Abstract: *Imaging Hemi-Thoracic Radiation Induced Lung Toxicity in Mice*

A thesis submitted for the degree of Doctor of Philosophy at the University of Oxford, Cynthia L. Eccles, CRUK/MRC Oxford Institute for Radiation Oncology and Biology and Wolfson College

Lung cancer is the second most frequently diagnosed cancer, and the leading cause of cancer deaths in the United Kingdom. Two thirds of patients present with advanced disease, which contributes significantly to the high mortality rate associated with this disease. Radiation therapy (RT) is used frequently in the treatment of lung cancer, however the delivery of tumouricidal doses is often limited by the risk of radiation induced lung toxicity (RILT). RILT occurs in an acute phase as pneumonitis followed by a later phase of fibrosis. Because the RILT symptoms are often similar to those of pulmonary infection or disease progression, diagnosis can be difficult.

Approaches to improving the outcome of RT for lung cancer include:

1. Detection of RILT at an early stage. This would allow for the timely introduction of treatment and, therefore, avoidance of severe episodes of RILT. Longitudinal imaging studies of patients undergoing lung RT, using ionizing radiation (IR) free imaging techniques, such as magnetic resonance imaging (MRI) may provide a means of early RILT detection.
2. Concurrent administration of targeted radiosensitizers. Several molecularly targeted agents have been explored in this context. For example, the PI3K inhibitors Nelfinavir, NVP-NBKM120, and NVP-BEZ235 cause radiosensitization in pre-clinical models of cancer. These agents are being introduced into clinical practise and so it is important to investigate their effect on normal lung tissues.

This thesis reports the development of a high throughput, multi-modality imaging programme for the longitudinal investigation of normal tissue toxicity in a pre-clinical model.

The aims of the research were:

1. To investigate novel, non-invasive imaging methods for the detection of RILT.
2. To compare the incidence and severity of RILT following radiation alone to that following radiation plus PI3K/AKT/mTOR inhibition.

Eight longitudinal imaging studies were undertaken in C57BL6 and CBA mice to detect the effects of single (right) lung irradiation with or without targeted radiosensitizers. Radiation was delivered in a single fraction (14, 18 or 20 Gy) using a Gulmay cabinet irradiator, or a small animal radiation research platform (SARRP) to deliver single (20 Gy) or fractionated (3 x 15 Gy) RT under image guidance. Plethysmography, computed tomography (CT) and magnetic resonance imaging (MRI) were undertaken in representative animals from each cohort at baseline and at pre-determined time points up to 20 weeks. Histological examination of lung tissue was undertaken for correlative studies. RILT was scored using a semi-quantitative system reflecting the percentage of the lung volume that was abnormal on imaging. CT densities, MRI signal intensities, and area under the curve (AUC) or initial area under the curve (iAUC) estimations, were used for t-tests and regression analyses to determine the statistical significance of lung changes. An exploratory analysis of dynamic

contrast enhanced MRI (DCE-MRI) as a tool to detect early radiation pneumonitis was undertaken using model- and non-model-based analyses.

The acquisition of high quality cardiorespiratory gated spin echo (SEMS), DCE, and high resolution MR angiography (ANGIO) on 4.7 and 7 T magnets was achieved by continuous optimization of imaging parameters and gating techniques.

The main findings from the work presented in this thesis were:

1. The incidence of RILT following hemi-thoracic radiation was similar whether the detection method used was MRI, CT or histological examination.
2. Severe RILT can be detected using cardiorespiratory gated DCE-MRI. Preliminary data suggest DCE-MRI may also detect mild to moderate RILT but this requires further validation.
3. The incidence and severity of RILT following RT + PI3K inhibitors was not significantly different to the incidence and severity of RILT following RT alone.