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

# Workshop report:

## Minimising animal use in target-to-function biology in preclinical oncology research

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# Background

In 2015 the NC3Rs, in collaboration with Medimmune, organised a one day workshop to explore how to use the 3Rs as a foundation for minimising both animal use and drug attrition in oncology research.

A copy of the workshop programme can be found in Annex 1. Topics covered included preclinical testing of immunotherapies and drug-drug combinations, the application of slice culture and microfluidic devices, and how *in vitro* modelling can be used to inform candidate drug selection, patient selection, scheduling and combinations. The workshop was attended by around 100 researchers from academia (60%), the pharmaceutical and biotechnology industries (23%) and other sectors active in preclinical oncology research (17%), including small and medium sized enterprises.

The workshop focused on how the use of animals could be reduced by improving the predictive value of models, both *in vivo* and *in vitro*. There were two main themes discussed. First, delegates identified opportunities to increase collaboration and data sharing between academia and industry to improve the predictive value of animal models of disease and efficacy testing. Second, delegates identified key steps to enable the development of biological complexity in *in vitro* models with relevance to human disease. This report summarises the discussions, including an overview of the models currently used, and provides a guide for future NC3Rs activities in this area.

# Defining the challenges – scientific, clinical and 3Rs

Treating cancer is one of the key unmet medical challenges of the 21st century, with the disease causing 29% of all deaths in the UK in 2014 [1]. Multiple diverse mechanisms of action contribute to tumour development and progression [2]. This results in tumour heterogeneity at both a cellular and population level [3, 4], and makes the disease difficult to treat effectively.

Research from the academic and pharmaceutical research communities continues to result in the approval of new cancer therapies [5], with 18 new compounds approved by the US Food and Drugs Administration in 2015 [6], and 14 by the European Medicines Agency [7]. Nevertheless, despite the fact that many of these new treatments may extend survival by a number of months or improve quality of life, patients with advanced disease often still have a poor prognosis.

The attrition rate of oncology drugs during clinical trials is high [8] and this has led to some pharmaceutical companies discontinuing research [9] or trading/consolidating assets [10] in this area. One major cause of drug attrition in oncology is the lack of efficacy of compounds despite extensive preclinical testing [11], and hence in recent years there has been an increase in the focus on the predictivity of preclinical models [12]. A range of test paradigms is typically used to piece together information on the physicochemical, biological and pharmacological properties of potential efficacious compounds. Through this, drug discovery programmes are used to generate a weight-of-evidence for company and regulatory decision making, using techniques ranging from monoculture and more complex preclinical models through to clinical expression and mechanism of action studies.

Rodent models are heavily relied upon in oncology preclinical development. The procedures involved can cause the animals pain, suffering and distress, and the cancer research community has been proactive in promoting refinements, including publication of welfare guidelines for cancer research [13] which outline best practice. Cancer is a disease area with one of the highest use of rodents in the UK, with 172,499 mice and rats used for oncology and cancer research in 2014 [14]. Advances in *in vitro* technologies bring the prospect of reduction in the number of animals used, as well as an opportunity to develop more predictive tools to address the challenge of drug attrition.

# Overview of model systems for cancer research

A range of published model systems are used for preclinical oncology research including 2D and 3D cell culture, invertebrates (e.g. *Drosophila*), "lower" vertebrate organisms (e.g. zebrafish), and rodents. The latter includes genetically engineered mouse models (GEMM), syngeneic, cell-derived xenografts (CDX) and patient-derived xenograft (PDX) models. A recent review from Genentech [15] illustrates how preclinical models fit into the drug development pipeline of a major organisation. Generally models are selected by their relevance to the target under consideration and the available biomarkers to confirm target engagement/modulation.

## Animal models

GEMMs are used in drug development to examine the effect of targeted mutations, and have advantages in that they harbour patient-relevant mutations, are immunocompetent and can demonstrate features of metastasis. Although GEMMs can model longitudinal tumour development effectively, they are limited by the restricted number of mutations within a tumour and by lack of human tissue components. In addition, practical challenges exist such as the slow development of tumours and difficulties assessing tumour growth [16].

CDX and PDX models allow the study of a whole tumour in situ, but are limited by the need to use immunodeficient mice to avoid xenograft rejection, by non-orthotopic implantation of the xenograft and by the absence or rapid resorption of human stromal components [17]. Published evidence shows that, far from being static isolated cell masses, tumours manipulate stromal components in their immediate environment and the immune system to evade destruction. Key differences exist in human and mouse stromal components which manifest themselves in differential reactions to anti-tumour drugs. For PDX models, selection of clones from a heterogeneous patient tumour can result in the use of minor clones for engraftment, which may not adequately represent the heterogeneity of mutations in the original tumour [18]. Despite such limitations the potential of personalised medicine and development of humanised mice [19] are likely to increase the use of PDX models in the future, particularly for patient/rodent co-clinical trials, and the selection of personalised treatments for patients offered as a service by some commercial organisations [20].

