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

Workshop report:

Human tissue-based models to reduce animal use in cancer research: barriers and opportunities

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Pioneering Better Science

Introduction

In 2015 in Great Britain, cancer research accounted for over 195,000 experimental procedures on animals [1]. This substantial level of research reflects the societal burden of cancer and the high level of funding this area attracts. Research into the origin and mechanisms of cancer pathology and cancer drug development has traditionally relied heavily on animal models, often mice. Genetically engineered mouse models (GEMMs), patient-derived xenograft and cell line-derived xenograft mice have found utility in both basic research and drug development [2-4], but do not adequately reflect human biology and the development, maintenance and spread of cancer [5]. Humanised mice are being developed which carry human immune system cells to allow for the testing of immunotherapies [6, 7], a growing area of interest for pharmaceutical companies, but are expensive to produce.

Attrition rates in cancer drug development have increased [8] despite a steep rise in investment in pharmaceutical R&D [9]. Likelihood of success in clinical trials was lowest in oncology among all indications analysed, with cancer drugs showing just a 25% success rate in Phase II [10]. During this Phase, development was most often stopped due to a failure to demonstrate efficacy, which can be traced back to a failure to correctly identify efficacy in animal models [11]. The translational relevance and predictivity-to-human of animal models used for efficacy testing of cancer therapeutics has therefore been called into doubt [12]. There is a great unmet need to develop alternative models which can recapitulate human cancer biology more closely and are highly predictive of drug responses in man.

Human tissue has been used for many years across a range of research areas in a fragmented manner. Collaborations are often based on personal connections between clinical and research staff to facilitate transfer of tissue, or local biobanks providing tissue to a small network of researchers. An increasing interest in the utility of human tissue for research purposes within the cancer research community has driven expansion of biobanking [13], and led to efforts to centralise information on biobanking more generally [14, 15]. Additionally, recent advances in human stem cell technologies have expanded the definition of human tissue to include pluripotent stem cell lines. However, significant blocks exist to the more widespread implementation of human tissue in cancer research laboratories.

The NC3Rs held a 1½ day workshop in March 2017 entitled 'Human Tissue Models for Cancer Research', in order to explore these blocks and ways in which the cancer research community can increase the implementation and uptake of human tissue models. The more widespread use of human tissue in cancer research would allow replacement of some animal models with more translationally-relevant human-based models, reducing animal numbers, improving basic research and increasing the probability of successful drug development.

The workshop brought together a multidisciplinary group of researchers and focused on the following themes:

- Engineering the microphysiology of cancer with human tissue;
- Fresh human tissue;
- Fixed human tissue for research and drug development.

For the purposes of the meeting, we defined a human tissue model as being a tissue explant or cell derived model of human origin which is maintained without the use of an animal. Speakers from academia and the pharmaceutical industry described a range of cutting-edge methods and processes using human tissue to study cancer (see Appendix 1). These included bioprinted tumour models, organoid biobanks, microfluidics, bioinformatics, molecular pathology and mass-spectrometry imaging. In addition, contributions from the UK Clinical Research Collaboration, the National Cancer Institute's Innovative Molecular Analysis Technologies division in the USA and the CRUK Edinburgh Drug Discovery group provided insight into relevant networks operating in the area.

Barriers to progress in implementing human tissue for cancer research

During the workshop, delegates were asked to detail the blocks to human tissue use they have faced in their work. Table 1 highlights the top responses, showing that collaboration between clinical and research staff, sharing of methods and data, and facilitating the development of new models/supporting technologies are important hurdles to the further uptake of human tissue in cancer research.

The most cited barriers to the increased uptake of human tissue models in cancer research provided by delegates broadly fall into the following categories:

- Multidisciplinary research team needed - The use of human tissue or cells requires a team of clinical and research staff with diverse skills and training to coordinate collection of tissue following clinical/pathology use. This often involves a surgeon, pathologist, research nurse and research scientists. Establishing and coordinating these teams is a barrier to the further implementation of human tissue in research, and can increase the cost and time resource required for human tissue based projects.
- Consent - A key aspect of the clinician/researcher partnership is the ability to gain consent from patients for their tissue or cell sample to be used in research – this is not an automatic assumption in the UK, as it is in several EU countries, where an 'opt-out' system is in place.
- Metadata - In addition to consent, researchers rely on clinical collaborators to supply high quality clinical metadata with tissue samples or cells.
- Availability of tissue - The sporadic nature of surgeries resulting in tissue samples contributes to a lack of certainty on when and how much tissue will be available.
- Maintaining tissue viability - Maintaining the viability and phenotypic characteristics of tissue from the patient through an experiment can be challenging due to technical limitations of technologies used for working with human tissue.
- Inertia - A long-standing cultural reliance on animal models in the cancer research community means that decades of data have been built up from these models. Researchers can be reluctant to replace an animal with a new model, in spite of evidence that animal models are often not predictive of responses in human cancer.

