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Preclinical models of radiation induced lung damage: Challenges and opportunities for small animal radiotherapy.

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ABSTRACT

Despite a major paradigm shift in radiotherapy planning and delivery over the past three decades with continuing refinements, radiation induced lung damage (RILD) remains a major dose limiting toxicity in patients receiving thoracic irradiations. Our current understanding of the biological processes involved in RILD which includes DNA damage, inflammation, senescence and fibrosis, is based on clinical observations and experimental studies in mouse models using conventional radiation exposures. Whilst these studies have provided vital information on the pulmonary radiation response, the current implementation of small animal irradiators is enabling refinements in the precision and accuracy of dose delivery to mice which can be applied to studies of RILD. This review presents the current landscape of preclinical studies in RILD using small animal irradiators and highlights the challenges and opportunities for the further development of this emerging technology in the study of normal tissue damage in the lung.

INTRODUCTION

Radiotherapy continues to play a critical role in the curative management of cancer patients with inoperable malignancies including the lung.[1] Despite a major paradigm shift in radiotherapy planning and delivery over the past 3 decades with continuing technical refinements, damage to surrounding normal tissue remains a major dose limiting toxicity in patients receiving thoracic irradiation[2,3].

CURRENT CLINICAL AND BIOLOGICAL FEATURES OF RADIATION-INDUCED LUNG DISEASE

Radiation induced lung damage (RILD) in the form of acute radiation pneumonitis (RP) or late occurring fibrosis (RF) can develop weeks to years after radiotherapy to significantly compromise quality-of-life and may be lethal in outcome [4]. RP is an early inflammatory driven acute toxicity that develops within weeks or months following radiotherapy with symptoms including dyspnoea, cough, chest pain and low grade fever, and in severe cases can cause oxygen dependency and may be life threatening. The incidence of moderate and severe RP ranges from 10–20% in NSCLC radiotherapy patients[5] and in severe cases (≥ Grade 3) the mortality rate associated with RP may approach 50%.[6]

Radiation fibrosis (RF) is a late toxicity which develops between 6 to 12 months following radiotherapy leading to permanent impairment of lung function. Fibrosis is one of the most common adverse effects of radiotherapy which is characterised by the progressive accumulation of extracellular matrix constituents replacing the normal functional parenchyma. Both RP and RF may occur not only following localised high dose lung irradiation but also from any clinical scenario involving irradiation of the thorax, including breast radiotherapy[7] and whole body irradiation for bone marrow ablation prior to transplantation.[8]

Current risk predictors of RILD are derived from population based estimates and the relative contributions of each of the multiple patient-related risk factors remains unclear.[9,10] These factors include treatment parameters, e.g. total dose, fractionation schedule, the percentage of lung receiving specific dose levels (e.g. V20, V30, etc.), and mean lung dose (MLD). In addition, patient-specific biological parameters, including individual radiosensitivity, age, smoking status and pre-existing lung disease,[9,10] are known to contribute to RILD risk, although, again, the mechanistic role of these factors remains to be fully determined.

At the cellular level, RILD, as with nearly all radiation-induced outcomes, is mediated by multiple cellular processes including DNA damage, inflammation and senescence that are coordinated through complex, sequential series of interactions between cellular and humoral factors, including immune and parenchymal cells, cytokines and adhesion molecules[11]. Our current understanding of the temporal nature of these processes is based both on clinical observations and experimental studies in mouse models which have been shown to recapitulate various physiologic or pathologic aspects of the radiation induced pulmonary sequelae observed in humans.[12,13] The sequential development of RILD in mouse models is shown schematically in Figure 1 where the immediate pulmonary radiation response is similar to that of canonical wound healing. This involves the rapid release of inflammatory cytokines by injured cells, leading to the activation and/or recruitment of innate immune leucocytes, predominantly macrophages and neutrophils, into the lumen.[14] This response quickly resolves, but in those volumes where the pulmonary tissue is not fully repaired, the lung enters a progressive and dysregulated process culminating in the acute and/or late endpoints of pneumonitis and fibrosis, respectively.[15]

