Overview

Evolution of the Supermodel: Progress in Modelling Radiotherapy Response in Mice

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Abstract

Mouse models are essential tools in cancer research that have been used to understand the genetic basis of tumorigenesis, cancer progression and to test the efficacies of anticancer treatments including radiotherapy. They have played a critical role in our understanding of radiotherapy response in tumours and normal tissues and continue to evolve to better recapitulate the underlying biology of humans. In addition, recent developments in small animal irradiators have significantly improved *in vivo* irradiation techniques, allowing previously unimaginable experimental approaches to be explored in the laboratory. The combination of contemporary mouse models with small animal irradiators represents a major step forward for the radiobiology field in being able to much more accurately replicate clinical exposure scenarios. As radiobiology studies become ever more sophisticated in reflecting developments in the clinic, it is increasingly important to understand the basis and potential limitations of extrapolating data from mice to humans. This review provides an overview of mouse models and small animal radiotherapy platforms currently being used as advanced radiobiological research tools towards improving the translational power of preclinical studies.
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Statement of Search Strategies Used and Sources of Information

Articles cited in this manuscript were sourced through a literature search on the Medline/Pubmed database using the search terms: radiotherapy, preclinical models, mouse model, radiation response, normal tissue and small animal radiotherapy. Full articles were sourced and retrieved when the abstract was deemed relevant. The bibliographies of retrieved papers were also searched and relevant articles included.

Introduction

Radiotherapy remains a major cancer treatment modality prescribed with curative intent in almost two-thirds of all cancers [1]. Since the pioneering use of X-rays in the treatment of cancer by Emil Grubbe in 1908 [2], clinical oncology has undergone multiple technological evolutions, largely driven by advances in technology and engineering. These achievements have resulted in the routine use of advanced, high-precision radiotherapy, offering unparalleled ability to delineate target volumes, conform dose and minimise normal tissue complications based on individual patient anatomy. In addition to physical developments, radiotherapy will continue to evolve and gain biological precision. This will involve the use of patient-specific genomic, transcriptomic, proteomic, radiomic or metabolomic data to stratify and biologically guide treatments in which parameters including dose, technique and fractionation schedule are optimised in individual patients [3].

The clinical benefits of precision radiotherapy have been supported by an improved understanding of radiotherapy response at the cell, tissue and whole-organism levels. Over the past century, improved knowledge of the biological basis of radiotherapy response in tumours and normal tissues has strongly influenced progress in the clinic. This is exemplified by the classical Rs of radiobiology (repair,

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reoxygenation, reassortment, redistribution) that have provided the biological basis of fractionation and response [4]. Furthermore, radiobiology research has led to many other fundamental discoveries that have potential to guide future innovations in the clinic, such as understanding the molecular basis of radiosensitivity and the DNA damage response, and novel paradigms such as non-targeted effects, abscopal effects and immunogenic cell death [5—9]. As our understanding of the complexity of radiotherapy response deepens, further opportunities will be identified to improve therapeutic index by targeted modulation of responses in tumours and normal tissues [10]. A critical step in successfully translating these discoveries is the development and application of advanced preclinical models that reflect patient scenarios both in terms of underlying biological response and physical radiation exposure conditions [11—13]. In a specialty as dynamic and ever-changing as clinical oncology, it follows that preclinical models should also evolve in being able to more accurately recapitulate aspects of patient biology.

Progress in targeted genome-editing technologies is allowing the development of next-generation mouse models to study the molecular mechanisms of carcinogenesis and response to therapy within a native tissue microenvironment [14,15]. In addition, the recent development of small animal irradiators capable of delivering small, highly conformal beam geometries under image guidance is enabling previously unimaginable approaches to be explored in the laboratory. The combination of contemporary mouse models with small animal irradiators represents a major step forward for the radiobiology field in being able to much more accurately replicate clinical exposure scenarios. This review aims to provide an overview of mouse models and small animal radiotherapy platforms currently being used as radiobiological research tools towards improving the translational power of preclinical studies.

In Vivo Models of Radiotherapy Response

Animal models of human cancers have played a major role in our current understanding of tumour biology and in the development of cancer therapies. In the context of radiotherapy response, the first documented in vivo model came from Regaud and Nogier in 1911 [16], who showed the tissue-sparing effects of fractionation in the testes of a ram. Since then, many species have been used to further our knowledge of the effects of radiation, including mice, rats, pigs and dogs [17—19]. However, it is the laboratory mouse, Mus musculus, that has become established as an invaluable experimental tool in radiobiology studies that require the complex microenvironment of a living mammal.