These barriers are not unique to the use of human tissue for cancer research, and have been cited as blocks to progress in other areas where human tissue is used, such as safety pharmacology [16] and asthma research [17]. In these areas, low or sporadic tissue availability, poor links between clinical and non-clinical staff, technical issues with preservation and storage of tissue and the need to change the culture of animal use hinder the progress of human tissue modelling. These parallels suggest that a holistic approach to human tissue provision for research in general may be possible, and could be explored as a mechanism to reduce animal use.

Reasoning for continued use of animals

Delegates were asked to provide information on why animal models remain the model of choice for many cancer researchers. Four key reasons were identified:

- An animal model is currently required to study systemic disease: A complete physiological system is required to study some aspects of cancer, and the mouse provides a convenient way to do this.
- Animals are used to validate the human tissue model: Validation is an important and ongoing phase of model development, during which physiological baseline data and responses to compounds are assessed and compared with clinical outcomes. However, validation against an animal which is not an accurate representation of human physiology or disease may result in the model closely representing mouse cancer, but being less relevant to humans.
- Animal models are the 'gold standard' in cancer research: Reliance on animal models has developed in the absence of alternatives for studying cancer, meaning that many previous studies have been based on animal data. New technologies are now maturing which can support the creation of a new gold standard based on human tissue, as outlined below. This could be perceived as a threat to those carrying out animal modelling.
- Regulators and journals request data from animal studies: Regulators currently require data from animal models during some phases of drug development, for instance to demonstrate safety prior to first-in-man studies. Journal reviewers also were reported as requesting animal data to support *in vitro* or ex vivo tissue culture experiments.

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Overcoming barriers to human tissue modelling in cancer research

Delegates provided viewpoints on how the barriers listed above can start to be overcome, summarised below in four main areas.

- Enabling collaboration between clinicians and researchers: The importance of collaboration between clinicians and researchers was highlighted throughout the workshop. Initial contact between relevant partners can be facilitated by disseminating details of clinicians who can provide tissue and setting up contact between the relevant collaborators. Publishing case studies of this mechanism will allow demonstration of the approach.
- Supporting and standardising biobanking: Biobanks can remove the need for direct contact with a clinical team, reducing the burden on resources this can create, and to some extent overcoming the sporadic nature of tissue provision. Biobanks can also impact on the reproducibility for human tissue research by collecting and providing tissue and cells according to established protocols, and can ensure high quality tissue-associated metadata is collected and the tissue is correctly consented. The standardisation of biobanking through provision of minimal requirements for collection, tissue/cell quality and clinical data ontologies could improve the utility and reproducibility of human tissue experiments. This approach is being examined by the UKCRC Tissue Directory and Coordination Centre [18].
- Driving technology development: New technologies will be key to providing the tools needed to harness human tissue for disease modelling. Recent developments in technologies in fresh tissue/cells and fixed tissue will allow the creation of human-based models for research, and encourage researchers to move away from animal models. Key state-of-the-art technologies identified by delegates are listed in Table 2, and included microfluidics, bioprinting and advanced microscopy techniques.
- Facilitating information exchange within the human tissue research community: The current reliance on animal models can start to be overcome by publishing and publicising experiments and case studies which demonstrate that a human tissue model is more predictive of responses in a human. Comparison with data from previous animal experiments would provide a mechanism for doing this without the need for further animal studies.

Conclusions

Tackling the issues outlined in this report will allow more widespread development and uptake of human tissue models for cancer. A range of resources are available to provide information on and sources of human tissue for research to enable this (Table 3). For example, the NC3Rs has recently established a Human Tissue Research hub (http://www.nc3rs.org.uk/ increasing-human-tissue-use) to centralise information and case studies to help researchers overcome some of the barriers described above [19]. By implementing human tissue-based research, predictivity to man and the quality of biological information will improve while reducing the number of animals used in this area. researchers to move away from animal models. Key state-of-the-art technologies identified by delegates are listed in Table 2, and included microfluidics, bioprinting and advanced microscopy techniques.

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Tables 1 and 2

Table 1: The barriers to increased uptake of human tissue models in cancer research	
General:	Requires a multidisciplinary team, with a link between clinical and research staff
	Cultural reliance on mouse models prevents researchers moving to human tissue models
	High cost of working with human tissue can be prohibitive
Clinical:	Routine and sustained access to high quality tissue is limited due to intermittent supply, and the need to first use harvested tissue for clinical diagnosis, leaving less for research.
	Missing or incomplete clinical data accompanying the tissue means that the history and provenance of the tissue is not known
	Difficulty with setting up and carrying out ethics/consenting procedure due to high levels of bureaucracy
Technical:	Working with human tissue is technically challenging and relevant tools and approaches need to be further developed and shared between researchers effectively.
	New model development is costly, can take a long time, and funding is lacking for the development of these approaches.