Classical studies using whole thorax irradiation (WTI) have provided vital information on the pulmonary radiation response of different rodent strains and defined dose thresholds for lung toxicities. Moreover, a recent study in Wistar rats characterized dose, region and time-

dependent changes in which 50% of the total lung volume was irradiated showing changes in pulmonary function correlated with the structural changes[16]. The implementation of small animal irradiators has enabled major refinements in the precision and accuracy of dose delivery to mice, allowing for more clinically relevant doses and fractionation schedules to be achieved experimentally.[17–19] Representative examples of the dose distributions achieved using WTI and a small animal irradiator targeting a 30% sub-volume of the lung for a prescribed dose of 20 Gy are shown in figure 2. It can be seen that for WTI, both lungs receive most of the prescribed dose at 15-20 Gy, with the organs at risk (OAR), specifically the heart and oesophagus receiving 15 Gy or more. These values can be viewed in contrast to the highly conformal delivery achieved using small animal irradiators, where in the representative example, a 5 x 5 mm beam was used to target a lung sub-volume with almost complete sparing of the OARs.

Currently, only a small number of published studies focussing on RILD have incorporated the use of small animal irradiators (summarised in table 1) [20–25]. This suggests that as preclinical techniques evolve, many challenges and opportunities remain for studies to advance our understanding of the key biological and physical factors underpinning radiation response in the lung.

CHALLENGES FOR SMALL ANIMAL RADIOTHERAPY

A recent review by the European Society for Radiotherapy and Oncology Advisory Committee in Radiation Oncology Practice (ESTRO ACROP) considered some of the key physics challenges for the optimal use of small animal image irradiators in translational radiation research.[26] Within this framework, commissioning, treatment planning, image registration and data processing were highlighted as areas for careful assessment in preclinical radiotherapy studies. Whilst these challenges are equally applicable to both tumour and normal tissue

targeting, preclinical radiotherapy studies focussing on radiation response in the lung have a number of additional complexities:

- The total capacity of the mouse lung is only around 1 mL compared to approximately 6 L in a human. [27] This leads to inherent challenges associated with the delivery of small beam sizes, in the order of several millimetres. Also, in this size range, photon scattering may result in high levels of uncertainty associated with non-Monte Carlo dose calculation engines and absolute dose measurements.[28,29] In addition, tissue segmentation based on imaging datasets acquired using 50-60 kV photons is challenging because of significant differences in the energy absorption of soft tissues as can be seen in Figure 2A and may lead to uncertainties in dose calculation. However, novel approaches using dual energy CT have demonstrated more accurate segmentation and dose calculation in small animal phantoms. [30,31]
- For many target sites within the thorax and abdomen, respiratory motion adds complexity to the provision of high accuracy targeting during treatment.[32,33] In the mouse lung, the degree of motion is in the order of 5 mm and likely to be greater in rats.[34] Using a four-dimensional (4D) digital mouse whole body phantom (MOBY), respiratory motion has been shown to result in an overestimation of mean tumour dose by up to 11%, with a maximum 3D tumour displacement of up to 4.1 mm found along the *y* and *z* axis, and no displacement along *x* axis for the four simulated cases.[35] These data, supported by the current absence of preclinical 4D-CT capabilities, suggest respiratory motion is an important aspect for the refinement of preclinical models.
- Although mice are considered relevant experimental models of RILD, direct extrapolation to humans is confounded by diverse strain-related differences that have been extensively characterised.[36,37] Importantly, individual strains have experimental utility for studying different aspects of the sequential pathologies that develop during RILD.[38] For example,

C3H/He mice develop classic early phase pneumonitis, but do not develop fibrosis below single doses of 20 Gy. In contrast, in C57BL/6 mice, 20 Gy is sufficient to cause fibrosis in all mice,[37] whilst other strains are intermediate between these extremes.[36,37] Selection of the appropriate experimental mouse strain is, therefore, particularly important and requires careful consideration of experimental endpoints when relating to early and late RILD, dose and target volume.

- Non-invasive pulmonary function tests in humans use established techniques to determine key parameters for spirometry, lung volumes and diffusing capacity such as tidal volume (TV), forced exhaled volume in 1 sec (FEV_1), and diffusing capacity and transfer factor of the lung for carbon monoxide (D_{LCO} and T_{LCO})[39]. These parameters cannot be directly measured in mice. Instead, unrestrained whole body plethysmography is used to longitudinally monitor respiratory frequency, TV and peak flows. However, this technique does not accurately measure airway resistance and should be complemented with invasive measures of pulmonary mechanics using forced ventilator systems in anaesthetized, tracheotomised animals[40]. Whilst these approaches provide important functional measurements of RILD, they are terminal and require significant numbers of animals for longitudinal effect studies which should be carefully considered into experimental designs.
- The vast majority of studies have been performed in non-tumour bearing mice as tumour growth in orthotopic or genetically engineered models, more often than not, results in mortality prior to any possible development of toxicities. A major challenge is to develop robust models for sequential evaluation of tumour and normal tissue response *in situ* accounting for the multifactorial interactions within the tumour-normal tissue microenvironment which are known to be important mediators of radiation response.[41] A number of orthotopic tumour models involving implantation of human NSCLC cells into immuno-deficient mice have recently been reported [42,43] and may provide further insight