The widespread use of mouse models in biomedical research is predicated on the genetic and physiological similarities between mouse and man [20]. Despite evolutionary divergence from a common ancestor around 85 million years ago [21], both species have common organs and system physiology, and share many pathologies due to large-scale synteny across most of the genomes. Comparative genome sequencing in humans and C57BL/6J mice has shown only around 300 unique genes to one species or the other [22]. A comparison of the main biological features in mouse and man is shown in Table 1, which highlights a number of important interspecies similarities and differences. In particular, the overall radiosensitivity of inbred mice is significantly different to man after total body irradiation. In mice, death typically occurs within 30 days and so the end point is defined as the $LD_{50/30}$, that is the dose expected to cause death to 50% of an exposed population within 30 days and has been reported to range from 6.5 to 9 Gy [23—25]. In comparison, humans are more radiosensitive, with the incidence of death after total body irradiation highest around 30 days after exposure, although deaths may continue up to 60 days. The end point is therefore

Table 1
Comparison of key biological and physiological characteristics of mice and humans. Information in this table has been collated from a number of sources [28—31]

	Mus musculus	Homo sapiens
Typical body mass	30 g	70 kg
Basal metabolic rate	High	Low
Lifespan (years)	Short (<3)	Long (<80)
Rate of aging	Fast	Slow
Rate of senescence	Slow	Fast
Haematopoiesis in the spleen	Active in adulthood	Ends before birth
Age to sexual maturity	6—8 weeks	15 years
Gestation period	20 days	9 months
Number of offspring	8—10 pups/litter	1 child/birth
Reproductive span	2 years	40 years
Rate of neoplastic transformation	High	Low
Estimated age for population to display 30% cancer incidence	2 years	70 years
Most frequent age-related neoplasia	Mesenchymal derived	Epithelial derived
Radiosensitivity	Strain dependent	Genotype dependent
LD_{50} (Gy)*	6.5—9	4.5

* LD_{50} is expressed as the total dose expected to cause death to 50% of an exposed population within a defined time period. In mice this is defined 30 days after exposure ($LD_{50/30}$) and in humans is defined 60 days after exposure ($LD_{50/60}$).

defined as the $LD_{50/60}$ and is around 4 Gy for healthy individuals exposed to low linear energy transfer (LET) sources [23–27].

Although these doses indicate up to a two-fold difference in radiosensitivity across species, it is important to note that variation may be significantly less for clinically relevant exposures where one type of target tissue may dominate response. Under these conditions, mice are more relevant as models of radiobiological response. Also, mice have many practical advantages (small, economical, short gestation periods) and can be routinely subjected to genome-editing and breeding strategies to better understand the basis of tumour initiation, progression and response to therapy [15,32]. These advantages have resulted in the widespread use of mice in radiobiology studies in dissecting underlying mechanisms of response and transitioning promising candidate therapies from bench to bedside. An overview of commonly used radiobiological response models and experimental irradiation techniques used in radiobiology studies is illustrated in Figure 1. The selection of the most appropriate tumour model, mouse strain and irradiation technique is dependent on overall experimental objectives. These should importantly be determined with extensively *a priori* knowledge of the approaches that can be used and appreciation of their associated advantages and limitations. It is also critical that *in vivo* experiments are carried out within the framework of the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) using both the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines in the respective planning and reporting of research involving animal models [33,34].

Murine Tumour Models

In studies of tumour response it is critical that murine tumour models display a number of key features, including replicating the underlying human tumour genetics of tumours, share common anatomy and histology to the human disease and have a preserved tumour microenvironment and immune cell function [32,35]. In reality, none of the currently available tumour models shown in Figure 1 meet all of these criteria and consequently investigations are carried out based on the availability of a particular mouse strain and on a known compatible tumour model. Commonly this involves transplantation of cell lines or tumour fragments into immunocompromised hosts, often with patient-derived stroma to better mimic the human tumour microenvironment.