Table 2: State of the art technologies to enable disease modelling with human tissue			
Fresh Tissue/Cells	Support technologies	Analytic tools	Fixed tissue
3D *in vitro* models	Microfluidics/Organ-on-chip technologies	Super-resolution microscopy	Automated digital pathology
Organotypic tissue slices	Bioprinting	Raman microscopy	Imaging mass spectrometry
Patient derived organoids	Scaffolds	Multiphoton microscopy	Immunohistochemical techniques
Circulating tumour cells	Live tissue culture sampling	Next Generation Sequencing	
Cell spheroids		Single cell 'omics	

Table 3

Table 3: Resources to Enable Human Tissue Use in Cancer Research		
	Role	Link
NC3Rs Human Tissue Hub	Provides information and case studies outlining resources and methods for working with human tissue.	https://www.nc3rs.org.uk/increasing-human-tissue-use
UKCRC Tissue Directory and Coordination Centre	Comprehensive and searchable register of sample collections in the UK covering multiple diseases.	https://www.biobankinguk.org/
National Cancer Research Institute Cellular Molecular Pathology (CM-Path)	Funding and support for the UK cellular molecular pathology community, including training in techniques and co-ordination with clinical trials.	http://cmpath.ncri.org.uk/
Edinburgh Drug Discovery	Edinburgh Drug Discovery is translating cancer drug discovery projects from target identification through to clinical evaluation using human cell-based models	http://www.ed.ac.uk/cancer-centre/impact-and-innovation/translational-science/edinburgh-drug-discovery
NIH NCI Cancer Research	International funding for technology development and cancer research, including using human tissue, from the NIH.	https://imat.cancer.gov/ https://cssi.cancer.gov/
Human Cancer Model Initiative, Sanger Centre	An international effort to develop the next-generation of cancer cell models that better represent the hallmarks and diversity of human cancer. Specifically working on organoid technology.	http://www.sanger.ac.uk/science/collaboration/human-cancer-model-initiative-hcmi
Hubrect Organoid Technology	A not-for-profit collaboration in the Netherlands which banks and provides organoids for research, and carried out training for researchers using organoid technology.	http://hub4organoids.eu/

Appendix 1: Workshop agenda

Agenda - Day 1	
08:30 – 09:00	**Registration and Coffee**
09:00 – 09:30	**Welcome and Introduction** *Professor Gareth Thomas, University of Southampton (Chair)*
09:30 – 10:30	**Keynote Lecture - Exploratory approaches to modeling human cancer: examples from the NCI IMAT portfolio** *Dr Tony Dickherber, Innovative Molecular Analysis Technologies (IMAT) Program, NIH National Cancer Institute*
10:30 – 11:00	**Coffee and poster viewing**
Theme 1: Engineering the microphysiology of cancer with human tissue	
11:00 – 11:30	**Tumour on a chip – application of human tissue to replace animal studies** *Professor John Greenman, University of Hull*
11:30 – 12:00	**3D bioprinting for brain tumour research** *Professor Will Shu, University of Strathclyde and Dr Nick Lesley, Heriot-Watt University*
12:00 – 12:30	**Towards the next-generation of cancer cell lines: derivation of an organoid biobank** *Dr Hayley Francies, Wellcome Trust Sanger Institute*
12:30 – 13:30	**Lunch and poster viewing**
Theme 2: Fresh human tissue	
13:30 – 14:00	**The collection and provision of human tissue for pharmaceutical development in cancer research** *Mr John Spaull, GlaxoSmithKline*
14:00 – 14:30	**The application of living tissue to improve predictivity over animal models in cancer research and drug development** *Dr David Bunton, ReproCELL Europe*
14:30 – 15:00	***In vitro* techniques to reduce the number of animals used in PDX mouse modelling during drug discovery** *Dr Larrisa Carnevalli, AstraZeneca*
15:00 – 15:30	**Coffee and poster viewing**
Breakout Session 1	
15:30 – 17:00	**Current status and definition of barriers to increased use of human tissue in cancer research**
17:00 – 17:25	**Feedback from breakout session**
17:25 – 17:30	**Wrap-up of day 1 and overview of day 2** *Professor Gareth Thomas, University of Southampton*

Appendix 1: Workshop agenda

Agenda - Day 2	
08:30 – 09:00	**Registration and Coffee**
09:00 – 09:30	**Welcome and Introduction** *Professor Gareth Thomas, University of Southampton (Chair)*
09:10 – 09:40	**Cancer tissue provision in the UK and the role of BBMRI-ERIC** *Dr Philip Quinlan, UK Clinical Research Collaboration and BBMRI-ERIC*
09:40 – 10:10	**Edinburgh CRUK centre – human tissue for drug screening consortium** *Professor Neil Carragher, University of Edinburgh*
Theme 3: Fixed human tissue for research and drug development	
10:10 – 10:40	**Applying bioinformatics and genomics to reduce animal use in cancer research** *Dr Christopher Woelk, University of Southampton*
10:40 – 11:10	**Molecular pathology/mass spectrometry imaging in cancer research** *Professor Malcolm Clench, Sheffield Hallam University*
11:10 – 11:30	**Lunch and poster viewing**
Breakout Session 2	
11:30 – 12:30	**Discussion of the barriers to development and implementation of human tissue models for cancer research**
12:30 – 12:55	**Feedback from breakout session**
12:55 – 13:00	**Meeting wrap-up** *Professor Gareth Thomas, University of Southampton*