Immunocompetent syngeneic models [21] and GEMMs [22] are being used to support the development of antibody-based therapeutics which modulate the immune system. For example, mouse syngeneic models were used to characterise the mechanism of action of anti-CTLA-4 in the B16 melanoma model [21, 23], which correlated with the successful development of ipilimumab, the T-cell targeted cancer immunotherapy which was FDA approved in 2011 [24].

Multiple differences in immune cell types and expression of key proteins in mice and humans [25] can however make establishing mechanism of action, efficacy and safety in animals complex. Mouse tumour cell lines used in syngeneic models are typically poorly annotated in terms of their mutation load and detailed phenotyping of the immune system in the mouse is necessary to select the appropriate model [15]. Some progress has been made in further investigating the role of the immune system in mouse syngeneic tumour models [26-28]. This is a developing field and further work will be required to fully characterise these models, to link mouse and human genetic and immunological features with treatment response data. Although

humanised mice are available which carry engrafted human immune cells, further work is required to characterise the immune/host cell interactions in these animals and confidently demonstrate immunomodulation-related effects and efficacy.

### *In vitro* technologies

Cell line panels have been effectively used to screen compounds during drug development [29], but have limited predictive and face validity to human tumours [30]. Emerging complex *in vitro* models which combine multiple tissue types in 3D have the potential to improve predictive validity by more closely recapitulating human biology. This includes capturing the complex dynamics between host and tumour cells, diversity of mutations, 3D structure and stromal components [31]. Recent developments in human stem cell and microphysiological device technologies allow 3D modelling of tumours with human cells, organoids or tissue in more physiologically-relevant environments. These models will require further development and characterisation to more fully define their usefulness in the drug development pipeline, and to build regulator confidence in the data they produce. Nonetheless, as the complexity of such systems evolves they will have the potential to reduce animal use in studies to define mechanisms of action, and improve compound selection [32].

## Themes emerging from the workshop

Animal models have been the 'workhorse' of cancer research for many years. The models are often generated and characterised by academic researchers, disseminated via a publication or presentation and subsequently adopted and validated by industry for a specific outcome measure or measures. The effective transfer of knowledge between academic and drug development or industry laboratories is therefore essential. Industry experience suggests that oncology studies are often not well reported in peer-reviewed publications [33]. Furthermore, scientists from a biotechnology company demonstrated that a high number of results from preclinical oncology studies from 'landmark studies' could not be reproduced in other laboratories [34]. Therefore there is a need for more direct collaboration and dialogue.

There are a number of consortium-based approaches to facilitate the sharing of information on animal models to help improve reproducibility and promote greater understanding of the models and their limitations. One example of this, the Cancer Research Technologies' Preclinical Models Network [35], is beginning to centralise information on preclinical models, including protocols. In addition, the SEARCH Breast project connects researchers working on breast cancer, and facilitates sharing of animal tissues and other resources [36]. In the EU, the EuroPDX consortium is creating a not-for-profit library to share patient-derived xenograft material for collaborative projects and multi-centre trials [37]. In addition, the Centre for Biomedical Informatics and Information Technology at the National Cancer Institute in the USA has established the Oncology Models Forum as a repository for models, to share information and reduce replication of model

development [38].

Preclinical and clinical data generated by commercial organisations during drug development could also help improve reproducibility, as clinical examples could be used to validate preclinical modelling. The sharing of industry data can be challenging because of issues around intellectual property and commercial advantage. Nevertheless, there are a number of successful initiatives that demonstrate the positive impact of cross-company data sharing, including the Innovative Medicines Initiative PREDECT consortium [31] and the Animal Model Framework project [39]. Additionally, many funding bodies and journals now require award holders/authors to place datasets in online repositories [40], and the pharmaceutical industry in Europe is increasingly called upon to make clinical trial data available [41].

### Increasing complexity and confidence in *in vitro* approaches

Advances in biology, material science and bioengineering are transforming the availability and utility of complex 3D models by using multiple human cell or tissue types and 3D cell culture [42] to produce organoids or spheroids which resemble functional units of organs [43]. This is being accelerated by developments in media, cell scaffolds, microphysiological devices and bioprinting technologies [44-46]. Stem cell, gene editing and next-generation sequencing technologies are also allowing novel human cancer-relevant assays to be built which allow studies that could only previously be carried out *in vivo* [32]. Nevertheless, there are significant barriers to the increased uptake and application of these models to reduce animal use across a range of disease areas including oncology, including lack of validation against human standard of care compounds.