Transplantation models can be generated by either ectopic or orthotopic implantation, depending on the requirements to preserve tumour phenotype and stromal interactions; both of which are important considerations in accurately determining radiotherapy response [36,37]. Xenograft tumour models offer some level of predictive insight into clinical outcomes when evaluating radiation response as some of the original tumour characteristics such phenotypes and genotypes and tissue architecture. However, it should be noted that studies involving human cell implantations commonly use SCID mice that are homozygous for the $Prkdc^{scid}$ mutation, which encodes a catalytic subunit of DNA-dependent protein kinase catalytic subunit (DNA-PKcs). This is a critical component of non-homologous end joining-mediated double-strand break (DSB) repair and V(D)J recombination involved in T and B cell maturation [16]. Consequently, SCID mice lack functional lymphocytes but are also highly radiosensitive, with an $LD_{50/30}$ of 3 Gy [17]. The first step in V(D)J recombination involves the creation of DSBs and subsequent hairpin formation by an enzymatic complex produced by the recombination activating genes (RAG) 1 and 2. Defects in RAG also produce T and B cells forms of SCID but without the radiosensitivity, suggesting that Rag1 knockout strains such as $Rag1^{tm1Mom}$ are more appropriate for use in radiation response studies [38].
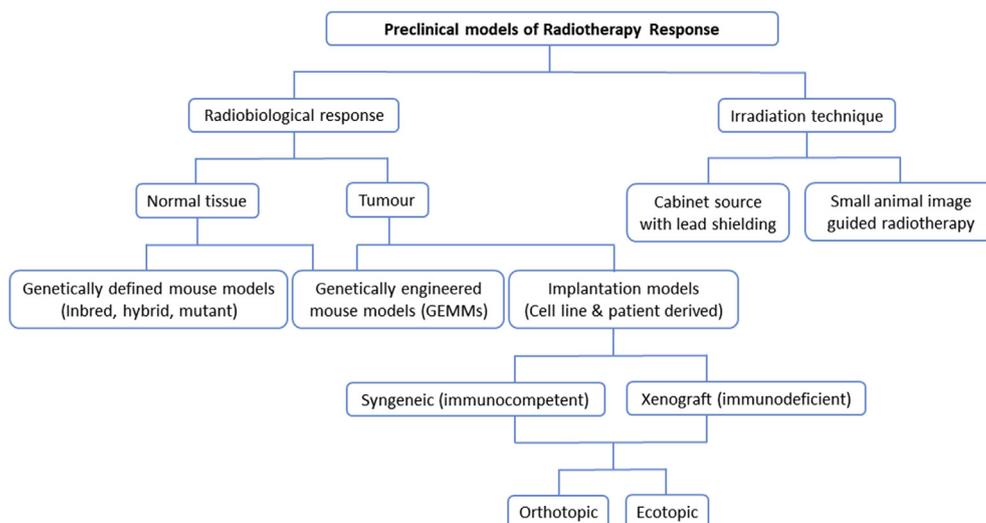

Fig 1. Summary of preclinical mouse models of tumour and normal tissue response and irradiation techniques used in radiobiology research.

Cell lines or tumour material of mouse origin can be allografted into syngeneic hosts when the experimental objective is to preserve a functional immune environment. In contrast to immunodeficient mice, immunocompetent inbred mouse strains, such as C3H, are more radioresistant, with a reported $LD_{50/30}$ of 7.8 Gy [17]. For syngeneic tumour models it should be noted that mouse embryonic stem cells, which give rise to all of the cells of the adult mouse, are known to be deficient in components of non-homologous end joining DSB repair [39], which may limit comparison with radiation responses observed in human cells.

Tumour transplantation models continue to be used as they are a readily accessible, cost-effective method to obtain preclinical tumour response data. Genetically engineered mouse models (GEMMs), in which tumours spontaneously develop due to oncogene activation or the somatic inactivation of tumour suppressor genes, are histologically and genetically accurate models of human cancers. GEMMs have been developed using conventional gene targeting approaches involving the insertion of targeted donor constructs into mouse embryonic stem cells. Once inserted into the genome, the stem cells are microinjected into blastocysts, which are implanted into pseudo-pregnant female recipients to allow extensive breeding of chimeric offspring [40]. This is a complex, time-consuming and costly process that has evolved due to the development of synthetic sequence-specific nucleases allowing the injection of gene-editing mRNA or protein directly into zygotes.