into radiation induced tumour-stromal interactions with the caveat of reduced B- and T-cell mediated function. To facilitate progression of preclinical models towards more clinically relevant disease and radiotherapy exposure scenarios, is important that significant efforts are made to address the pertinent challenges described above, that could improve translational success and ultimately delivering benefit to patients.

OPPORTUNITIES FOR SMALL ANIMAL RADIOTHERAPY

Despite the improvements that have been made in cancer therapy, overall, the outcome for lung cancer patients remains poor along with the quality of life experienced by survivors. The advent of small animal irradiators and their potential to improve clinical translation of basic science modelling offers a number of opportunities:

1. Perhaps the greatest opportunity for small animal irradiators applied to studies of RILD lies in their ability to deliver localised irradiation, targeting small volumes of the lung. This approach is a significant refinement over WTI techniques that have been used (of necessity) since the pioneering studies of Travis and colleagues[12,13] . The significant dosimetric advantage of small animal irradiators (highlighted in figure 2) more accurately represents clinical scenarios and so extends current experimental capabilities. Interestingly, overall improvements in the outcomes for patients with lung cancer following radiotherapy and better characterisation of late toxicities have led to an estimated incidence of late cardiac toxicity in up to 33% of patients.[44] Currently, there is a lack of data underpinning the causes and consequences of cardiac toxicity in lung cancer radiotherapy practice; this offers potential areas for investigation, such as identifying the critical structures within the heart, dose volume effects, along with biological mechanisms of response.

2. Numerous motion management technologies have been implemented clinically to reduce acute toxicity and improve quality of life and survival outcomes.[33] Similarly, a preclinical beam gating assembly has been developed, incorporating a fast rotating X-ray

shutter along with optical breathing monitoring technology and associated adaptive gating control.[45] This system allows beam delivery to take place only during the stationary resting phase of the breathing cycle, which improves conformity during animal movement and is an important new tool in moving preclinical studies closer to clinical practice.

3. Other opportunities exist in combining small animal irradiators with preclinical molecular and functional imaging, e.g. using micro-positron emission tomography (micro-PET), magnetic resonance imaging (MRI) and bioluminescence imaging (BLI). Whilst these approaches have not yet been demonstrated in studies of RILD, a recent report has shown the feasibility in monitoring cerebral blood flow, vascular endothelial function and cellular metabolism in a hemi-irradiated brain normal tissue response model.[46] Opportunities may include assessment of pulmonary changes during or after radiotherapy using PET, functional lung avoidance and response-adaptive escalation (FLARE) and dose boosting to metabolically active sub-volumes, all of which are being explored in the clinic.[47–49] Research efforts using combined imaging modalities have focussed on tumour response in defining target sub-volumes for dose painting and adaptive treatments. The combination of a small animal irradiator with PET and MRI has applied to demonstrate PET-guided sub-volume boosting in a glioblastoma rat model.[50] In addition, the impact of differential PET based dose boosting has been reported in a linac irradiated rhabdomyosarcoma model. [51]

4. Finally, despite the physical and biological advantages offered by proton therapy, several unknowns remain regarding the radiobiology of proton beams in tumours and normal tissues.[52] The recent development of an image-guided low-energy proton system for preclinical radiotherapy studies[53] will undoubtedly enable further exploration of key aspects of radiobiological response to protons in proving their promise for patients with NSCLC.[54]

In conclusion, small animal irradiators coupled with disease appropriate mouse models have high potential to further our understanding of radiation response in the lung. These approaches may help to address long-standing questions regarding the mechanistic principles and inter-relationship of acute and late effects in models that more faithfully simulate the clinical situation. This would further facilitate the development of novel therapies that can provide therapeutic advantage whilst preserving lung function and quality of life after radiotherapy.

Figure legends

Figure 1: Generalised schema of sequential inflammatory and fibrotic driven changes in the mouse lung following irradiation. Dependent on strain, mice may show some or all of these toxicities following exposure to ionising radiation. Based on Travis et al[13].