Stem cell maturity and the relevance of organoids and spheroids to the whole organ need to be fully demonstrated. Some tissues or cell populations are technically more challenging to reconstitute *in vitro* than others, such as the immune system. In addition, the combination of multiple tissues in parallel or within the same device has yet to be demonstrated with most tissue types. Programmes such as the NC3Rs CRACK IT Challenges funding competition provide a mechanism for multi-disciplinary and cross-sector collaboration. An oncology-based CRACK IT Challenge would be timely in terms of providing a test case for developing novel cancer models and embedding them in an industrial setting.

The scientific community, including industry and regulators, needs to build confidence in using these models to make decisions, and demonstrating that they provide information that translates to the clinic or is at least as 'good' as the animal model is critical. Human (and in some instances animal) data against which to benchmark and validate *in vitro* models is essential. A harmonised approach across research groups in terms of data collection and reporting would potentially be game changing in identifying which models recapitulate human physiology and disease based on defined technical and/or performance standards. Critical information would include basal characteristics of the *in vitro* models, for example, the growth rate of cells, production of appropriate proteins and appropriate metabolism. In addition, data describing responses to drugs/compounds would give context to the *in vitro* model that could be compared with clinical data, including responses of key biomarkers in the *in vitro* model to the most

## Themes emerging from the workshop

commonly used standard-of-care treatments for cancer. Access to biobanking and libraries of defined compounds will underpin proof-of-concept in the models and ultimately drive uptake [47].

Ultimately there needs to be regulatory confidence and acceptance in *in vitro* technologies. In cases where there is no relevant animal model, *in vitro* models and functional cross-reactivity studies have been used to build the case for efficacy and safety. This is illustrated by the development pathway for Immunocore's ImmTAC molecules [48]. These are human bi-specific proteins which combine a T-cell receptor based targeting system and an anti-CD3 effector function to activate a T-cell response against cancer cells. Given the human specific nature of these molecules, regulators accepted a preclinical data package which contained no *in vivo* data, but instead relied upon human *in vitro* data to demonstrate safety and toxicology. It is possible that this principle can be extended to other cases where the value of the animal model is unproven. European medicines regulators have introduced the 'safe harbour' concept [49] to allow companies to submit data obtained using new approaches in parallel with data generated using existing studies, typically *in vivo*. The former is not used as part of the regulatory decision making process but instead for consideration of its possible future regulatory acceptance, in effect helping to build regulatory confidence in new approaches whilst minimising the risk to the companies. It will be important to exploit these opportunities as capacity in *in vitro* modelling for oncology increases.

## Conclusions

While animal models continue to be an important component of oncology drug discovery programmes, new technologies and developments in bioengineering are leading to opportunities to increase the human relevance of preclinical modelling, and reduce the number of animals used. Maximising this potential will require a coordinated strategy, new ways of working and partnerships between *in vivo* and *in vitro* scientists, different disciplines, and academia, industry and regulators. There are a number of relevant and ongoing initiatives but more could be done particularly to facilitate data sharing to accelerate the development of *in vitro* models. Increasing the interaction between *in vitro* and *in vivo* researchers through face-to-face meetings such as this workshop and online activities is required to achieve this.