Genome editing uses designer nucleases, such as zinc finger nucleases, transcription activator-like effector nucleases and, more recently, the RNA-guided clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated genes (CRISPR/Cas9) system to introduce targeted mutations into the mouse germ line [13,26,37]. CRISPR/Cas9 has become established as the genome-editing tool of choice due to the ease and speed with which the technology can be used to experimentally investigate specific genetic modifications, allowing more objective study of carcinogenesis [15,41]. GEMMs have broad utility in preclinical radiotherapy for evaluating tumour responses in defined genomic backgrounds similar to their human counterparts, but are equally applicable to studies in normal tissues and radiation-induced carcinogenesis where the impact of specific genomic alterations on a defined experimental end point can be assessed. A summary of the main advantages and limitations of mouse tumour models commonly used in radiobiology studies is presented in Table 2.

Models of Normal Tissue Response

Laboratory mice have been used extensively to investigate the radiation responses of normal tissues after wholebody, hemi-body and targeted irradiation of specific organs. Mouse tissues have similar turnover rates and organisational structures to human tissues and so develop similar pathologies in terms of early and late occurring radiation toxicities. Normal tissue injuries involve a complex series pathogenic cascade, which affects tissue homeostasis and is dependent on immune status, vascular integrity, cytokine signalling and oxidative stress levels [40,42]. A particularly important consideration in the context of assessing the translational power of mice in modelling normal tissue responses is the functional similarity of the immune system and features of response to challenge.

Mice have been used as a central tool in immunological research and in many respects accurately mirror characteristics of human biology and so data concerning the

Table 2
Summary of the main advantages and limitations of mouse tumour models used in radiobiology research

Tumour model	Advantages	Limitations
Implantation models Xenograft (cell line derived)	Robust, reproducible and synchronous growth	Immunodeficient, non-human hosts
	Large number of annotated cell lines available	Not representative of genetic heterogeneity
	Easy to establish large cohorts and assess response	Perturbed tissue architecture and microenvironment
		Expensive and require pathogen-free housing
Xenograft (patient derived)	Preserved genetic, histological and microenvironmental features of human tumour	Immunodeficient or partially immunocompetent, non-human hosts
	Can be predictive of human tumour response	Can have low engraftment rates
	Representative of genetic heterogeneity	Expensive and technically challenging
Syngeneic	Robust, reproducible and synchronous growth	Not representative of genetic heterogeneity
	Easy to establish large cohorts and assess response	Limited number of poorly annotated cell lines
	Immunocompetent host	Can be fast growing
	Preserved tumour–stroma interactions	Poorly representative of human phenotypes
Genetically engineered mouse models (GEMMs)	*De novo* tumorigenesis in tissue of origin	Limited genetic heterogeneity (to number of target genes)
	Preserved genetic, histological and microenvironmental features of human tumour	Slow and variable growth rates
	Can be predictive of human tumour response	Expensive and time consuming
	Immunocompetent host and *in situ* tumour–stroma interactions	

structure and function of the immune system can be readily translated across species [43]. However, there are many significant differences across species relating to the development and activation of the innate and adaptive immune systems. Specific examples include differences in myeloid and lymphocyte populations, such as higher neutrophil numbers in adult humans (50–70% neutrophils compared with 10–25% in mice), mechanisms of T cell activation and differentiation such interferon-α-mediated differentiation of T helper cells, and differential expression of proinflammatory cytokines and their cognate receptors [28,44].

In the context of radiation response, it is important to consider differences following challenge, which have been debated over the past few years. A controversial study by Seok *et al.* [45] compared correlations of gene expression changes in a total whole white blood cell sample following acute inflammatory stresses (trauma, burn and endotoxemia) between humans and mice. They reported highly similar genomic responses in humans that were not reproduced in mouse models, which showed close to random matching to their human counterparts (R^2 values <0.1) [45]. These provocative data challenged the validity of mouse models of human disease due to low biological similarity and caused widespread debate across the scientific community.

In response, a series of prolific editorials raised concerns regarding the failure of the study to examine responses in more than a single immune-polarised mouse strain, that there was lack of correction for differentially abundant cell types, the data did not reflect patient variations and that the data analysis did not consider these confounding factors [46–48]. Furthermore, re-evaluation of the same gene expression datasets focused on genes significantly altered in humans and mice, providing directly contradicting conclusions. The analysis showed that transcriptional changes in mouse models had extraordinarily significant correlations with those of the human conditions (Spearman's rank correlation coefficient: 0.43–0.68; genes changed in the same direction: 77–93%; $P = 6.5 \times 10^{-11}$ to 1.2×10^{-35}), indicating commonly regulated pathways in mice that closely recapitulate those in human inflammatory conditions [49]. These data highlight potential challenges in drawing definitive conclusions regarding immune responses in mice and humans. These caveats should be carefully considered when interpreting and attempting to translate data across species, as mice will probably continue to be the primary *in vivo* model for experimentally evaluating radiotherapy response in normal tissues.

