greater sensitivity than the animal test being able to distinguish between the different Botulinum neurotoxin serotypes. It has been taken in-house by two toxin manufacturers and a major contract research organisation. With further NC3Rs funding, the team are now developing the assay for tetanus vaccine and antitoxin production to replace current *in vivo* mouse tests which also use thousands of animals each year. The UK's medicine regulator, the MHRA[2], has been a collaborator on both the Botulinum and tetanus projects, a key partnership given that it produces 90% of the WHO's[3] global reference standards against which all biologics must be benchmarked. The MHRA is currently evaluating the tetanus cell-based assay using historical animal data as well as validating the assay for use with International Reference Standard Tetanus Antitoxin. The Sheffield team have formed a spin-out company, Sansanima Ltd, to commercialise the assays.

We have invested in human-based complex *in vitro* models of bone formation and disease to replace the use of animal models, including rodents, rabbits and sheep, in research that often requires surgical procedures for example to study bone injury and healing. This includes funding for Professor Martin Knight at Queen Mary University of London who was supported through a joint BBSRC[4]-NC3Rs call to produce the first *in vitro* model of the human growth plate (the cartilage at the end of bones) and its development into mature osteochondral tissue. By partnering with the organ-on-a-chip biotech company, Emulate, the model has been further developed to incorporate vascularisation and mechanical loading, with Emulate providing the chips, financial support and staff resource for the project. The model enables improved understanding of musculoskeletal health, ageing and disease, permits drug efficacy testing and has the potential to replace the use of thousands of mice each year.

[2] Medicines and Healthcare products Regulatory Agency.

[3] World Health Organization.

[4] Biotechnology and Biological Sciences Research Council.

Supporting preclinical model qualification and uptake

Integrating new *in vitro* models into research and development can be challenging due to concerns about how results compare with traditional animal models, and because their use may not yet be widely accepted by peers, regulators or collaborators. We fund projects (including with co-funding partners) and build networks to help overcome these barriers, supporting efforts to benchmark new human-based *in vitro* approaches against established gold standards, assess how easily they can be used in other labs, and confirm that they produce reliable, repeatable results. We also promote the sharing of skills, knowledge and resources between labs to support broader adoption.

With joint CRUK[5]-NC3Rs funding, Dr David Fernandez-Antoran's team at the University of Cambridge have transferred the technical know-how for generating an epithelioid model to six other cancer research groups so that the model can be optimised and applied to additional epithelial tissue types including the oesophagus, trachea, skin, ovary and bladder. The methodology allows for the first time the generation of long-term, self-maintaining and expandable primary 3D cultures of mouse and human epithelial tissues from different origins (termed epitheliods). The epithelioids permit detailed studies of cancer mechanisms, are amenable to gene editing, and can be used to study ageing and regeneration. David's lab has replaced its use of mice (by 200 per year) by generating epithelioids from human tissues, with the collaborator labs also either fully replacing or reducing their animal use.

With joint BHF[6]-NC3Rs funding Dr Sarah Jones at the Manchester Metropolitan University has developed two physiologically-relevant, endothelialised organ-on-a-chip models of atherothrombosis to replace the use of mice in arterial thrombus studies which involve ferric chloride or laser-induced damage to the blood vessel lining. The *in vivo* models have limited relevance to human biology due to differences in platelets, vessels, and blood flow dynamics and studies can be poorly reproducible. The complex *in vitro* models developed incorporate human endothelial cells and blood under coronary artery-like flow conditions to replicate the vessel wall environment following plaque rupture or erosion. They offer a basic platform to assess endothelial cell contributions to thrombosis and drug efficacy, and a more advanced model designed to mimic the ferric chloride mouse model. The organ-on-a-chip models have replaced the use of 180 mice in Sarah's lab over the past two years, and with further NC3Rs funding the models are being transferred to two other research groups that are the main animal users in the thrombosis research field in the UK. This will enable independent replication and application to other areas including for stroke research – and allow up to 2,500 animals per year to be replaced.

[5] Cancer Research UK.

[6] British Heart Foundation.

We have used our convening power to bring together industry, academia and SMEs through specialist networks that facilitate the sharing of expertise and knowledge to drive the preclinical application of *in vitro* models. Our networks focus on the areas where there are currently the greatest opportunities for change based on the use of animals and need for improved translation – oncology, cardiovascular sciences and new approach methodologies for safety testing purposes – in just over a year since their launch we already have more than 1,000 network members, a new programme to increase the use of human tissue for cardiovascular research and a new collaboration with the BBSRC to provide business interaction vouchers for collaborations between industry and academic members.