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# References


1. Office of National Statistics. Available from: http://www.ons.gov.uk/ons/rel/vsob1/death-reg-sum-tables/2014/sb-deaths-first-release--2014.html. Accessed 28/9/15;
2. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5) 646-74.
3. Bedard PL, Hansen AR, Ratain MJ, et al. (2013) Tumour heterogeneity in the clinic. Nature 501(7467) 355-364.
4. Meacham CE, Morrison SJ (2013) Tumour heterogeneity and cancer cell plasticity. Nature 501(7467) 328-337.
5. Centerwatch. Available from: https://www.centerwatch.com/drug-information/fda-approved-drugs/therapeutic-area/12/oncology. Accessed 10-12-2015;
6. Centerwatch. Available from: http://www.centerwatch.com/drug-information/fda-approved-drugs/year/2014. Accessed 11/2/16;
7. Hoffmans R. Available from: http://sms-oncology.com/blog/ema-anticancer-drug-approval-in-2014/. Accessed 11/2/16;
8. Arrowsmith J, Miller P (2013) Trial watch: phase II and phase III attrition rates 2011-2012. Nat Rev Drug Discov 12(8) 569.
9. Carroll J. Available from: http://www.fiercebiotech.com/story/high-cost-failure-drives-drug-development-costs-stratosphere/2012-02-10. Accessed 5/3/15;
10. Slaoui M, Mullard A (2015) Moncef Slaoui. Nat Rev Drug Discov 14(7) 452-3.
11. Seruga B, Ocana A, Amir E, et al. (2015) Failures in Phase III: Causes and Consequences. Clin Cancer Res 21(20) 4552-4560.
12. Paul SM, Mytelka DS, Dunwiddie CT, et al. (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov 9(3) 203-14.
13. Workman P, Aboagye EO, Balkwill F, et al. (2010) Guidelines for the welfare and use of animals in cancer research. Br J Cancer 102(11) 1555-77.
14. Home Office. Available from: https://www.gov.uk/government/collections/statistics-of-scientific-procedures-on-living-animals. Accessed 20/9/15;
15. Gould SE, Junttila MR, de Sauvage FJ (2015) Translational value of mouse models in oncology drug development. Nat Med 21(5) 431-9.
16. Richmond A, Su Y (2008) Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech 1(2-3) 78-82.
17. Finak G, Bertos N, Pepin F, et al. (2008) Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14(5) 518-27.
18. Eirew P, Steif A, Khattra J, et al. (2015) Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature 518(7539) 422-6.
19. Garcia S, Freitas AA (2012) Humanized mice: current states and perspectives. Immunol Lett 146(1-2) 1-7.
20. Malaney P, Nicosia SV, Dave V (2014) One mouse, one patient paradigm: New avatars of personalized cancer therapy. Cancer Lett 344(1) 1-12.
21. Liakou CI, Kamat A, Tang DN, et al. (2008) CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc Natl Acad Sci U S A 105(39) 14987-92.
22. Ostrand-Rosenberg S (2004) Animal models of tumor immunity, immunotherapy and cancer vaccines. Curr Opin Immunol 16(2) 143-50.
23. Quezada SA, Peggs KS, Curran MA, et al. (2006) CTLA4 blockade and GM-CSF combination immunotherapy

# References


alters the intratumor balance of effector and regulatory T cells. J Clin Invest 116(7) 1935-45.

24. Maio M, Grob JJ, Aamdal S, et al. (2015) Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. J Clin Oncol 33(10) 1191-6.
25. Mestas J, Hughes CC (2004) Of mice and not men: differences between mouse and human immunology. J Immunol 172(5) 2731-8.
26. Castle JC, Loewer M, Boegel S, et al. (2014) Immunomic, genomic and transcriptomic characterization of CT26 colorectal carcinoma. BMC Genomics 15 190.
27. Kim K, Skora AD, Li Z, et al. (2014) Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc Natl Acad Sci U S A 111(32) 11774-9.
28. Lechner MG, Karimi SS, Barry-Holson K, et al. (2013) Immunogenicity of murine solid tumor models as a defining feature of *in vivo* behavior and response to immunotherapy. J Immunother 36(9) 477-89.
29. Wilding JL, Bodmer WF (2014) Cancer cell lines for drug discovery and development. Cancer Res 74(9) 2377-84.
30. Grainger DW (2014) Cell-based drug testing; this world is not flat. Adv Drug Deliv Rev 69-70 vii-xi.
31. Hickman JA, Graeser R, de Hoogt R, et al. (2014) Three-dimensional models of cancer for pharmacology and cancer cell biology: capturing tumor complexity *in vitro*/ex vivo. Biotechnol J 9(9) 1115-28.
32. van de Wetering M, Francies HE, Francis JM, et al. (2015) Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161(4) 933-45.
33. Kilkenny C, Browne WJ, Cuthill IC, et al. (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 8(6) e1000412.
34. Begley CG, Ellis LM (2012) Drug development: Raise standards for preclinical cancer research. Nature 483(7391) 531-3.
35. Cancer Research UK . Available from: http://www.cancertechnology.co.uk/preclinical-models-network-taking-shape. Accessed 01/10/2015;
36. SearchBreast. Available from: https://searchbreast.org/. Accessed 14/3/16;
37. Hidalgo M, Amant F, Biankin AV, et al. (2014) Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov 4(9) 998-1013.
38. Lu T. Available from: http://ncip.nci.nih.gov/blog/oncology-models-forum/. Accessed 24/11/15;
39. Valentin JP, Bialecki R, Ewart L, et al. (2009) A framework to assess the translation of safety pharmacology data to humans. J Pharmacol Toxicol Methods 60(2) 152-8.
40. McNutt M (2014) Journals unite for reproducibility. Science 346(6210) 679.
41. Cressey D. Available from: http://blogs.nature.com/news/2014/10/europes-milestone-medical-data-transparency-rules-finally-confirmed.html. Accessed 1/10/2015;
42. Chambers KF, Mosaad EM, Russell PJ, et al. (2014) 3D Cultures of prostate cancer cells cultured in a novel high-throughput culture platform are more resistant to chemotherapeutics compared to cells cultured in monolayer. PLoS One 9(11) e111029.
43. Gao D, Vela I, Sboner A, et al. (2014) Organoid cultures derived from patients with advanced prostate cancer. Cell 159(1) 176-87.
44. Li X, Zhang X, Zhao S, et al. (2014) Micro-scaffold array chip for upgrading cell-based high-throughput drug testing to 3D using benchtop equipment. Lab Chip 14(3) 471-81.
45. Maschmeyer I, Lorenz AK, Schimek K, et al. (2015) A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 15(12) 2688-99.