Preclinical Radiotherapy Techniques

Another critical limitation towards accurately modelling radiotherapy response in mice is the ability to irradiate small target volumes with high precision in a manner analogous to the clinical techniques. Historically, *in vivo* radiobiology experiments have been carried out using broad fields generated from fixed, kilovoltage radiation sources with *in situ* lead shielding to target the volume of interest. Although some of these experiments involved the irradiation of large volumes (usually whole body or whole thorax) and did not require precision image guidance, these procedures have high levels of uncertainty due to inaccurate beam targeting and had no treatment planning system or dose verification.

Similar to advances in the clinic, preclinical technologies have also evolved to allow the delivery of conformal irradiation beam geometries to small target volumes under image guidance. Over the past decade, several commercially available and research small animal irradiator systems have been implemented in radiobiology laboratories around the world. These systems consist of rotating or fixed gantries with cone beam computed tomography (CBCT) detectors or conversions of micro-CT devices with dedicated treatment planning systems [50]. A summary of these devices and their features is presented in Table 3. An illustrated example of a small animal irradiator (the Small Animal Radiotherapy Research Platform [SARRP], Xstrahl Life Sciences, UK; panel A) is shown in Figure 2, set against a clinical linear accelerator (Varian TrueBeam, Varian Medical Systems Inc, USA; panel B), highlighting the similar features of the two systems.

Numerous motion management technologies have been implemented clinically to improve delivery accuracy, reduce acute toxicity and improve survival outcomes [63]. Similar approaches have been adopted by small animal irradiators, such as the recent development of a beam-gating assembly incorporating a fast, rotating X-ray shutter together with optical breathing monitoring technology for adaptive gating control. This system allows the beam delivery to take place only during the stationary resting phase of the breathing cycle [64]. A small animal proton therapy device has also been achieved using a 50 MeV proton beam from an existing cyclotron to produce 2 mm collimated beam coupled to SARRP [65] that will offer new opportunities in proton therapy research [66]. Finally, most systems now include on-board integration of optical imaging methods, including bioluminescence imaging and tomography for tumour targeting and response monitoring [51,67].

The implementation of small animal irradiators into the laboratory is a significant step forward in the development of translationally relevant preclinical radiotherapy models. Researchers are now empowered to conduct experimental studies in a manner that more accurately reflects clinical technique scenario using millimetre-sized beams under precision CBCT image guidance. The striking similarities in workflows that are now present between the clinical and experimental settings are illustrated in Figure 3, using a representative example of prostate tumour irradiation in mouse and man.

Perhaps the greatest opportunity for the application of small animal irradiators lies in their ability to deliver localised irradiation, targeting small volumes that were not previously achievable using conventional lead shielding. Figure 4 illustrates the dosimetric advantages offered by small animal irradiators by comparing a small animal radiotherapy treatment plan (panels A–C) and dose volume

Table 3
Summary and characteristics of currently available small animal image-guided radiotherapy systems

Device	Vendor/institute	Beam energy (KeV)	Dose rate (Gy min)	Image guidance	Treatment planning system	Reference
Commercial						
Small Animal Radiotherapy Research Platform (SARRP)	Xstrahl Life Sciences, UK	5—225	1—4	CBCT BLT	Muriplan	[51—53]
X-RAD 225Cx SmART	Precision X-ray Inc, USA	5—225	0.01—4	CBCT BLI	SmART-Plan	[45,54]
Academic						
Image-guided small animal arc radiation treatment system (iSMAART)	University of Miami, USA	45—225	2.5—4	CBCT	In house	[55—57]
Small animal image-guided radiotherapy system (SAIGRT)	Technical University of Dresden, Germany	10—225	1—4	CBCT	In house	[58]
Small animal conformal radiation therapy device (SACRTD)	University of Arkansas, USA	60—225	0.4—3	CBCT	In house	[59]
Micro-CT based devices	Stanford University, USA	70—120	2	CBCT	In house	[60]
	Heidelberg University, Germany	10—160	4.5—6.4	CBCT	In house	[61]
	University of Western Ontario, Canada	70—140	2	CBCT	In house	[62]