Bridging the translational gap through collaboration with industry

R&D collaborations are key to catalysing the deployment of preclinical models from academics and SMEs into the hands of industry scientists. We have a strong track record of enabling effective partnerships between multinational organisations and SMEs through our unique CRACK IT Challenges open innovation programme which funds the development of preclinical products and services that are fit-for-purpose for industry use. We have invested £25.6M in 21 innovation awards to develop human-relevant *in vitro* models for translational research into various diseases and for safety and toxicology studies, collaborating with 35 industry partners and government organisations including companies such as AstraZeneca, GSK and Unilever, which have provided co-funding and/or in-kind contributions of around £0.75M. We have supported 46 SMEs working on human-based *in vitro* models, including four that were formed as a direct result of our investment. The models funded include the generation of patient derived stem cells of Alzheimer's and bipolar affective disorder, organ-on-a-chip models of osteoarthritis, kidney and neuronal toxicity and the development of retinal organoids for use across disease modelling, efficacy and toxicity testing. The latter were developed by the Newcastle-based SME Newcells Biotech from human-induced pluripotent stem cells to replace the use of rodents and rabbits in ocular disease research and toxicity testing.

Newcells Biotech's retinal organoids contain key cell types and mimic physiological features of the retina *in vivo*. They are light responsive, stable for over 150 days in culture and can be produced at scale. The engagement of Merck, Novartis and Roche in the award from the outset has been key to the success of this project. Newcells Biotech now produces thousands of retinal organoids a month for screening next generation therapies on behalf of more than 100 biotech customers globally. The organoids are also shipped around the world for customers to study retinal diseases and generate efficacy and safety data prior to first-in-human studies. The retinal platform accounts for a significant proportion of Newcells Biotech's total revenue and has driven the growth of the company which has over 40 employees. This success has allowed Newcells Biotech to access further multi-million pounds of external investment from venture capital investors, including Merica and Northstar Ventures.

Providing the underpinning infrastructure for preclinical models

The widescale adoption of human-based *in vitro* models requires equitable access to equipment, reagents and specialist services and expertise. In 2024, with extra funding allocated to the NC3Rs from DSIT[7] we committed £3.95M for infrastructure to help underpin the growth in interest and activity in the use of non-animal methods. We made 11 awards, of which ten focused on facilitating the use of human-based *in vitro* models. This included funding to Dr Ildem Akerman at the University of Birmingham's BetaCell facility to expand its production capacity for insulin-secreting organoids derived from human stem cells to replace the use of mouse-derived beta cells in diabetes research – with tens of thousands of animals used in this area each year. The award supported the purchase of a larger perfusion-based suspension bioreactor and the introduction of automation to streamline production such that the facility can meet the high demand from users locally and nationally. The facility is prioritising the supply of human-derived beta cells to replace the use of animals, with the cells being used to develop cell replacement therapies for type 1 diabetes.

We have also supported NC3Rs grant holders to access specialist expertise that enables their models to be further developed and tested for industry use and commercialisation. Working with the Milner Therapeutics Institute in Cambridge, we funded Dr Deepali Pal from the University of Bristol to optimise an *ex vivo* organoid culture method for expanding patient-derived leukaemia samples using induced pluripotent stem cell technology to better represent the *in vivo* environment and support the long-term proliferation of primary leukaemia cells. The model which has allowed Deepali to replace the use of over 2,500 mice in her laboratory, has now been established at the Milner Therapeutics Institute in both 96- and 384-well plate formats. It is being used to investigate the molecular mechanisms underlying leukaemia-bone marrow niche interactions, with a focus on identifying genes involved in cancer treatment resistance.

Many *in vitro* models are dependent on animal-derived products such as matrices and fetal calf serum to support the growth and differentiation of cells. Not only do these require the use of animals but they are associated with batch-to-batch variation that compromises the reproducibility of studies. We funded Professor Cathy Merry at the University of Nottingham to develop a novel synthetic animal-free extracellular matrix (a peptide hydrogel) that can be used as an alternative to the commonly used Matrigel which is derived from mouse connective tissue cancers. We funded access to the specialist facilities at the Medicines Discovery Catapult so that the transferability and reproducibility of the peptide hydrogel could be independently assessed – the data from this was used to support the spinning out of the company PeptiMatrix and the hydrogel is now available commercially for a range of applications including for complex *in vitro* models used in cancer research.

[7] Department for Science, Innovation and Technology.

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