# References


46. Leonard F, Godin B (2016) 3D *In Vitro* Model for Breast Cancer Research Using Magnetic Levitation and Bioprinting Method. Methods Mol Biol 1406 239-51.
47. Clevers H, Bender E (2015) Q&A: Hans Clevers. Banking on organoids. Nature 521(7551) S15.
48. Oates J, Hassan NJ, Jakobsen BK (2015) ImmTACs for targeted cancer therapy: Why, what, how, and which. Mol Immunol 67(2 PtA) 67-74
49. European Medicines Agency (2014) Guideline on regulatory acceptance of 3R (replacement, reduction, refinement) testing approaches. EMA/CHMP/CVMP/JEG-3Rs/450091/2012.

# Annex 1: Workshop Programme

| | |
|---|---|
| 9.00–9.30 | Registration and refreshments |
| 9.30– 9.50 | **Welcome and introduction**<br>Chair: Professor John Hickman, IMI PREDECT Consortium |
| Understanding the challenges in functional cancer biology | |
| 9:50-10:25 | **Selecting the right preclinical models for cancer immunotherapy**<br>Dr Michelle Morrow, MedImmune Ltd |
| 10:25-11:00 | **Value of preclinical models for progressing oncology combinations**<br>Dr Hazel Jones, Cancer Research UK |
| 11:00-11:20 | Refreshments and poster/exhibitor viewing |
| 11:20– 11.55 | **A new Dutch precompetitive Research Institute for *in vitro* human organ and disease models: Human Organ and Disease Model Technologies (hDMT), - towards *in vitro* (pre-) clinical trials "on chips"**<br>Dr Anja van de Stolpe, Philips Research (Philips Group Innovation), The Netherlands |
| Application of new and emerging technologies to oncology research | |
| 11:55-12:20 | **Imaging in animal models of cancer and translation to the clinic**<br>Professor Kevin Brindle, University of Cambridge |
| 12:20 - 12:45 | **Developing immunotherapeutic biologics using *in vitro* approaches: a positive experience**<br>Dr Namir Hassan, Immunocore |
| 12:45 – 13:45 | Lunch and poster/exhibitor viewing |
| 13:45 – 14:10 | **Screening in cultured human tissues to improve selection of pre-clinical candidates**<br>Dr Leo Price, Ocello, The Netherlands. |
| Use of alternative models in preclinical oncology research | |
| 14.10 – 14.35 | **Using pre-clinical models to model patient selection, scheduling and combinations, to optimize therapeutic index of cancer therapies**<br>Dr Simon Barry, AstraZeneca |
| 14.35 – 15.00 | **Precision-cut tumour slices: towards capturing tumour complexity and heterogeneity *in vitro***<br>Professor John Hickman, IMI PREDECT Consortium |
| 15.00 – 15.25 | **Use of zebrafish to study the effects of cytotoxic drugs on inflammatory cells in primary and metastatic tumours**<br>Professor Claire Lewis, University of Sheffield |
| 15.25 – 16.00 | Refreshments and poster/exhibitor viewing |
| Focus and feedback sessions; *In vitro* models and collaborative approaches | |
| 16.00 – 16.30 | **Discussion and vote : Blocks to the adoption of *in vitro* models for preclinical oncology research**<br>Introducer: Dr Namir Hassan |
| 16.30 – 17.00 | **Discussion and vote : Collaboration to improve predictive value of preclinical models in oncology**<br>Introducer: Dr Hazel Jones |
| 17.00 – 17.30 | **Q&A and audience vote** |
| 17.30 – 18.30 | Networking and poster/exhibitor viewing |