CBCT, cone beam computed tomography; BLT, bioluminescence tomography; BLI, bioluminescence imaging.

histograms targeting an orthotopic prostate tumour with conventional lead shielding (panel D) and using a small animal irradiator (panel E) for a prescribed dose of 15 Gy. It can be seen that for the shielded configuration the critical organs at risk (bladder and rectum) receive most of the prescribed dose of 15 Gy. This can be viewed in contrast to the highly conformal delivery achieved with a small animal irradiator, as delivering four parallel-opposed 5 × 5 mm beams shows almost complete sparing of the bladder and rectum.

The extended capabilities offered by small animal irradiators continues to be leveraged by investigators in delivering advanced preclinical protocols with wide-ranging experimental objectives. Treatment plans are being developed by combining CBTC datasets with online or sequential functional and anatomical imaging, which have been demonstrated in orthotopic pancreatic, lung and brain tumour models [54,68,69]. Other recent examples of innovative applications in the field include evaluation of radiosensitising strategies, such as hyperthermia [70] and drug
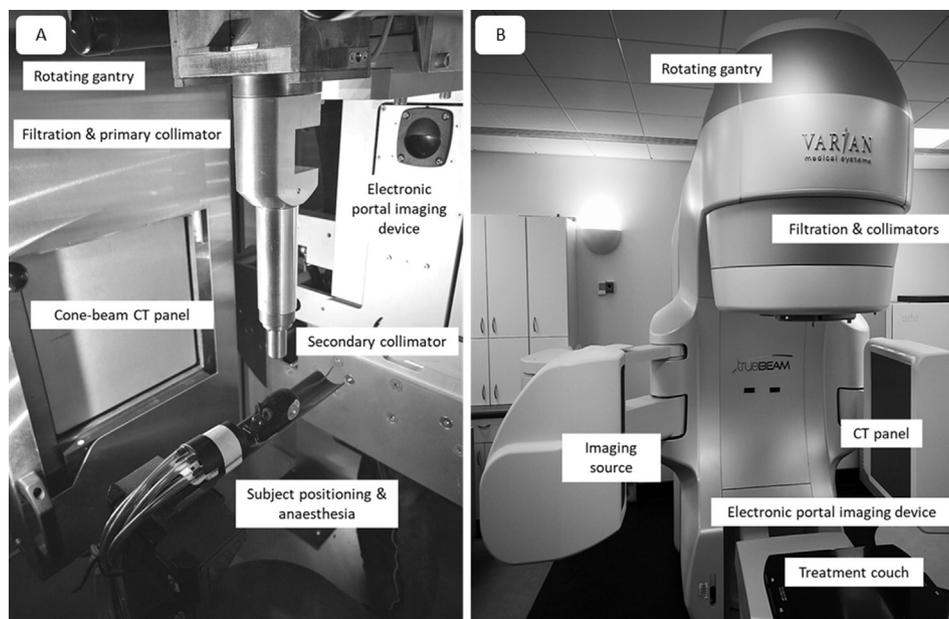