Figure 2: A- Representative CBCT (coronal and axial section) with outlined target (healthy lung) and organs at risk (oesophagus and heart) and isocentre. B- Representative dose volume histograms (DVHs) of the lungs, heart and oesophagus for a prescription dose of 20 Gy delivered using a small animal irradiator targeting a 30% sub-volume of the lung (panel i). whole thorax irradiation (WTI) (panel ii) The significant dosimetric advantage of small animal irradiators is highlighted with the critical organs at risk (OARs) receiving minimal dose compared to WTI where the OARs receive most of the prescribed dose.

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Figure 1

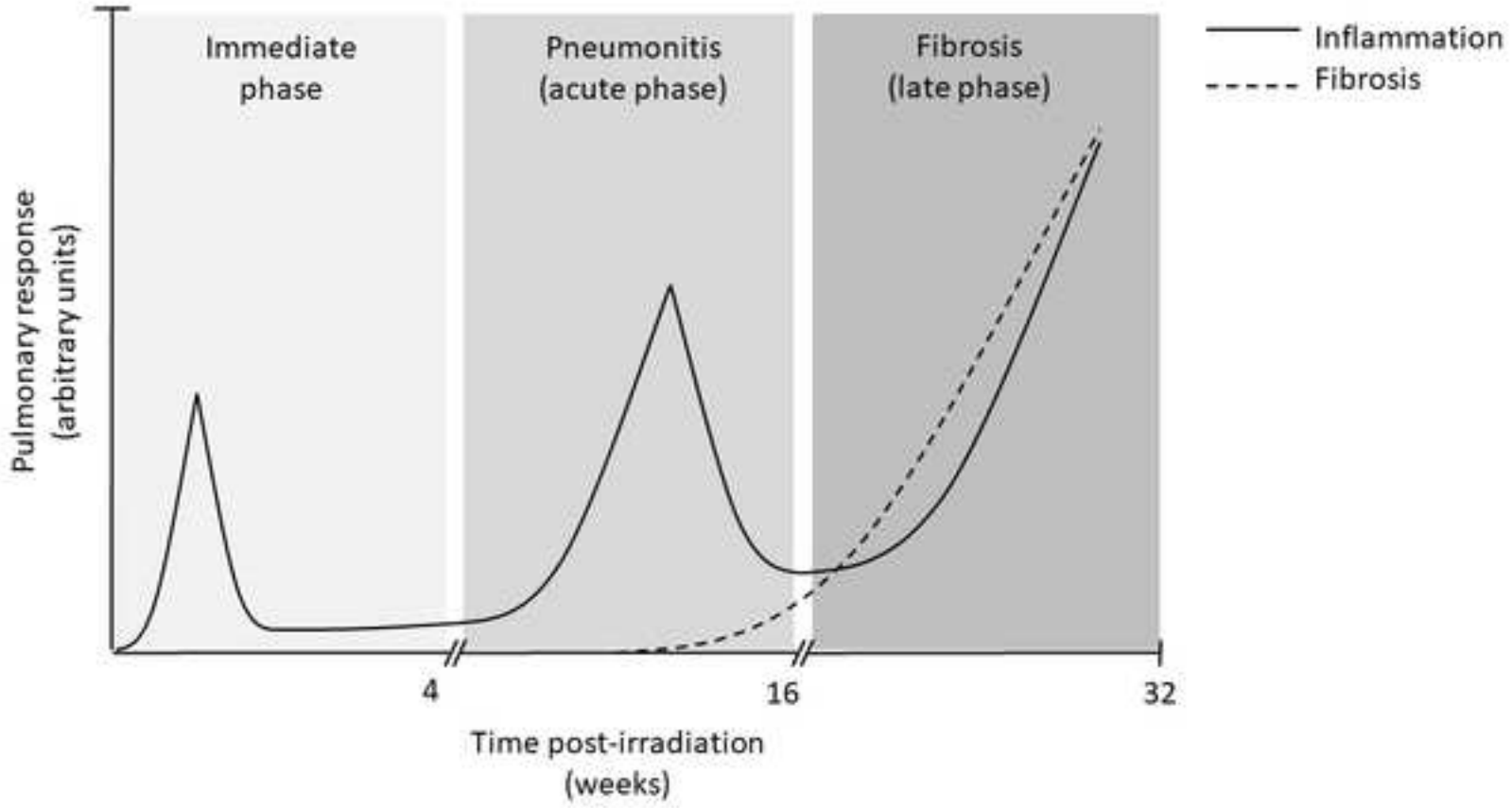
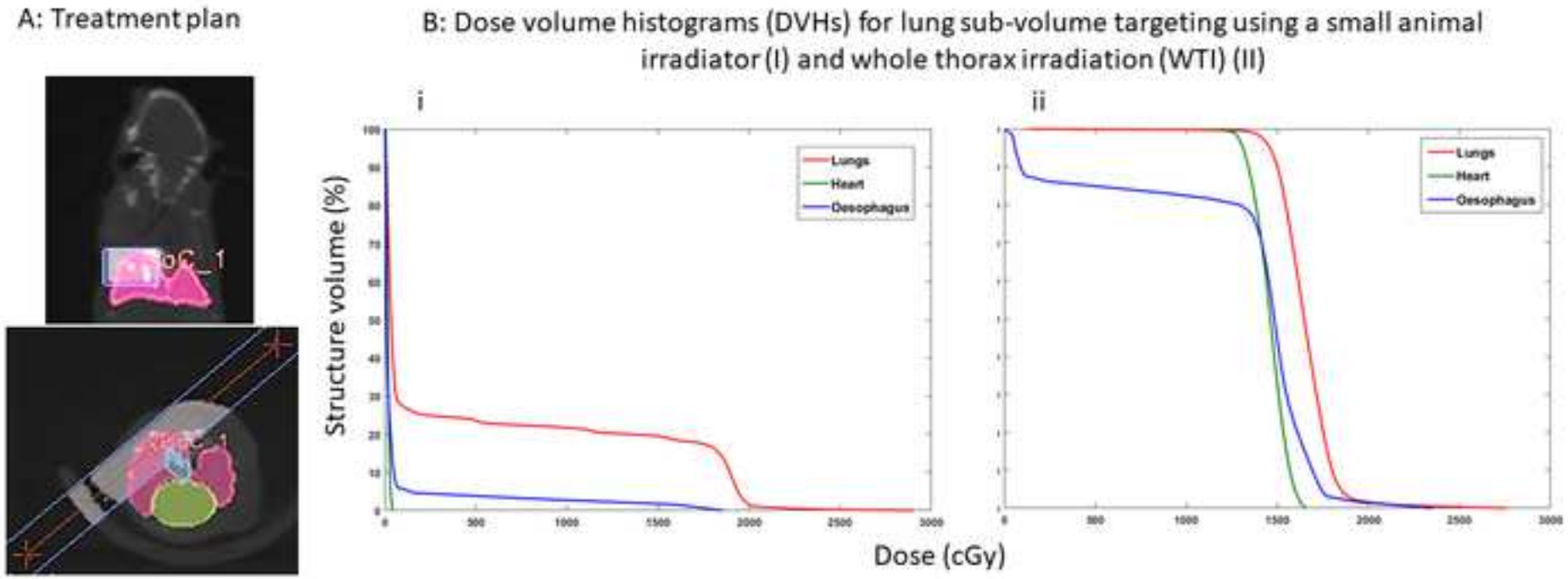


Figure 2



Table

Author	Strain	Irradiation source	Beam geometry	Dose (Gy)	Time-points (weeks)		Experimental end-points
Ghita et al. 2018	C57BL/6 C3H/He	SARRP	5 x 5 mm POP	20 3 x 6.67	72 hours- 26 weeks	RP, RF	Neutophil Macrophage CBCT Masson's Trichrome
Sievert et al. 2018	C57BL/6	SARRP	8 x 6 mm POP	8, 16	20-50	RF Survival	CBCT Elastica van Gieson
De Ruysscher et al. 2017	C57BL/6	XRAD 225Cx	5 mm diameter POP	20	4 - 39	RF	Micro-CT Masson's trichrome
Dunne et al. 2017	C57BL/6J	SARRP	5 x 5 mm POP	20 3 x 6.67	4 – 26	RF	CBCT Masson's trichrome
Granton et al. 2014	C57BL/6	XRAD 225Cx	5 mm diameter POP	4 - 20	4 - 39	RF	Micro-CT
Hill et al. 2011	Sprague Dawley rats C57BL/6 C57BL/6 TNFR1KO C57BL/6 TNFR2KO	Co-60 XRAD 225Cx	Whole thorax POP	10 Gy	4 -28 <24		Micronuclei Breathing rate