Fig 2. Illustration of the main features of the Small Animal Radiotherapy Research Platform (SARRP; A) with a clinical linear accelerator (B).
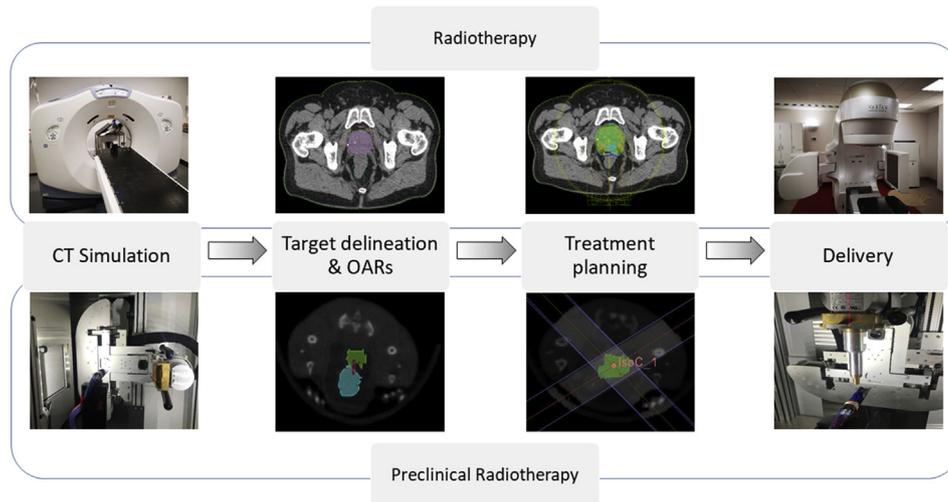
Fig 3. Schematic representation showing similarities between clinical and preclinical radiotherapy workflows enabled by small animal irradiators.
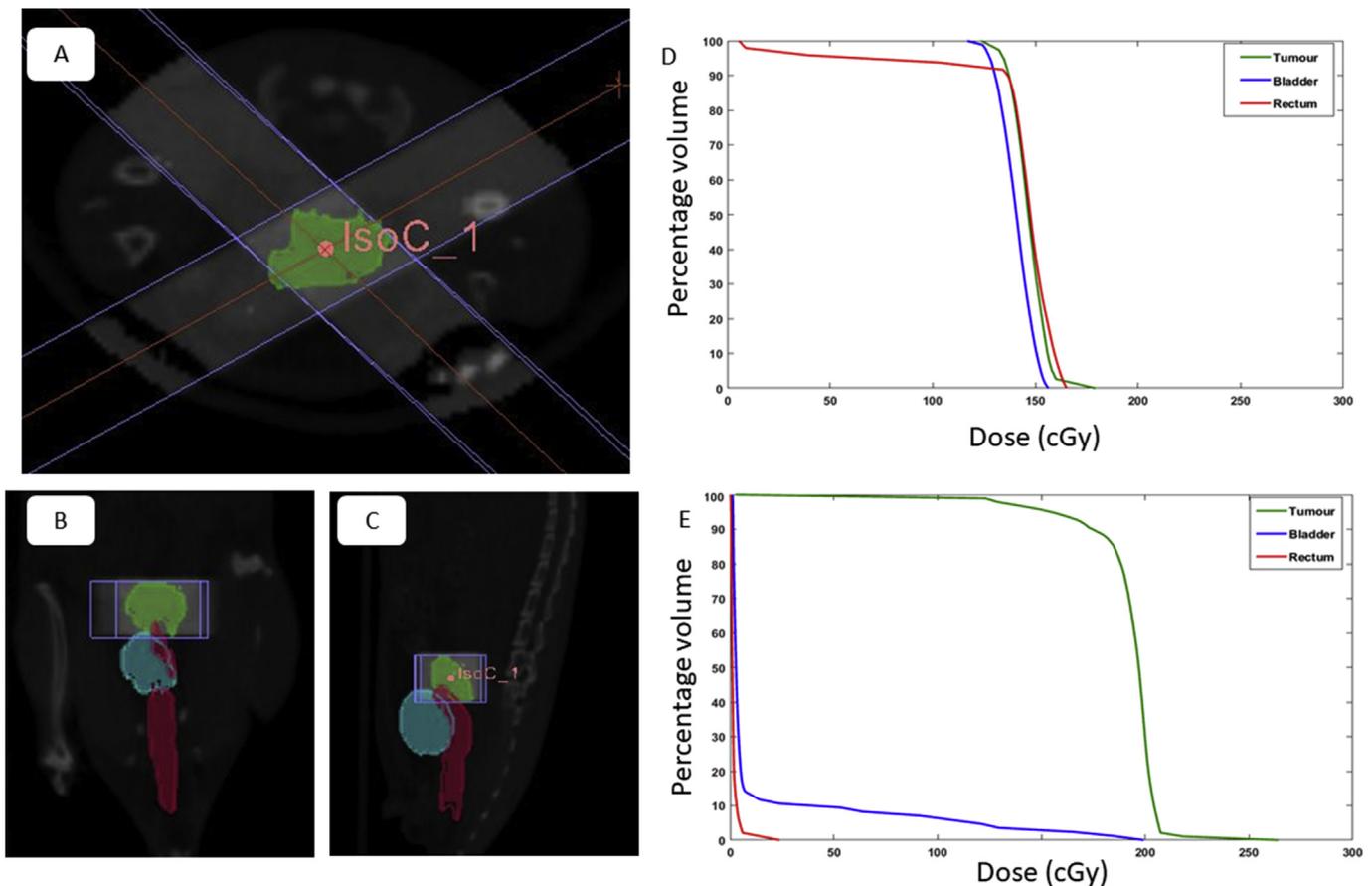

Fig 4. Representative example of a small animal radiotherapy treatment plan generated in Muriplan (Xstrahl Life Sciences, UK) and dose volume histograms targeting an orthotopic prostate tumour using conventional lead shielding and using a small animal irradiator. The treatment plan from cone beam computed tomography was generated for an isocentre of within a tumour implanted into the anterior lobe of the mouse prostate (green) and organs at risk (OARs), the bladder (blue) and rectum (red). The plan shows irradiation of the target volume using four parallel-opposed beams with a 5×5 mm geometry in the axial (A), coronal (B) and sagittal (C) planes. Dose volume histograms are shown for conventional targeting of the prostate using lead shielding (D) and using a small animal irradiator to deliver the illustrated plan (E). The significant dosimetric advantage of the small animal irradiator is highlighted with the critical OARs receiving minimal dose compared with the conventional approach in which the OARs receive most of the prescribed dose of 2 Gy.

radiotherapy combinations [71−74], studies of normal tissue damage in the lung, bladder and heart [72,75−78] and effects of spatial fractionation, including dose painting [79] and intensity-modulated radiotherapy [79−81].

Realisation of the Preclinical Supermodel

Radiation biology continues to play a crucial role in the advancement of clinical oncology by providing a rationale for the implementation of new treatment strategies and clinical concepts. Preclinical models are essential tools in radiation biology that have been widely used to interrogate underlying molecular mechanisms of response and because of the many similarities between mouse and man, *Mus musculus* remains a widely used experimental system. Without doubt, mouse models have made a major contribution to our understanding of radiotherapy response, yet their utility in translating to improvements in the clinic is based on the extent to which they accurately replicate human biology and predict clinical outcome. The process of accurately modelling radiotherapy response in mice remains challenging, based not only on the heterogeneity of cancer but on the complexity and variability of radiotherapy responses in tumours and normal tissues. The ideal model does not exist and undoubtedly there are aspects of human radiotherapy response that cannot be modelled in mice. However, when used with an appreciation of their biological and physical limitations, mouse models can provide essential datasets supporting translation of novel therapies to the clinic.

In terms of tumour response, advances in genome editing using CRISPR/Cas9 have enabled spatial and temporal control over gene regulation to delineate the dynamics of carcinogenesis and inform the basis of radiosensitivity. Spontaneous tumour models harbouring multiple mutations have been shown where the top three most significantly mutated genes in lung adenocarcinoma (p53, Lkb1 and Kras^{G12D}) have been modulated by loss-of-function mutations in p53 and LKB1 and homology-directed repair-mediated Kras^{G12D} mutations [82]. Humanised xenograft models involving implantation of human tissue into NOD-scid-γ mice with injection of peripheral blood or bone marrow cells, allow for an almost complete reconstitution of the immune response, which is also a major evolution in trying to accurately recapitulate aspects of patient immune response during treatment [83]. These models are also important in the context of immune checkpoint therapies and may play a central role in optimising radio-immunotherapy combinations, an area of intense experimental and clinical research [84].

The application of contemporary mouse models used with small animal irradiators has much potential to deliver translationally relevant data leading to better outcomes in radiotherapy trials. A caveat to this approach is that most mouse models used in radiobiology studies are young and treatment naïve, and do not have other pre-existing comorbidities that may impact on radiotherapy response. An important next step is to develop systems that imitate patient-specific risk factors, such as age, previous interventions and comorbidities such as cardiovascular disease or diabetes. Further refinement of mouse models and irradiation techniques will probably improve their power in predicting clinical outcome, advancing radiobiology ever closer towards the preclinical supermodel.

Conclusions

Major advances have been made in the quest to deliver translationally relevant mouse models of radiotherapy response that have focused on recreating the underling biology of human disease and irradiation techniques reflecting those used the clinic. Although the radiobiological supermodel may not yet have been fully realised, or may even be achievable, advanced experimental strategies now hold much potential to improve translational success. With continued multidisciplinary team science across biology, physics and clinical oncology, these tools should inform the next evolution of radiation-based cancer therapies, leading to improved survival and quality of life of patients receiving radiotherapy

Conflict of interest

The author declares no conflict of interest.

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